Molecular Psychiatry (2015), 1–7

www.nature.com/mp

ORIGINAL ARTICLE

SKA2 methylation is associated with decreased prefrontal cortical thickness and greater PTSD severity among trauma-exposed veterans

N Sadeh[1,2], JM Spielberg[2,3], MW Logue[4,5,6], EJ Wolf[1,2], AK Smith[7], J Lusk[1], JP Hayes[1,2,3], E Sperbeck[1], WP Milberg[8,9], RE McGlinchey[8,9], DH Salat[3,10], WC Carter[11], A Stone[11], SA Schichman[11], DE Humphries[12] and MW Miller[1,2]

Methylation of the *SKA2* (spindle and kinetochore-associated complex subunit 2) gene has recently been identified as a promising biomarker of suicide risk. Based on this finding, we examined associations between *SKA2* methylation, cortical thickness and psychiatric phenotypes linked to suicide in trauma-exposed veterans. About 200 trauma-exposed white non-Hispanic veterans of the recent conflicts in Iraq and Afghanistan (91% male) underwent clinical assessment and had blood drawn for genotyping and methylation analysis. Of all, 145 participants also had neuroimaging data available. Based on previous research, we examined DNA methylation at the cytosine–guanine locus cg13989295 as well as DNA methylation adjusted for genotype at the methylation-associated single nucleotide polymorphism (rs7208505) in relationship to whole-brain cortical thickness, posttraumatic stress disorder symptoms (PTSD) and depression symptoms. Whole-brain vertex-wise analyses identified three clusters in prefrontal cortex that were associated with genotype-adjusted *SKA2* DNA methylation (methylation$_{adj}$). Specifically, DNA methylation$_{adj}$ was associated with bilateral reductions of cortical thickness in frontal pole and superior frontal gyrus, and similar effects were found in the right orbitofrontal cortex and right inferior frontal gyrus. PTSD symptom severity was positively correlated with *SKA2* DNA methylation$_{adj}$ and negatively correlated with cortical thickness in these regions. Mediation analyses showed a significant indirect effect of PTSD on cortical thickness via *SKA2* methylation status. Results suggest that DNA methylation$_{adj}$ of *SKA2* in blood indexes stress-related psychiatric phenotypes and neurobiology, pointing to its potential value as a biomarker of stress exposure and susceptibility.

Molecular Psychiatry advance online publication, 1 September 2015; doi:10.1038/mp.2015.134

INTRODUCTION

The suicide rate among US service members is alarmingly high, with a recent study of 1.3 million veterans reporting that the suicide risk is 41–61% higher for veterans than civilians.[1] Studies suggest that warzone trauma, posttraumatic stress disorder (PTSD) symptoms and other post-deployment mental health problems put veterans at heightened risk for suicide relative to the general population.[2] Although the biological mechanisms involved in this association are not yet well-understood, converging lines of evidence implicate stress-related molecular alterations of the hypothalamic-pituitary-adrenal (HPA) axis, as well as changes in brain morphology and neural function in the etiology of suicide.[3] Furthermore, early adversity and exposure to traumatic stress have been linked to changes in the methylation status and transcriptional activity of genes that regulate HPA-axis function.[4,5] These changes are associated with lower glucocorticoid receptor expression, decreased glucocorticoid negative feedback responses to stress and hypercortisolism.[6,7] Brain regions densely populated with glucocorticoid receptors, such as the prefrontal cortex and hippocampus, are especially vulnerable to neuronal damage and cell death as a result of these effects.[8–11]

One recently identified gene that may have a critical role in this process is *SKA2* (spindle and kinetochore-associated complex subunit 2). The SKA2 protein is implicated in chaperoning the glucocorticoid receptor from the cytoplasm into the nucleus and, by doing so, it serves as a moderator of negative feedback inhibition of the HPA axis and a neuroprotective function by enhancing activation of glucocorticoid receptors.[12] The clinical relevance of the *SKA2* locus was recently demonstrated by an epigenome-wide analysis of post-mortem brain tissue conducted to identify genes associated with suicide. Guintivano *et al.*[13] showed that methylation of probe cg13989295 in the 3′ untranslated region of *SKA2* in prefrontal neurons was associated with suicide, with suicide decedents showing greater DNA methylation and less *SKA2* expression in surrounding tissue than controls. They found that *SKA2* methylation in blood predicted

[1]National Center for PTSD, Behavioral Science Division, VA Boston Healthcare System, Boston, MA, USA; [2]Department of Psychiatry, Boston University School of Medicine, Boston, MA, USA; [3]Neuroimaging Research for Veterans Center, VA Boston Healthcare System, Boston, MA, USA; [4]Research Service, VA Boston Healthcare System, Boston, MA, USA; [5]Department of Biomedical Genetics, Boston University School of Medicine, Boston, MA, USA; [6]Department of Biostatistics, Boston University School of Public Health, Boston, MA, USA; [7]Department of Psychiatry & Behavioral Sciences, Emory University School of Medicine, Atlanta, GA, USA; [8]Translational Research Center for TBI and Stress Disorders and Geriatric Research, Educational and Clinical Center, VA Boston Healthcare System, Boston, MA, USA; [9]Department of Psychiatry, Harvard Medical School, Boston, MA, USA; [10]Department of Radiology, Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA; [11]Pharmacogenomics Analysis Laboratory, Research Service, Central Arkansas Veterans Healthcare System, Little Rock, AR, USA and [12]Massachusetts Veterans Epidemiology Research and Information Center, VA Boston Healthcare System, Boston, MA, USA. Correspondence: Dr N Sadeh, National Center for PTSD, Behavioral Science Division, VA Boston Healthcare System, 150 South Huntington Avenue, Boston, MA 02130, USA.
E-mail: Naomi.SamimiSadeh@va.gov
Received 2 April 2015; revised 28 July 2015; accepted 29 July 2015

suicidal ideation and reduced suppression of waking cortisol in clinical samples after controlling for genotype (which is correlated with cg13989295 methylation), concluding that methylation is a more proximal predictor than genotype. These findings provide preliminary evidence that DNA methylation of *SKA2* is an indicator of glucocorticoid signaling dysregulation and a potentially useful biomarker of suicide vulnerability. A recent independent study found decreased expression of *SKA2* in blood from violent suicide completers compared with non-suicidal controls,[14] replicating the findings of Guintivano *et al.*[13] An important next step is to determine whether epigenetic variability at *SKA2* is also associated with identifiable alterations in neural integrity in psychiatrically relevant regions of the brain and/or stress-related psychiatric disorders linked to suicide.

Thus, building on previous research on *SKA2*, the primary aims of this study were to examine whether *SKA2* methylation is associated with alterations in cortical thickness and suicide-related psychiatric symptoms, specifically PTSD and depression. We focused on cortical rather than subcortical structures (for example, hippocampus), based on evidence of reduced expression of *SKA2* in prefrontal tissue of suicide patients. Based on evidence that the SKA2 protein may be involved in mitigating the neurotoxic effects of stress, we hypothesized that greater DNA methylation of the cytosine–guanine (CpG) site identified by Guintivano *et al.*[13] would be associated with decreased cortical thickness in prefrontal brain regions. We also expected DNA methylation to be associated with greater PTSD and depression symptoms given their associations with suicide risk.

MATERIALS AND METHODS

Sample

The sample consisted of 200 white non-Hispanic service members of Operations Enduring Freedom, Iraqi Freedom and New Dawn (OEF/OIF/OND) (see Table 1 for sample characteristics).[1] Participants were consecutively enrolled in the Translational Research Center for Traumatic Brain Injury (TBI) and Stress Disorders, a VA RR&D Traumatic Brain Injury Center of Excellence at VA Boston Healthcare System. DNA methylation data were available for all 200 participants, and neuroimaging data were available for 152 of those participants. The final sample for neuroimaging analyses was 145 after excluding individuals with a history of moderate or severe TBI. Approval for the study was obtained from all relevant Institutional Review Boards and regulatory committees. After a complete description of the study was given to participants, informed consent was obtained.

Table 1. Characteristics of OEF/OIF/OND trauma-exposed veteran study sample

	Mean (s.d.)	N *(%)*
Age (years)	31.8 (8.4)	
Sex (male)	—	182 (91%)
Military deployment duration (months)	12.9 (8.7)	—
Psychiatric medication use[a]		
No		108 (54%)
Yes		88 (44%)
Current PTSD[b]	—	
No		83 (41%)
Yes		116 (58%)
Current MDD[b]	—	
No		150 (75%)
Yes		49 (25%)
Current SUD[b]	—	
No		171 (86%)
Yes		28 (14%)

Abbreviations: MDD, major depressive disorder; OEF/OIF/OND, Operations Enduring Freedom, Iraqi Freedom and New Dawn; PTSD, posttraumatic stress disorder; SUD, substance use disorder. [a]Four missing cases. [b]One missing case.

Procedures

DNA genotyping and methylation. DNA was extracted from peripheral blood samples. Whole-genome genotyping data were obtained by hybridizing DNA samples to Illumina HumanOmni 2.5–8 microarrays and scanning with an Illumina iScan System (Illumina, San Diego, CA, USA). Single nucleotide polymorphism (SNP) imputation was performed using Impute2 (ref. 15) and 1000 genomes reference data (The 1000 Genomes Project Consortium). DNA methylation data were obtained by hybridizing bisulfite-modified DNA to Illumina HumanMethylation450 K microarrays and scanning with an Illumina iScan System (Illumina). Details on genotyping and methylation methods are available in the Supplementary Materials.

Methylation analyses focused on the CpG dinucleotide implicated by Guintivano *et al.*;[13] Illumina probe cg13989295 and the intervening SNP rs7208505. Because the cytosine at this position of the CpG site measured by cg13989295 is one allele of the (C–T) SNP rs7208505, cg13989295 methylation is highly correlated with rs7208505 genotype. Given that the cytosine measured by cg13989295 is one allele of the SNP rs7208505, the number of alleles that can be methylated depends on an individual's genotype (for example, for the C/T genotype, methylation is possible at one allele). Thus, before evaluating whether methylation associates with the phenotypes of interest, it was prudent to take genotype into account (that is, adjust for genotype), because the range of possible methylation levels (0–100%, typically) was correlated with genotype and thus restricted in some participants. See Supplementary Materials for associations between SNP and methylation (Supplementary Figure S1). The estimated proportion of methylation (Beta-value) of cg13989295 was logit transformed prior to analysis.

Morphometric acquisition and processing. Structural imaging data were acquired on a 3T Siemens TIM TRIO whole-body MRI scanner (Siemens Healthcare, Erlangen, Germany). Two T1-weighted anatomical scans (voxel size = 1 mm^3, time to repeat = 2530 ms, time to echo = 3.32 ms, field of view = 256x256, no. of slices = 176) were acquired and averaged to create a single high contrast-to-noise image. The standard FreeSurfer v5.1 (Martinos Center for Biomedical Imaging, Boston, MA, USA) morphometric pipeline was computed, including reconstruction of the cortical mantle and spatial smoothing of 20 mm full width at half maximum. Cortical surface models were manually checked slice-by-slice and edited for accuracy.

Psychiatric symptoms

Posttraumatic stress disorder. Current PTSD symptom severity and PTSD diagnosis were assessed by doctoral level psychologists using the Clinician Administered PTSD Scale.[16] The frequency and intensity of each DSM-IV PTSD criterion were assessed for the month preceding the assessment. Severity scores were calculated by summing the frequency and intensity scores for all 17 symptoms. All participants endorsed a DSM-IV PTSD Criterion A event. One participant was missing PTSD data.

Depression. Current depression symptom severity was assessed via the 14-item total depression subscale of the self-report depression anxiety stress scale.[17] Current major depressive disorder (MDD) diagnosis was assessed by doctoral level psychologists using the Structured Clinical Interview for DSM-IV Axis I Disorders.[18] Twelve participants were missing the depression anxiety stress scale, and one participant was missing MDD diagnosis information.

Data analysis

The data analytic approach was based on the study by Guintivano *et al.*[13] For each phenotype, we examined associations with rs7208505 genotype, cg13989295 methylation and methylation adjusted for genotype. Methylation adjusted for genotype ($methylation_{adj}$) was calculated by taking the residuals of a linear model of *SKA2* 3′ untranslated region DNA methylation as a function of rs7208505 genotype. All statistical models were adjusted for age, sex and population substructure (as indexed by the first three

ancestry principal components). Associations between methylation level (unadjusted) and each psychiatric/brain phenotype are presented separately by genotype in the Supplementary Materials for reference (Supplementary Table S1 and Supplementary Figure S2). Cell counts were estimated from the methylation data, and analyses using these cell counts were performed to rule out cell composition as a potential confound. Unless otherwise specified, reported methylation$_{adj}$ results remained significant when cell composition estimates were included in the model. All tests were two-tailed.

We computed vertex-wise analyses across the entire cortex to test the hypothesis that *SKA2* methylation would be associated with reduced cortical integrity in prefrontal brain regions. General linear model analyses were run using FreeSurfer's Qdec. Vertex-wise significance threshold was set at $P < 0.01$. Monte Carlo simulations (10 000 iterations) were used to correct for multiple comparisons, resulting in a whole-brain-corrected threshold of $P < 0.05$. Only regions surviving correction for multiple comparisons are reported.

SKA2 methylation associations with suicide-related psychiatric phenotypes were assessed using hierarchical linear regression models in SPSS v22 (SPSS, Chicago, IL, USA). Mediation analyses were conducted in Mplus 7.11 (ref. 19) in the subsample with complete data ($n = 144$) using the maximum likelihood estimator with bootstrapped s.e. and confidence intervals (CI). All brain region phenotypes were examined simultaneously in the model such that each was regressed on methylation, psychiatric phenotype and rs7208505 genotype. Methylation was also regressed on genotype and psychiatric phenotype. The indirect effect of psychiatric phenotype on the brain phenotypes via methylation was assessed using the 'model indirect' procedure.

RESULTS

SKA2 associations with cortical thickness

The genotype at rs7208505 did not relate to cortical thickness in the whole-brain analyses nor did methylation. As hypothesized, however, *SKA2* methylation$_{adj}$ was associated with reduced cortical thickness in prefrontal cortex. Three clusters survived correction for multiple comparisons (Table 2 and Figure 1). A left hemisphere cluster spanned frontal pole and the anterior aspects of superior frontal gyrus (SFG) and rostral middle frontal gyrus (MFG) (Brodmann's area (BA) 9/10). Two clusters also emerged in right PFC: one spanning right frontal pole and anterior SFG (BA 9/10) and one in (lateral and medial) orbitofrontal cortex (OFC) and the anterior aspects of inferior frontal gyrus pars orbitalis and rostral MFG (BA 10/11). Greater *SKA2* methylation$_{adj}$ was associated with decreased cortical thickness in all clusters ($rs < -0.28$, $ps < 0.001$). These results were consistent with those of a similar analysis in rs7208505 genotype groups (Supplementary Figure S2 and Supplementary Table S1).

To examine the clinical significance of the alterations in brain morphology associated with *SKA2* epigenetic variation, we correlated mean cortical thickness for each cluster with measures of PTSD and depression. PTSD symptom severity was negatively correlated with cortical thickness in both right prefrontal clusters ($rs < -0.20$, $ps < 0.018$) and showed a trend for the left prefrontal cluster ($r = -0.16$, $P = 0.06$). In contrast, current depression symptom severity was unrelated to cortical thickness in any of the clusters. Analyses performed using dichotomous indices of PTSD and MDD diagnosis produced a similar pattern of results.[2]

To rule out potential confounds, we tested whether findings were due to the influence of comorbid substance use disorders, psychiatric medication use or mild TBI (individuals with moderate/severe TBI were already excluded from analyses). We extracted each brain cluster and ran a hierarchical linear regression analysis with these variables added to the statistical models. The associations between DNA methylation$_{adj}$ and cortical thickness for each of the clusters remained significant when these potential confounds were included in the models ($ps < 0.002$).

SKA2 associations with psychiatric symptoms

We next examined whether genetic and epigenetic variation at *SKA2* were associated with psychiatric symptoms that increase risk of suicide—specifically PTSD and depression. Linear regression models revealed that rs7208505 genotype was associated with current PTSD symptom severity ($\beta = -0.16$, $P = 0.028$), such that individuals homozygous for the C minor allele had lower PTSD symptom severity than heterozygotes ($M_{Difference} = -16.5$, $P = 0.013$) and T/T carriers ($M_{Difference} = -15.8$, $P = 0.024$). Methylation was not associated with PTSD, but methylation$_{adj}$ was associated with PTSD symptom severity over and above the effect of genotype ($\beta = 0.37$, $P = 0.021$), with methylation$_{adj}$ correlating positively with current PTSD symptoms (see Table 3). Genetic and epigenetic variations at *SKA2* were not associated with depression symptoms. Analyses performed using PTSD and MDD diagnostic status yielded a similar pattern of results.[3]

SKA2 epigenetic variation as a mediator of brain–behavior relationships

Based on the findings above, we tested whether *SKA2* methylation$_{adj}$ mediated the observed association between PTSD symptom severity and reduced cortical thickness in the prefrontal clusters. All phenotypes were examined simultaneously in one model. The indirect effect of PTSD on the right OFC/rostral MFG cluster through DNA methylation$_{adj}$ was significant (standardized $\beta = -0.067$, $P = 0.039$, 95% CI $= -0.131$ to -0.003), as was the corresponding effect on the left prefrontal cluster (standardized $\beta = -0.063$, $P = 0.043$, 95% CI $= -0.125$ to -0.002). The effect was not statistically significant for the right frontal pole/SFG cluster, although the direction of association was consistent (standardized $\beta = -0.061$, $P = 0.056$, 95% CI $= -0.124$ to 0.002). In total, the model explained 82% of the variance in methylation, and 16% of the variance in each of the three brain clusters.

DISCUSSION

This study extended the link between *SKA2* DNA methylation and suicide by examining associations between this novel epigenetic risk locus, cortical thickness and suicide-related psychiatric symptoms. We hypothesized that greater DNA methylation at *SKA2* would be associated with decreased cortical thickness in prefrontal brain regions in trauma-exposed veterans. Whole-brain vertex-wise analyses revealed that *SKA2* methylation$_{adj}$ at this locus was associated with diminished cortical thickness in three regions of prefrontal cortex, and reduced thickness in these

Table 2. Reductions in cortical thickness in frontal cortex associated with SKA2 3′ UTR DNA methylation adjusting for genotype.

	Peak F-*value*	*Peak* (x, y, z)	*No. of vertices*	*Cluster size* (mm^2)
L frontal pole/SFG/rostral MFG	−2.87	−8, 61, 12	121 563	1832
R frontal pole/SFG	−3.49	7, 55, 25	64 286	1347
R OFC/IFG pars orbitalis/rostral MFG	−3.17	27, 49, -12	64 226	1411

Abbreviations: IFG, inferior frontal gyrus; L, left hemisphere; MFG, middle frontal gyrus; OFC, orbitofrontal cortex; R, right hemisphere; SFG, superior frontal gyrus; UTR, untranslated region. $N = 145$. All clusters survived Monte Carlo Simulation correction for multiple comparisons.

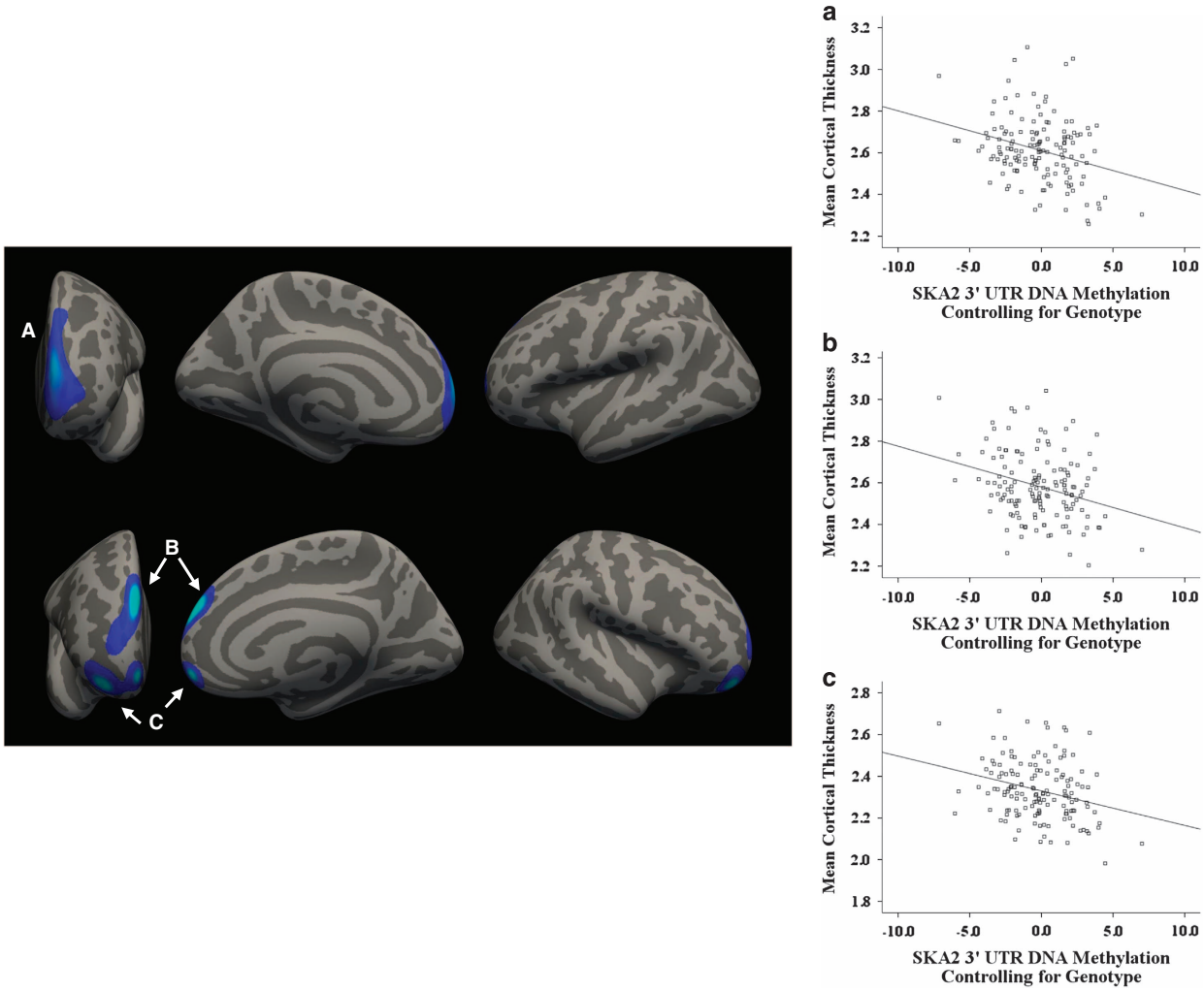


Figure 1. SKA2 methylation adjusting for genotype relates to reduced cortical thickness in frontal cortex. (**a**) Frontal pole, superior frontal gyrus and rostral middle frontal gyrus. (**b**) Frontal pole and superior frontal gyrus. (**c**) Orbitofrontal cortex, inferior frontal gyrus and rostral middle frontal gyrus.

regions corresponded to more severe current PTSD symptoms. Although causality cannot be inferred from this cross-sectional data set, path analysis showed that *SKA2* methylation$_{adj}$ significantly mediated the relationship between PTSD and neural integrity in prefrontal cortex. These findings extend previous work by linking *SKA2* methylation$_{adj}$ to alterations in cortical thickness—a possible brain endophenotype of stress-related suicide risk—and broaden its potential clinical utility by pointing to its value as a possible PTSD biomarker.

The association between *SKA2* methylation$_{adj}$ and reductions in cortical thickness provides novel insight into how alterations in stress-sensitive molecular pathways impact neural integrity and function. In both hemispheres, greater methylation$_{adj}$ was associated with reduced cortical thickness in frontal pole and the anterior aspects of SFG and rostral MFG, areas of the brain crucial for several high-level cognitive-control processes. Specifically, meta-analytic evidence[20] indicates that the observed clusters (that is, BA 9/10) are involved in multitask control (for example, holding one task online while performing a separate task), which is necessary for evaluating multiple choices at once, complex reasoning and cognitive flexibility. Burgess *et al.*[21] have proposed a 'gateway' hypothesis, in which these regions coordinate behavior by biasing attention toward internal mental stimuli

Table 3. Hierarchical linear regression analysis of genetic and epigenetic variation at SKA2 and current PTSD symptom severity

	Model 1 β *(s.e.)*	*Model 2* β *(s.e.)*	*Model 3* β *(s.e.)*
Step1			
Age	−0.10 (0.3)	−0.10 (0.3)	−0.10 (0.3)
Sex	0.04 (7.3)	0.04 (7.3)	0.04 (7.3)
Ancestry PC1	0.02 (29.9)	0.02 (29.9)	0.02 (29.9)
Ancestry PC2	0.08 (30.1)	0.08 (30.1)	0.08 (30.1)
Ancestry PC3	−0.16 (30.1)*	−0.16 (30.1)*	−0.16 (30.1)*
Step2			
rs7208505 genotype	−0.16 (2.9)*	—	−0.16 (2.9)*
cg13989295 methylation	—	−0.07 (0.4)	—
Step3			
cg13989295 methylation (methylation$_{adj}$)	—	-—	0.37 (0.9)*

Abbreviations: PC, principal component; PTSD, posttraumatic stress disorder; s.e., standard error. *$P < 0.05$. Note: $N = 199$. Model 1. Step1: $R^2 = 0.043$, Step2: $\Delta R^2 = 0.024$*. Model 2. Step1: $R^2 = 0.043$, Step2: $\Delta R^2 = 0.01$. Model 3. Step1: $R^2 = 0.043$, Step2: $\Delta R^2 = 0.024$*, Step3: $\Delta R^2 = 0.026$*. *$P < 0.05$.

(thoughts and emotions) or external sensory information. In addition, frontal pole in particular has been implicated in prospective thinking and introspection.[22,23] Disruption of these higher-order control processes may lead to impulsive decision-making, difficulty imagining future prospects and emotional dysregulation, which are all risk factors for suicidal behavior.[3,24,25] An additional cluster emerged in regions of right OFC and inferior frontal gyrus pars orbitalis (Figure 1) thought to be crucial for maintaining, updating, and adjusting ongoing behavior based on reward and punishment contingencies.[26] Reduced integrity in these regions has been linked to emotional dysregulation and impulsivity.[24,27,28] Given the cognitive functions subserved, reduced cortical integrity in the identified prefrontal clusters may represent a significant neurobiological vulnerability for suicidal behavior in the context of stress-related psychiatric illnesses, an important question that can be addressed in future research that explicitly examines suicidal behavior.

Given that the SKA2 protein has not been studied extensively, the exact mechanisms by which *SKA2* may impact cortical integrity are unknown. However, studies suggest that it interacts with the glucocorticoid receptor by chaperoning it from the cytoplasm to the nucleus and is a moderator of negative feedback inhibition of cortisol by the glucocorticoid receptor.[12] In prefrontal cortex, where glucocorticoid receptor density is enriched,[9] sustained elevations of cortisol can cause neuronal damage and cell death either directly[29–31] or indirectly through mechanisms such as oxidative stress.[32] Thus, *SKA2* expression may serve a neuroprotective function by enhancing activation of the glucocorticoid receptor. This, in turn, upregulates the expression of anti-inflammatory proteins in neuronal nuclei, represses the expression of pro-inflammatory proteins in the cytosol and mitigates oxidative stress. These functions are similar to that of the better-known FK506-binding protein 5 (FKBP5), a co-chaperone of the glucocorticoid receptor, which has been linked in genetic association studies to a variety of relevant phenotypes including PTSD,[33] alterations of brain morphology[34,35] and suicide.[36,37] Epigenetic variation at *SKA2,* therefore, may represent an important additional locus that confers individual differences in risk and resilience to stress.

Although there are compelling data that *SKA2* has a role in suicide,[13,14] the present findings suggest it is also relevant for understanding the pathophysiology of stress-related disorders more broadly. By associating methylation at this locus with reduced thickness in prefrontal cortex, this study implicates a molecular and neurobiological pathway by which prolonged or extreme stress exposure impacts susceptibility for psychiatric disorders. More precisely, *SKA2* may index a transdiagnostic susceptibility to neural deterioration from stress exposure, with suicide and PTSD being two clinical outcomes in which stress exposure is a potent etiological factor, and thus highly relevant. Although novel to *SKA2*, these hypotheses are consistent with research on the neurotoxic effects of stress exposure,[9,38] including in relation to suicide and PTSD.[32,39]

Moving forward, an important next step will be to focus on clarifying how alterations in stress-sensitive molecular and neurobiological pathways interact with other biological, psychological and social risk processes to promote suicidal behavior. Not all individuals with stress-related disorders ultimately die by suicide, and thus examination of more comprehensive etiological models will be needed to individualize risk assessment measures and intervene effectively. For example, present findings suggest that epigenetic modifications from stress exposure may compromise the neural circuits that typically support adaptive decision-making and self-regulation. Expansion of this work to clarify how these molecular and neural mechanisms interact with putative endophenotypes for suicide risk, including HPA-axis dysregulation, serotonin dysfunction and aberrant cognitive-affective processes (for example, impulsive-aggressive traits and disadvantageous decision-making), as well as stressful life events and functional genetic/epigenetic influences[40,41] could elucidate heterogeneity in pathways to suicidal behavior and improve the precision of risk models.

The findings and conclusions need to be considered in the context of the study limitations. First, our results indicate that adjusting methylation levels by the methylation-associated SNP revealed associations that were not apparent for unadjusted methylation or the local SNP. Indeed, no significant relationships were observed for unadjusted methylation at the cg13989295 probe, and no new results emerged for rs7208505 that were not also present for adjusted methylation (that is, a main effect of SNP was only observed for PTSD). Although we did not observe effects for unadjusted methylation, Guintivano *et al.*[13] reported associations between DNA methylation and *SKA2* expression in brain tissue both before and after controlling for genotype. These discrepant findings for unadjusted methylation may be due to differences in measuring DNA methylation in peripheral blood samples versus brain tissue, which is beyond the scope of the present study but an important avenue of future inquiry. Further, given that it is not clear what adjusting methylation for genotype implies on a biological level and research on *SKA2* is in its infancy, further investigation is needed to clarify the functional significance and biological implications of this analytic method. In particular, whether adjusted methylation in blood reflects a specific causal mechanism underlying alterations in neural structure/function or rather serve as useful indicators of biological processes related to stress exposure and susceptibility more broadly has yet to be determined.

Second, the cross-sectional nature of our data prohibits strong conclusions regarding the direction of the proposed effects, and other mediational models are certainly possibly. We tested *SKA2* methylation as a mediator of the association between PTSD (as a measure of ongoing stress) on neural integrity (effects of stress on the brain), but it is also possible that brain phenotypes may mediate the association between methylation and PTSD or that alterations in cortical thickness cause HPA-axis dysregulation and thus alter *SKA2* methylation. Thus, prospective research is needed to ascertain the mechanism(s) by which traumatic stress impacts epigenetic variation and neural integrity. Third, the absence of an association between depression symptoms and *SKA2* variation in this sample should be interpreted in the context of both the modest size and the makeup of our sample. Given that *SKA2* purportedly measures a stress-specific molecular pathway, the high levels of trauma exposure in this sample may have strengthened our ability to detect PTSD effects. However, it is also possible that methylation at *SKA2* is less predictive of depression than PTSD phenotypes, and this hypothesis requires testing in larger and more diverse samples. Fourth, we do not have information about the stability of the methylation level of this gene, and prospective studies are needed to ascertain how methylation levels vary over time. Finally, it is important to note that a measure of suicidal behavior was not included in this study, and thus the findings can only speak to the implications for understanding suicide-related risk processes indirectly, through connections from previous research on *SKA2*.[13,14] Despite these limitations, this study also has several strengths, most notably the clinically-relevant sample of veterans with trauma exposure and the innovative research design that integrates neuroimaging and genetic methods to study the biology of stress-related psychiatric phenotypes.

In summary, findings advance our understanding of stress susceptibility for psychiatric disorders by identifying *SKA2* as a potential biomarker of the effects of stress exposure on cortical thickness in psychiatrically relevant brain regions. As a putative molecular measure of cumulative dysregulation in stress response systems, *SKA2* epigenetic variation may be a useful blood biomarker for screening military personnel prior to deployment

to identify individuals with a high lifetime burden of stress who are at risk for developing PTSD and suicide following exposure to warzone stress.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

This research was supported in part by the NIMH grant R21MH102834 'Neuroimaging Genetics of PTSD' and the Translational Research Center for TBI and Stress Disorders (TRACTS), a VA Rehabilitation Research and Development Traumatic Brain Injury Center of Excellence (B9254-C) and the Cooperative Studies Program, Department of Veterans Affairs. This research is the result of work supported with resources and the use of facilities at the Pharmacogenomics Analysis Laboratory, Research and Development Service, Central Arkansas Veterans Healthcare System, Little Rock, Arkansas. This work was also supported by a Career Development Award to EJW from the United States Department of Veterans Affairs, Clinical Sciences Research and Development Program. The views expressed in this article are those of the authors and do not necessarily reflect the position or policy of the Department of Veterans Affairs or the United States Government.

ENDNOTES

(1) Three individuals with methylation levels >3 s.d. from the sample mean for each genotype were excluded from the study, resulting in a final *N* of 200. Supplemental analyses indicated that including these cases did not change the study findings.

(2) Current PTSD diagnosis was associated with decreased cortical thickness in the right frontal pole/SFG cluster ($F_{1,143}=4.75$, $P=0.031$) and right OFC/IFG/rostral MFG cluster ($F_{1,143}=6.25$, $P=0.014$), with a trend emerging for the left frontal cluster ($F_{1,143}=3.39$, $P=0.068$). Cortical thickness in these clusters did not differ by MDD diagnostic status ($F_{1,143}<1.42$, ps >0.23).

(3) DNA methylation$_{adj}$ was associated with a greater likelihood of current PTSD diagnosis (Wald $\chi2=4.35$, OR $=1.2$, $P=0.037$), but not current MDD diagnosis. However, the relationship between DNA methylation$_{adj}$ and PTSD diagnosis were reduced to a trend-level when cell counts were entered in the model (Wald $\chi2=2.75$, OR $=1.1$, $P=0.097$).

REFERENCES

1 Kang HK, Bullman TA, Smolenski DJ, Skopp NA, Gahm GA, Reger MA. Suicide risk among 1.3 million veterans who were on active duty during the Iraq and Afghanistan wars. *Ann Epidemiol* 2014; **25**: 96–100.
2 Nock MK, Deming CA, Fullerton CS, Gilman SE, Goldenberg M, Kessler RC *et al.* Suicide among soldiers: a review of psychosocial risk and protective factors. *Psychiatry* 2013; **7**: 97–125.
3 Mann JJ, Arango VA, Avenevoli S, Brent DA, Champagne FA, Clayton P *et al.* Candidate endophenotypes for genetic studies of suicidal behavior. *Biol Psychiatry* 2009; **65**: 556–563.
4 McGowan PO, Sasaki A, D'Alessio AC, Dymov S, Labonte B, Szyf M *et al.* Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat Neurosci* 2009; **12**: 342–348.
5 Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR. Epigenetic programming by maternal behavior. *Nat Neurosci* 2004; **7**: 847–854.
6 Perroud N, Paoloni-Giacobino A, Prada P, Olie E, Salzmann A, Nicastro R *et al.* Increased methylation of glucocorticoid receptor gene (NR3C1) in adults with a history of childhood maltreatment: a link with the severity and type of trauma. *Transl Psychiatry* 2011; **1**: e59.
7 Logue MW, Smith AK, Baldwin C, Wolf EJ, Guffanti G, Ratanatharathorn A *et al.* An analysis of gene expression in PTSD implicates genes involved in the glucocorticoid receptor pathway and neural responses to stress. *Psychoneuroendocrinology* 2015; **57**: 1–13.
8 de Kloet ER. Hormones, brain, and stress. *Endocr Regul* 2003; **37**: 51–68.
9 Diorio D, Viau V, Meaney MJ. The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. *J Neurosci* 1993; **13**: 3839–3847.
10 Sato H, Takahashi T, Sumitani K, Takatsu H, Urano S. Glucocorticoid generates ROS to induce oxidative injury in the hippocampus, leading to impairment of cognitive function of rats. *J Clin Biochem Nutr* 2010; **47**: 224–232.
11 Sapolsky RM. Glucocorticoid and hippocampal atrophy in neuropsychiatric disorders. *Arch Gen Psychiatry* 2000; **57**: 925–935.
12 Rice L, Waters CE, Eccles J, Garside H, Sommer P, Kay P *et al.* Identification and functional analysis of SKA2 interaction with the glucocorticoid receptor. *J Endocrinol* 2008; **198**: 499–509.
13 Guintivano J, Brown T, Newcomer A, Jones M, Cox O, Maher BS *et al.* Identification and replication of a combined epigenetic and genetic biomarker predicting suicide and suicidal behaviors. *Am J Psychiatry* 2014; **17**: 1287–1296.
14 Niculescu AB, Levey D, Le-Niculescu H, Niculescu E, Kurian SM, Salomon D. Psychiatric blood biomarkers: avoiding jumping to premature negative or positive conclusions. *Mol Psychiatry* 2015; **20**: 286–288.
15 Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE *et al.* An integrated map of genetic variation from 1,092 human genomes. *Nature* 2012; **491**: 56–65.
16 Blake DD, Weathers FW, Nagy LM. A clinical rating scale for assessing current and lifetime PTSD: the CAPS-1. *Behav Ther* 1993; **18**: 187–188.
17 Lovibond SH, Lovibond PF. Manual for the depression anxiety stress scales, 2nd edn. Psychology Foundation: Sydney, NSW, Australia, 1995.
18 First MB, Spitzer RL, Gibbon M, Williams JBW. Structured Clinical Interview for DSM-IV Axis I Disorders Research Version (SCID-I). New York State Psychiatric Institute: New York, NY, USA, 1996.
19 Muthén LK, Muthén BO. Mplus User's Guide, (6th edn). Muthén & Muthén: Los Angeles, CA, USA.
20 Gilbert SJ, Spengler S, Simons JS, Steele JD, Lawrie SM, Frith CD *et al.* Functional Specialization within rostral prefrontal cortex (area 10): a meta-analysis. *J Cogn Neurosci* 2006; **18**: 932–948.
21 Burgess PW, Simons JS, Dumontheil I, Gilbert SJ. The gateway hypothesis of rostral prefrontal cortex (area 10) function. In: Duncan J, Phillips L, McLeod P (eds). *Measuring the mind: speed, control, and age*. Oxford University Press: Oxford, UK, 2005.
22 Okuda J, Fujii T, Ohtake H, Tsukiura T, Tanji K, Suzuki K *et al.* Thinking of the future and past: The roles of the frontal pole and the medial temporal lobes. *Neuroimage* 2003; **19**: 1369–1380.
23 Tsujimoto S, Genovesio A, Wise SP. Frontal pole cortex: encoding ends at the end of the endbrain. *Trends Cogn Sci* 2011; **15**: 169–176.
24 Matsuo K, Nicoletti M, Nemoto K, Hatch JP, Peluso MA, Nery FG *et al.* A voxel-based morphometry study of frontal gray matter correlates of impulsivity. *Hum Brain Mapp* 2009; **30**: 1188–1195.
25 Jollant F, Bellivier F, Leboyer M, Astruc B, Torres S, Verdier R *et al.* Impaired decision making in suicide attempters. *Am J Psychiatry* 2005; **162**: 304–310.
26 Keilp JG, Sackeim HA, Brodsky BS, Oquendo MA, Malone KM, Mann JJ. Neuropsychological dysfunction in depressed suicide attempters. *Am J Psychiatry* 2001; **158**: 735–741.
27 Kringelbach ML, Rolls ET. The functional neuroanatomy of the human orbitofrontal cortex: evidence from neuroimaging and neuropsychology. *Prog Neurobiol* 2004; **72**: 341–372.
28 Davidson RJ, Putnam KM, Larson CL. Dysfunction in the neural circuitry of emotion regulation--a possible prelude to violence. *Science* 2000; **289**: 591–594.
29 Cook SC, Willman CL. Chronic stress alters dendritic morphology in rat medial prefrontal cortex. *J Neurobiol* 2004; **60**: 236–248.
30 Goldwater DS, Pavlides C, Hunter RG, Bloss EB, Hof PR, McEwen BS *et al.* Structural and functional alterations to rat medial prefrontal cortex following chronic restraint stress and recovery. *Neuroscience* 2009; **164**: 798–808.
31 Wellman CL. Dendritic reorganization in pyramidal neurons in medial prefrontal cortex after chronic corticosterone administration. *J Neurobiol* 2001; **49**: 245–253.
32 Miller MW, Sadeh N. Traumatic stress, oxidative stress and posttraumatic stress disorder: Neurodegeneration and the accelerated-aging hypothesis. *Mol Psychiatry* 2014; **19**: 1156–1162.
33 Klengel T, Mehta D, Anacker C, Rex-Haffner M, Pruessner JC, Pariante CM *et al.* Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. *Nat Neurosci* 2013; **16**: 33–4110.
34 Fani N, King TZ, Binder EB, Jovanovic T, Bradley B, Ressler KJ. FKBP5 genotype and structural integrity of the posterior cingulum. *Neuropsychopharmacology* 2014; **39**: 1206–1213.
35 Fujii T, Ota M, Hori H, Hattori K, Teraishi T, Sasayama D *et al.* Association between the common functional FKBP5 variant (rs1360780) and brain structure in a non-clinical population. *J Psychiatr Res* 2014; **58**: 96–101.
36 Brent D, Melhem N, Ferrell R, Emslie G, Wagner KD, Ryan N *et al.* Association of FKBP5 polymorphisms with suicidal events in the treatment of resistant depression in adolescents (TORDIA) study. *Am J Psychiatry* 2010; **167**: 190–197.
37 Pérez-Ortiz JM, García-Gutiérrez MS, Navarrete F, Giner S, Manzanares J. Gene and protein alterations of FKBP5 and glucocorticoid receptor in the amygdala of suicide victims. *Psychoneuroendocrinology* 2013; **38**: 1251–1258.

38 Arnsten AF. Stress signalling pathways that impair prefrontal cortex structure and function. *Nat Rev Neurosci* 2009; **10**: 410–422.
39 Jollant F, Lawrence NL, Olié E, Guillaume S, Courtet P. The suicidal mind and brain: a review of neuropsychological and neuroimaging studies. *World J Biol Psychiatry* 2011; **12**: 319–339.
40 Courtet P, Gottesman II, Jollant F, Gould TD. The neuroscience of suicidal behaviors: what can we expect from endophenotype strategies? *Transl Psychiatry* 2011; **1**: e7.
41 Oquendo MA, Perez-Rodriguez MM, Poh E, Sullivan G, Burke AK, Sublette ME *et al.* Life events: a complex role in the timing of suicidal behavior among depressed patients. *Mol Psychiatry* 2014; **19**: 902–909.

Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)

Supplementary Methods

Inclusion/Exclusion Criteria

Pre-determined eligibility criteria included no: history of seizures, prior serious medical illness (e.g., cerebrovascular accident, myocardial infarction, diabetes), imminent risk of suicide or homicide requiring immediate intervention (3 veterans were deemed ineligible due to imminent risk of suicide), current DSM-IV-TR diagnosis of bipolar disorder, schizophrenia or other psychotic disorder, or cognitive disorder due to a general medical condition other than a traumatic brain injury (TBI). For MRI acquisition, individuals were excluded if they had any metal implant, shrapnel, aneurysm clip, pacemaker, or were pregnant.

Genotyping: Laboratory Procedures

DNA was isolated from peripheral blood samples on a Qiagen AutoPure instrument with Qiagen reagents; concentrations were normalized using the Quant-iT™ PicoGreen dsDNA fluorescent assay (Invitrogen). DNA quality and quantity were ascertained by the TaqMan® RNase P Detection assay (Applied Biosystems Assay, Life Technologies, Carlsbad, CA) with fluorescence detection on a 7900 Fast Real Time PCR System (Applied Biosystems, Life Technologies, Carlsbad, CA) according to the manufacturer's protocol. DNA samples were whole-genome amplified, fragmented, precipitated and resuspended prior to hybridization on Illumina HumanOmni2.5-8 beadchips for 20 hours at 48^0C according to the manufacturer's protocol (Illumina, San Diego, CA). After hybridization, a single-base extension followed by a multi-layered staining process was performed. Beadchips were imaged using the Illumina iScan System and analyzed with Illumina GenomeStudio v2011.1 software containing Genotyping v1.9.4 module. A GenomeStudio project was created with a custom genotyping

cluster file, and call rates were >0.994 for all samples. Technical replicates had genotyping reproducibility error rates <0.0005 prior to SNP data cleaning.

Genotyping: Statistical Procedures

SNP data cleaning and manipulation was performed using PLINK[8]. X-chromsome genotypes were concordant with self-report sex in all cases. IBD analysis was used to check for cryptic relatedness in the sample. Only 218 subjects with self-reported white non-Hispanic (WNH) were analyzed. Concordance between self-reported and genetically predicted ancestry was investigated using principal components (PC) analysis as implemented in EIGENSTRAT[9], based on the genotypes of 100,000 common SNPs. First, PC analysis of the WNH data was performed together with 1,000 Genomes[10] reference data for the EUR, AFR, ASN, and AMR samples. Of the 218 self-reported WNH subjects, 4 were more than 6 SD away from the WNH group mean on the generated PC were removed from the analysis, leaving a sample of 214 WNH subjects. Next, PC were calculated for use as analysis covariates using the remaining 214 subjects. None of the subjects were outliers (>6SD) in the PC analysis. For these subjects, genotypes for SNPs not covered on the 2.5-8 array (including rs7208505) were generated using Impute2[11] and 1000 genomes reference data. Prior to imputation, all C/G and A/T SNPs, SNPs with missing rates > 1%, and SNPs with Hardy-Weinberg equilibrium (HWE) test p-values $<10^{-6}$ were removed. Imputation was done using 1000 Genomes phase 1 integrated haplotypes (June 2014), based on only the haplotypes for the 379 EUR reference-panel subjects in order to reduce computational cost. The imputation quality of rs7208505 was very high (info = 1.00). Of the remaining 214 samples, 11 samples were excluded, because we did not have permission to use them in this study, resulting in a final sample size of 203.

Methylation: Laboratory Procedures

Sample integrity and quantity were determined by TaqMan® RNase P Detection assay (Applied Biosystems Assay, Life Technologies, Carlsbad, CA) with fluorescence detection on a 7900 Fast Real Time PCR System (Applied Biosystems, Life Technologies, Carlsbad, CA) according to the manufacturer's protocol. Samples were bisulfite-modified using Zymo EZ-96 DNA Methylation Kits (D5004). The efficiency of bisulfite-mediated conversion was determined by PCR with DAPK1 primers (Zymo) and gel electrophoresis of PCR-products. Bisulfite-modified DNA samples were whole-genome amplified, fragmented, precipitated, resuspended, and hybridized to Illumina HumanMethylation 450 beadchips. Following single-base extension and staining, beadchips were scanned using the Illumina iScan System. Preliminary data analyses to assess internal quality control and performance were performed with GenomeStudio v2011.1 software using Methylation module v1.9.0.

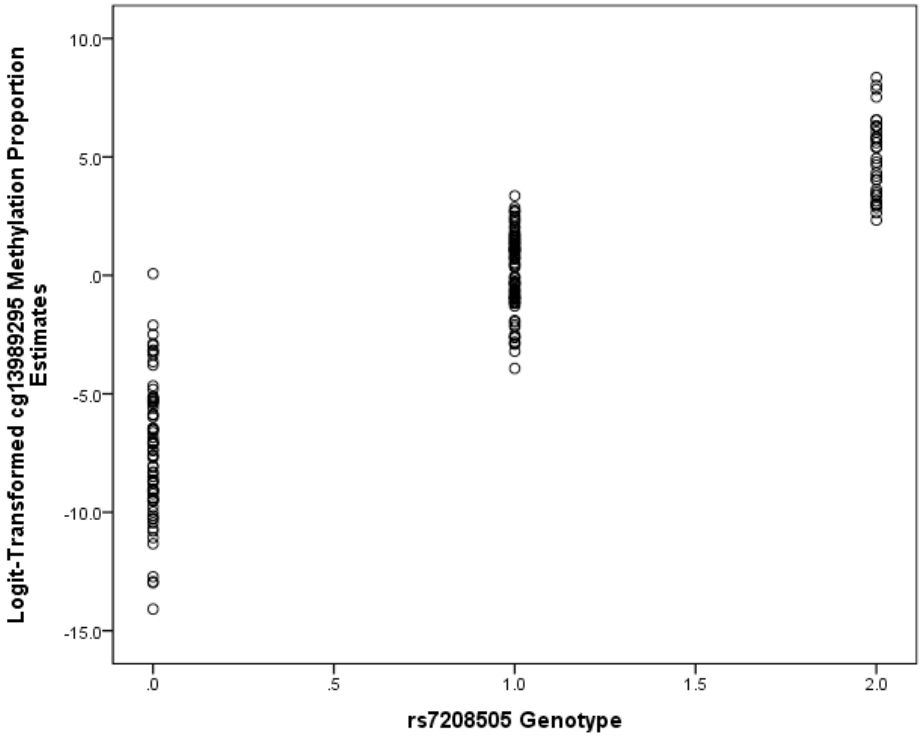
Methylation: Statistical Procedures

Individual-level background-corrected probe data were output from GenomeStudio. Methylation values were received for 332 samples. Cleaning was performed within the CpGassoc package Bioconductor package in R[1]. Two subjects with signal intensity of less than 50% of the experiment-wide mean were excluded, leaving 330 methylation profiles for analysis. Probe normalization was performed using beta mixture quantile dilation (BMIQ) method[2] as implemented in the wateRmelon[3; 4] R package. Removal of batch and chip effects were performed using an empirical Bayes batch-correction method (ComBat)[5] as implemented in the Bioconductor sva package[6]. As differential white-cell counts were not available for our samples, cell counts were estimated from the methylation data itself using the R minfi[7]

package. Methylation proportions were transformed using a logit function prior to analysis. Only the 203 WNH who had SNP data were analyzed and duplicates were removed prior to analysis.

Analytic Plan

The sample size was chosen based on the expected magnitude of the association between *SKA2* and psychiatric phenotypes, which was based on previous work by Guintivano and colleagues showing a medium effect size (Cohen's $f^2 = 0.18$). Our primary analyses used multiple regression models; thus, we calculated the required sample size needed to test the significance of an individual regression beta, with $\alpha = 0.05$ and power = 0.95 to be N = 89, making our smallest sample size of 145 more than adequately powered. To ensure the results were not an artifact of sampling, we examined the data for outliers and non-linear distributions. As described in Footnote 1 of the main text, three individuals with methylation levels greater than 3 SDs from the sample mean for each genotype were excluded from the study. No other outliers were detected in the explanatory or dependent variables, which were not found to be excessively skewed or kurtotic (values ranged from −1.1 to 1.8). To test for equality of variances for the main effect of genotype analyses, we conducted Levene's Test of Equality of Error Variances and found this assumption was not violated for PTSD symptoms, depression symptoms, and the extracted cortical thickness clusters. However, given the correlation between *SKA2* genotype and DNA methylation, we did not expect the variance in methylation levels to be equal across the genotypes and they were not ($F_{2, 197} = 12.3$, $p < .001$). Consequently, we adjusted methylation values for genotype in our primary analyses, and we genotype-stratified analyses post hoc to evaluate the direction of association when indicated.



Supplementary Results

Figure S1. Relationship between rs7208505 Genotype and cg13989295 Methylation
Note. 0 = T/T genotype. 1 = C/T genotype. 2 = C/C genotype. T/T: $n = 75$, $M = -7.4$, $SD = 2.8$. C/T: $n = 91$, $M = 0.2$, $SD = 1.6$. C/C: $n = 34$, $M = 4.9$, $SD = 1.7$.

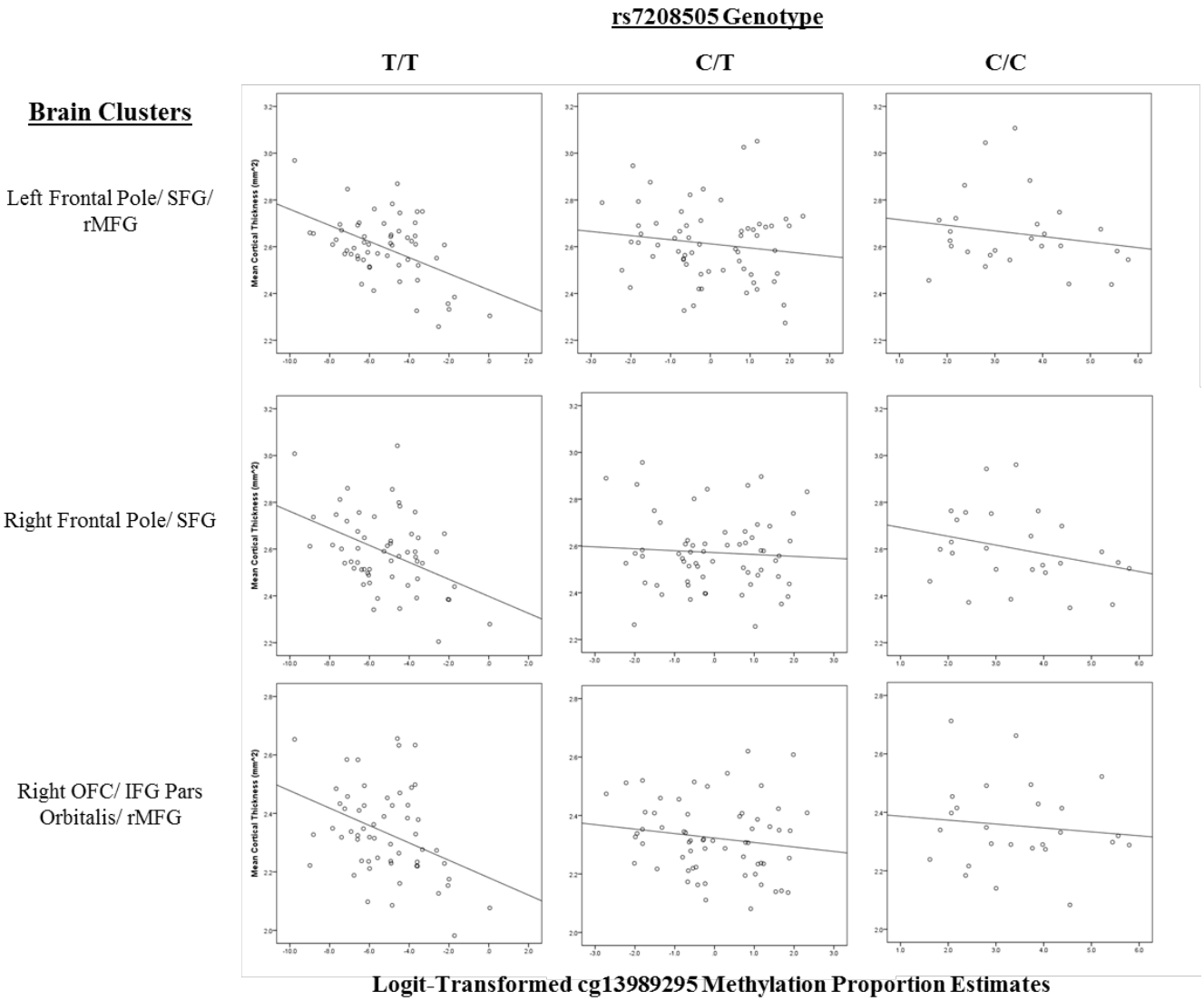


Figure S2. Associations between cg13989295 Methylation and Brain Phenotypes by rs7208505 genotype. Scatterplots of the association between cortical thickness in each of the prefrontal brain clusters and *SKA2* methylation as a function of genotype. SFG = superior frontal gyrus. rMFG = rostral middle frontal gyrus. OFC = orbitofrontal cortex. IFG = inferior frontal gyrus.

Table S1. Correlations between cg13989295 methylation (unadjusted for genotype) and psychiatric/ brain phenotypes by rs7208505 genotype

T/T Genotype	cg13989295 Methylation
Psychiatric Symptoms	
PTSD Symptom Severity	.10
Depression Severity	.01
Cortical Thickness	
R Frontal Pole/ SFG Cluster	-.43
R OFC/ IFG/ rMFG Cluster	-.40
L Frontal Pole/SFG Cluster	-.48
C/T Genotype	
Psychiatric Symptoms	
PTSD Symptom Severity	.15
Depression Severity	-.07
Cortical Thickness	
R Frontal Pole/ SFG Cluster	-.07
R OFC/ IFG/ rMFG Cluster	-.15
L Frontal Pole/ SFG Cluster	-.15
C/C Genotype	
Psychiatric Symptoms	
PTSD Symptom Severity	.22
Depression Severity	.02
Cortical Thickness	
R Frontal Pole/ SFG Cluster	-.36
R OFC/ IFG/ rMFG Cluster	-.21
L Frontal Pole/ SFG Cluster	-.22

Note. PTSD = posttraumatic stress disorder. SFG = superior frontal gyrus. rMFG = rostral middle frontal gyrus. OFC = orbitofrontal cortex. IFG = inferior frontal gyrus. Values represent partial correlations adjusting for age, sex, and population substructure (as indexed by the first three ancestry principal components).

Table S2. Descriptive statistics for phenotypes of interest separately by rs7208505 genotype.

T/T Genotype	*M (SD)*
Psychiatric Symptoms	
PTSD Symptom Severity	50.0 (30.1)
Depression Severity	9.0 (10.0)
Cortical Thickness	
R Frontal Pole/ SFG Cluster	2.6 (0.1)
R OFC/ IFG/ rMFG Cluster	2.3 (0.1)
L Frontal Pole/SFG Cluster	2.6 (0.1)
C/T Genotype	
Psychiatric Symptoms	
PTSD Symptom Severity	50.5 (28.7)
Depression Severity	8.6 (8.6)
Cortical Thickness	
R Frontal Pole/ SFG Cluster	2.6 (0.1)
R OFC/ IFG/ rMFG Cluster	2.3 (0.1)
L Frontal Pole/ SFG Cluster	2.6 (0.1)
C/C Genotype	
Psychiatric Symptoms	
PTSD Symptom Severity	35.0 (28.7)
Depression Severity	4.5 (7.9)
Cortical Thickness	
R Frontal Pole/ SFG Cluster	2.6 (0.1)
R OFC/ IFG/ rMFG Cluster	2.4 (0.1)
L Frontal Pole/ SFG Cluster	2.6 (0.1)

Note. PTSD = posttraumatic stress disorder. SFG = superior frontal gyrus. rMFG = rostral middle frontal gyrus. OFC = orbitofrontal cortex. IFG = inferior frontal gyrus.

References

1. R Development Core Team. R: A language and environment for statistical computing, 2008

2. Teschendorff AE, Marabita F, Lechner M, Bartlett T, Tegner J, Gomez-Cabrero D, Beck S: A beta-mixture quantile normalization method for correcting probe design bias in Illumina Infinium 450 k DNA methylation data. *Bioinformatics* 2013; 29: 189-196

3. Touleimat N, Tost J: Complete pipeline for Infinium((R)) Human Methylation 450K BeadChip data processing using subset quantile normalization for accurate DNA methylation estimation. *Epigenomics* 2012; 4: 325-341

4. Pidsley R, Wong CC, Volta M, Lunnon K, Mill J, Schalkwyk LC: A data-driven approach to preprocessing Illumina 450K methylation array data. *BMC Genomics* 2013; 14: 293

5. Johnson WE, Li C, Rabinovic A: Adjusting batch effects in microarray expression data using empirical Bayes methods. *Biostatistics* 2007; 8: 118-127

6. Leek JT, Johnson WE, Parker HS, Jaffe AE, Storey J: sva: Surrogate Variable Analysis. R package version 3.10.0

7. Jaffe AE, Irizarry RA: Accounting for cellular heterogeneity is critical in epigenome-wide association studies. *Genome Biol* 2014; 15: R31

8. Purcell S, Neale B, Todd-Brown, K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC: PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007; 81: 559-575

9. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D: Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* 2006; 38: 904-909

10. The 1000 Genomes Project Consortium, Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, Kang HM, Marth GT, McVean GA: An integrated map of genetic variation from 1,092 human genomes. *Nature* 2012; 491: 56-65

11. Howie BN, Donnelly P, Marchini J: A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet* 2009; 5: e1000529