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Identification and replication of a combined epigenetic and genetic biomarker predicting suicide and suicidal behaviors

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Abstract

Objective—Suicide is a major preventable public health problem. Reliable identification of individuals at increased suicide risk remains an unfulfilled priority for suicide prevention. The primary purpose of this study was to identify genes exhibiting epigenetic variation associated with suicide and suicidal behaviors.

Methods—Genome-wide DNA methylation profiling was employed separately on neuronal and glial nuclei in a discovery set of *post mortem* brains from the National Institutes of Child Health and Development (NICHD) cohort to identify associations with suicide. Pyrosequencing based validation was conducted in prefrontal cortical tissue from the Stanley Medical Research Institute (SMRI) and Harvard Brain Bank at McLean Hospital (McL) cohorts and peripheral blood from three independent living samples. Functional associations with gene expression, self-reported stress and anxiety, and salivary cortisol measurements were assessed.

Results—The genome-wide DNA methylation scan identified an additive epigenetic and genetic association with suicide at rs7208505 within the 3′UTR of the *SKA2* gene independently in the three brain cohorts. This finding was replicated with suicidal ideation using peripheral blood in three diverse retrospective and prospective cohorts. *SKA2* gene expression was significantly reduced in suicide decedents and was associated with genetic and epigenetic variation of rs7208505, possibly mediated by long-range interaction with miR-301a. Analysis of salivary cortisol measurements suggested that *SKA2* epigenetic and genetic variation may modulate suppression of cortisol, consistent with its implicated role in glucocorticoid receptor transactivation. *SKA2* significantly interacted with anxiety and stress metrics to explain between 79 and 84% of suicidal behavior and the progression from suicidal ideation to suicide attempt.

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Competing financial interests

Z.A.K. and H.C.W. are listed as co-inventors on a patent to evaluate risk of suicidal behavior using genetic and epigenetic variation at the *SKA2* locus. J.P. received legal consulting fees from Pfizer, Astra Zeneca and Johnson and Johnson and research support from Corcept Therapeutics. No additional conflicts of interest are noted.

Conclusions—Our findings implicate *SKA2* as a novel genetic and epigenetic target involved in the etiology of suicide and suicidal behaviors.

Keywords

Suicide; DNA methylation; epigenetic; biomarker; *SKA2*; rs7208505; miR-301a

Introduction

Suicide is a complex, heterogeneous phenotype as well as an intractable public health problem with the overall annual suicide rate remaining stable over the past 60 years at around 10 to 12 per 100,000 (1). The National Action Alliance for Suicide Prevention (NAASP) has set out to build a research agenda with the potential to reduce our national suicide rate by 20% within five years (2). One strategy proposed is to identify and target subgroups at greatest risk. Heuristic models outlining the chain of events leading to suicide often include biological or genetic characteristics, early life trauma/life events/stressors, impulsive aggressive traits, psychopathology, inadequate social support, and access to lethal means (3–5).

A growing body of evidence suggests that suicide vulnerability may be due to epigenetic alterations in molecular pathways important for hypothalamic pituitary adrenal (HPA) axis function. For example, DNA methylation changes in the *NR3C1* gene that encodes the glucocorticoid receptor (GR) are altered by maternal behavior in rats (6) and are elevated in the hippocampus of suicide decedents that experienced early life trauma (7). The cumulative effect of these epigenetically mediated events is a reduction in GR levels possibly leading to impaired responses to stressors. Suicidal individuals exhibit a reduced ability to suppress the experimentally administered synthetic glucocorticoid, dexamethasone (8), and the cortisol stress response has been identified as one of the most promising candidate suicide endophenotypes (3). Other studies have demonstrated evidence that first degree relatives of suicide victims fail to mount a proper HPA axis response to stress (9). Such findings are consistent with the diathesis-stress or dual risk hypothesis, whereby an underlying biological state moderates an aberrant response to stress (10–14). Identification of the underlying genetic and epigenetic factors influencing vulnerability to suicidal behaviors in the context of stressors is needed to maximize suicide prevention efforts.

The objective of this study was to use genome-wide screening techniques to identify novel epigenetic associations in *post mortem* brain tissue of suicide decedents, followed by replication and functional assessment of identified loci. A secondary objective was to assess the degree to which identified loci would be present in peripheral blood samples and to evaluate their biomarker efficacy in the context of stress and anxiety.

Materials and Methods

Human Samples

Post mortem prefrontal cortical tissue samples were obtained from the National Institutes of Child Health and Development (NICHD) University of Maryland Brain Bank of

Developmental Disorders (15), the Stanley Medical Research Institute (SMRI), and the Harvard Brain Bank at McLean Hospital (McL). Peripheral blood was obtained from three Johns Hopkins studies of participants who consented to blood draw for future research including the GenRED Offspring (16, 17), The Prevention Research Center (PRC) study (18, 19), and a prospective cohort of pregnant women described previously (20). A description of the cohorts can be found in Table 1 and Figure S1 and the supplementary methods.

Experimental procedures

Genome-wide DNA methylation data were obtained from Illumina HM450 microarrays previously generated by our group (15) for which data is located under Gene Expression Omnibus accession: GSE15745. A discovery set of NICHD prefrontal cortical tissue data was generated from N=10 Caucasian individuals with Major Depression who did (N=7) and did not (N=3) die by suicide for whom bulk tissue data was available. A replication set consisted of the remaining N= 8 suicide and N= 4 non-suicide samples from Caucasians with Major Depression in the NICHD cohort not originally interrogated.

Pyrosequencing was conducted in microarray identified loci in all individuals in Table 1. All *SKA2* gene expression data and rs7208505 genotyping was performed using quantitative real time PCR (RT-qPCR). Detailed methods in addition to salivary cortisol analysis for the GenRED offspring are available in the online supplementary methods and Table S1.

Statistical analysis

Unless otherwise stated, reported statistics derive from linear regression analysis, adjusted for age, sex, race, and post mortem interval (in brain cohorts) generated in R(http://www.r-project.org/). Relevant additional covariates were adjusted according to the strategy presented in the supplementary methods (Table S2). Using the Cramer-von Mises test, all data distributions that rejected the null hypothesis of normality were subsequently evaluated with non-parametric tests. All statistical tests were two tailed, $p \leq 0.05$ denotes statistical significance, and ± denotes the standard deviation. Microarray analysis employed False Discovery Rate correction for multiple testing. Where specified, genotype correction of *SKA2* 3′UTR DNA methylation was achieved by taking the residuals of a linear model of *SKA2* 3′UTR DNA methylation as a function of rs7208505 genotype. Randomization was employed within all experimental processing batches. Personnel performing laboratory experiments were blind to caseness.

Results

Genome-wide DNA methylation analysis and replication

We performed a genome-wide screen for DNA methylation variation associated with suicide in a small discovery sample of *post mortem* prefrontal cortical tissue from the NICHD sample. Using a linear model adjusting for age and sex as covariates, we identified four loci significantly associated with suicide after correction for multiple testing corresponding to the *ATP8A1* (cg24533989), *SKA2* (cg13989295), *LOC153328* (cg15918259), and *KCNAB2* (cg17106415) genes (Fig 1A). Using fluorescence activated cell sorting (FACS), we separated neuronal and glial nuclei as described previously (15), after which only *SKA2*

exhibited nominal significance in the neuronal and glial fractions of both the discovery and replication sets (Fig 1B). The identified CpG is located on the antisense strand of chromosome 17 at position 57187729 (hg19) within the 3′UTR of the *SKA2* (spindle and kinetochore associated complex subunit 2) gene which encodes a scaffold protein implicated in chaperoning the glucocordicoid receptor (GR) into the nucleus (21). Importantly, the cytosine (C) at this position represents the alternative allele of SNP, rs7208505, while the reference allele is a thymine (T). Importantly, T allele abrogates the CpG dinucleotide and can not be methylated. The assessment of rs7208505 epigenetic and genetic variation in an additive linear model demonstrated significant associations of both model terms with suicide across the entire NICHD cohort of N= 23 suicide cases and N=35 controls independent of ethnicity or DSM-IV diagnosis (Table 2). These associations replicated in two independent cohorts of *post mortem* prefrontal cortical samples from the Stanley Medical Research Institute (SMRI) cohort and the Harvard Brain Bank at McLean Hospital (McL) cohort (Fig 1E, Table 2) and did not appear to be related to the mode of death (Result S1).

Gene expression and functional relevance of the identified locus

In the NICHD brain cohort, *SKA2* exhibited significantly lower gene expression values in suicide cases as compared to controls (Fig 1C). *SKA2* expression was significantly negatively associated with DNA methylation both before and after controlling for rs7208505 variation while genotype alone was not associated (Fig S2A, Table S3).

To understand the effects of 3′UTR epigenetic variation on expression, we correlated rs7208505 variation to all other CpGs located across *SKA2* using available microarray data. Significant correlations were observed in neuronal but not glial DNA in two regions including promoter CpGs flanking a CREB binding site and upstream of an intronic microRNA, miR-301a (Fig S2, Result S2). Epigenetic variation in these regions showed opposing effects on *SKA2* gene expression (Fig S2, Result S2). Average levels of DNA methylation of CpGs in the region upstream of miR-301a were lower in glial relative to neuronal DNA, possibly accounting for the neuron specific associations observed in this region (Fig S2F). Together, rs7208505 epigenetic and genetic variation interacted with promoter and miR-301a proximal epigenetic variation to explain 39.52% of *SKA2* gene expression as compared to the 16% explained by the model with rs7208505 variation alone (Table S3).

Replication in peripheral tissues

We assessed the association of *SKA2* variation with suicidal ideation in peripheral blood samples from the three living cohorts. Across all samples, significant rs7208505 DNA methylation elevations were observed consistent with the brain findings (Fig 1D,E, Table 2, Table S4, Result S4). Model factors were significant among the 30 women in the prospective cohort who provided blood samples months prior to the suicidal ideation measurement (Table S5, Result S4), suggesting suicidal ideation relevant *SKA2* 3′UTR DNA methylation variation preceded the transition to suicidal ideation. Weighted gene co-expression network analysis (WGCNA) (22) in brain and blood derived data provided supporting evidence that peripheral epigenetic variation is a marker of primarily neuronal processes (Result S5).

Association of *SKA2* with salivary cortisol

Using prospectively collected cortisol measurements in the GenRED offspring cohort, we assessed the ability of *SKA2* 3′UTR epigenetic and genetic variation to mediate suppression of cortisol levels. Waking cortisol was significantly associated with suicidal ideation in this cohort; however, cortisol taken at 30 min and 60 min was not associated nor were CpGs in the region directly upstream of rs7208505 (Fig S4A, Result S6). Only waking cortisol was significantly associated with epigenetic variation at rs7208505 (Table S6, Fig S4B); however, as *SKA2* is implicated in glucocorticoid signaling, we reasoned that cortisol levels may interact with *SKA2* to mediate suppression of future cortisol. Prospective investigation of the interaction of waking cortisol with *SKA2* 3′UTR epigenetic and genetic variation was significantly associated with the reduced suppression of cortisol from the 30 to 60 minute time points (Table S6). *SKA2* mediated changes in glucocorticoid signaling may influence and interact with other suicidal ideation related biological variation, such as promoter CpGs in the *SAT1* gene (Result S7, Fig S4C,D) where gene expression has been previously implicated in suicidal behavior (23).

Interaction of *SKA2* with anxiety and stress

We observed a significant interaction of both perceived stress scores and anxiety scores with rs7208505 genotype and DNA methylation in predicting suicidal ideation in the prospective cohort (Table 3, Fig S5). This association was replicated in the GenRED offspring cohort, where anxiety interacted with rs7208505 epigenetic and genetic status to moderate suicide attempt, but not suicidal ideation (Table 3). In the PRC cohort, the anxiety interaction model was associated with suicidal ideation; however, the association became even stronger in the subset with suicide attempt (Table 3, Fig S5). The *SKA2* 3′UTR DNA methylation interaction with anxiety was significantly associated suicide attempt in those with suicidal ideation (Table S6). Finally, a DNA methylation anxiety interaction non-significant trend was observed in distinguishing those with suicide attempt and intent to die from those with suicide attempt without intent to die (Table S6). Cumulatively, our data suggest that epigenetic variation at *SKA2* could increase risk for suicidal ideation and, among ideators with anxiety or stress, suicide attempt is more likely.

Prediction of suicidal behavior

We assessed the ability of our statistical model to predict suicidal ideation in peripheral tissues of living samples. We used suicide attempt data from the PRC cohort to generate an additive linear model of rs7208505 genotype and *SKA2* 3′UTR DNA methylation interacting with anxiety status, controlling for age and sex as covariates. Using anxiety status as the interactive covariate, the GenRED cohort predicted suicidal ideation with an area under the of the receiver operator characteristic curve of 0.71 (95% CI= 0.42 : 1); however, use of salivary cortisol levels as the interactive covariate improved the area under the curve to 0.82 (95% CI= 0.60 : 1) (Fig. 2A). In the prospective sample, the perceived stress metric at the time of blood draw was used as the interactive covariate, resulting in suicidal ideation prediction AUC of 0.80 (95% CI= 0.64 : 0.97) (Fig. 2A). Limiting the sample to prospective prediction of those 30 women where 3rd trimester suicidal ideation was predicted from 1st or 2nd trimester blood generated an AUC of 0.79 (95% CI= 0.42 : 1) (Fig. 2B). Increasing the

stringency of the threshold to define suicidal ideation (see Supplementary Methods) resulted in improved model performance across both comparisons in this cohort (All women, N=51, AUC= 0.91, 95% CI= 0.8 : 1 ; 1st or 2nd trimester women, N=30, AUC = 0.96, 95% CI= 0.89 : 1). In the GenRED cohort, the model predicted those N=4 suicide attempters from the sample with an AUC of 0.97 (95% CI= 0.89 : 1) (Fig. 2B).

Discussion

Using microarray technology to scan for epigenetic suicide associations, we identified a significant effect in a very small population of suicide decedents. The effect size of ~55% DNA methylation difference at *SKA2* enabled this small sample size to have adequate power to survive correction for multiple testing, which was driven by the underlying genetic status of the rs7208505 SNP that abrogates the CpG dinucleotide. While microarray analysis was performed only in Caucasians, incorporation of both the genetic and epigenetic variation at this locus enabled replication across the entirety of the NICHD cohort, two additional *post mortem* brain cohorts, and three blood cohorts. Despite the striking consistency of the findings, the relatively small sample sizes of the studied cohorts suggest they represent promising but preliminary results warranting further study. The presented linear models implicated that DNA methylation and rs7208505 genotype may have opposing effects on suicidal behavior; however, as these metrics were highly correlated, the protective effects of rs7208505 may represent a statistical artifact. Analysis of genetic and epigenetic effects on suicidal behavior and gene expression separately indicated that DNA methylation alone may be the primary factor conferring risk. Importantly, the overall proportion of DNA methylation at rs720505 increases significantly with each successive C containing allele, suggesting that the underlying genetic architecture at rs7208505 may confer vulnerability by providing a genetic template for methylation changes to occur. This risk template would be expected to vary as a function of ethnicity, as allele frequencies for the C containing allele of rs7208505 are reportedly much lower in African Americans (~18%) compared to other ethnicities (~50–60%). Cumulatively, numerous consistent associations were observed with suicidal ideation, suicide attempt, and suicide completion, independent of variation in ethnicity and psychiatric diagnosis suggesting that variation in *SKA2* may mediate risk for suicidal behaviors that progress from ideation, to attempt, to suicide.

SKA2 may influence suicidal phenotypes through its role in chaperoning the GR from the cytoplasm to the nucleus (21). Rice et al., demonstrated that knockdown of *SKA2* eliminated GR transactivation and response to dexamethasone treatment *in vitro* and that protein levels of SKA2 were decreased by glucocorticoid treatment (*ibid*), suggesting *SKA2* gene expression may be a component of the glucocorticoid feedback inhibition system. In our data, *SKA2* genetic and epigenetic differences were associated with reduced suppression of salivary cortisol after waking in the GenRED cohort. As blood was not drawn at the same time as salivary cortisol sampling, the causative role of DNA methylation must be interpreted cautiously. While DNA methylation variation at rs7208505 might be important for suicidal ideation etiology, it remains possible that this variation is a reflection of cortisol variation.

In the above model, *SKA2* epigenetic and genetic variation represents an underlying state increasing suicide risk in the presence of a stressor. *SKA2* epigenetic and genetic variation interacted with stress and anxiety metrics to mediate suicidal ideation in the prospective cohort, while in the PRC and GenRED offspring cohorts, the same model distinguished between individuals with suicidal ideation who transitioned to suicide attempt. It is important to note that the suicidal ideation, suicide attempt and suicide phenotypes are not interchangeable; however, in some individuals, they represent progressive stages of suicidal behavior that share many etiological factors. The proportion of variance accounted by our models was very high in some cohorts and leaves little room for the involvement of other factors. While our data suggest that *SKA2* may be etiologically relevant to glucocorticoid signaling, it is possible that the detected epigenetic variation at *SKA2* also represents a molecular record of suicide dysregulated glucocorticoid load over time and thus may be reflective of other sources of etiologically relevant variation at other suicide implicated loci. A recent report identified blood gene expression at *SAT1* prospectively predicted both suicidal ideation and suicide attempt(23). Our supplemental analysis demonstrated an interaction between *SKA2* variation and DNA methylation at a CpG in the *SAT1* promoter located within a region enriched for GR binding. *SKA2* mediated failure to suppress normal stress response may play a role in *SAT1* gene expression variation and could contribute to the transition from suicidal ideation to suicide attempt. Cumulatively, our data is consistent with an epidemiological study of 108,664 individuals in 21 countries that found disorders characterized by anxiety and poor impulse control predict the transition from suicidal ideation to suicide attempt(24).

One caveat with these analyses is that different metrics of suicidal ideation, suicide attempt, stress and anxiety were available across the studied cohorts. In the PRC and prospective samples, anxiety was reported by response to a single question, while the SCARED scale was used for the GenRED offspring. In the prospective cohort, we showed that anxiety, as measured by the Edinburgh Postnatal Depression Scale, was highly associated with perceived stress, which in another study was correlated with salivary cortisol levels (25). Thus, it is possible the observed interactions of *SKA2* with anxiety across cohorts are a reflection of underlying differences in stress and HPA axis response in anxious individuals. Despite these limitations, the ability of the PRC generated linear model to accurately predict both suicidal ideation and suicide attempt in the prospective and GenRED cohorts suggests that consistency was captured by these diverse metrics.

SKA2 gene expression decreases with suicide in NICHD brains associated primarily with isolated neuronal nuclei, suggesting the epigenetic dysregulation may be confined to neurons. The *post mortem* brain data was generated in the prefrontal cortex, a brain region with inhibitory connections to the HPA axis (26, 27) and responsible for decision making, inhibition of negative thoughts, and impulsivity (28, 29). Reduction of GR transactivation is consistent with current models of suicide diathesis as GR gene expression reduction experiments in rodents mimic suicidal human characteristics (8), exhibiting increases in corticosterone and helplessness in response to stress (30).

The influence of *SKA2* 3′UTR epigenetic variation on gene expression appeared to be mediated by interaction with epigenetic variation within the gene promoter and proximal to

intronic miR-301a, which has previously been shown to be reduced in *post mortem* prefrontal cortex of suicide completers (31). Critically, miR-301a modulates *SKA2* gene expression in A549 cell models by indirectly inhibiting CREB binding to the *SKA2* promoter (32), while the promoter CpGs shown to correlate with rs7208505 DNA methylation above directly flank this CREB binding site. As would be expected given the model, we observed a significant interaction of miR-301a and promoter CpG variation on *SKA2* expression. The observed correlations of *SKA2* 3′UTR DNA methylation with other CpGs across the gene could result from common epigenetic reprogramming effects of GR binding, as the regions demonstrating correlations were located within GR immunoprecipitation peaks identified from ENCODE data. It is also possible that miR-301a proximal genetic variation in linkage disequilibrium with rs7208505 serve to alter GR recruitment, subsequently reprogramming DNA methylation in the region as discussed in the supplementary material. The miR-301a is an intronic microRNA and requires mRNA transcription of *SKA2* to be generated by Drosha (33). DNA methylation upstream of miR-301a may therefore result in co-transcriptional slowing and allow for spliceosomal interaction as occurs with inclusion of methylated alternative exons in alternatively spliced genes (34). Elevated neuronal but not glial DNA methylation levels proximal to miR-301a suggests a possible functionally different effect of miR-301a in these two cell types. Importantly, while epigenetic variation proximal to the miR-301a and CREB binding site was associated with *SKA2* gene expression, it was not associated with suicidal phenotypes.

While a growing number of studies are investigating epigenetic alterations in suicide (23, 35–40), few studies report biomarkers with high prediction accuracy. To our knowledge, the identified biomarker represents the first genetic and epigenetic biomarker capable of predicting suicidal ideation and suicide attempt in a prospective manner with over 80% accuracy from blood. The model performed remarkably well at predicting suicide attempt in the GenRED cohort; however, with 4 attempters, this result should be interpreted with caution. While the PRC cohort contained many more suicide attempt cases, we did not attempt prediction in this cohort as the time between suicide attempt and blood draw was greater than 10 years on average. Accumulating epigenetic change due to stochastic drift, substance use, and errors in retrospective reporting would call into question the reliability of the prediction. However, this highlights the fact that the cause vs. effect of prediction accuracy in the GenRED offspring sample must also be interpreted with care as the blood was taken after suicide attempt. Nevertheless, our data demonstrate very similar accuracies when predicting suicidal ideation in a prospective manner, suggesting *SKA2* epigenetic and genetic variation may represent a trait influencing underlying suicide risk when interacting with stress. Cumulatively, the clinical implications of this finding are that early screening of those at risk for suicidal ideation and suicide attempt may be possible, allowing for the identification of individuals at risk, proactive treatment, and stress and anxiety reduction. The potential biomarker efficacy of our findings have relevance to numerous populations, for example, the military, where the identification of an underlying vulnerability may identify those individuals at risk for developing suicidal behaviors when exposed to the stress of war time situations. Future studies should be carried out to further evaluate the prospective efficacy of this finding in additional populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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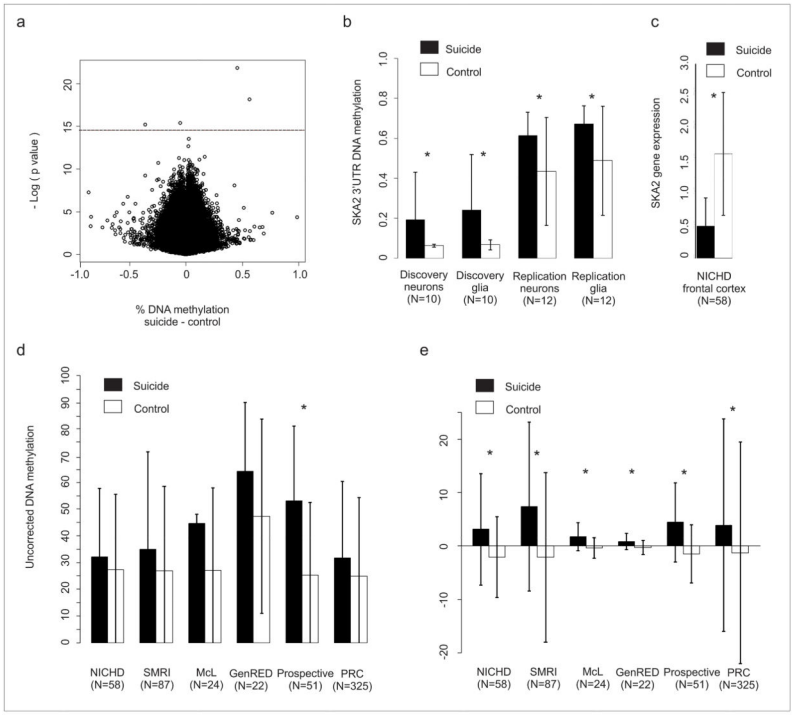

Figure 1. *SKA2* discovery and functional analysis

a.)Volcano plot of linear model based DNA methylation differences (x axis) and negative natural log of the p value (y axis) generated for N=7 suicide and N= 3 non-suicide Major Depression National Institute of Child Health and Development (NICHD samples) generated in bulk tissue. False Discovery Rate significant loci appear above the dashed line identifying significant hits at *ATP8A1* (F= 3424.53, df=3/6, p= 3.2×10^{-10}), *SKA2* (F= 1101.663, df= 3/6, p= 1.3×10^{-8}), *LOC153328* (F= 384.31, df= 3/6, p= 2.5×10^{-7}), and *KCNAB2* (F= 243.51, df=3/6, p= 2.09×10^{-7}). b.) Bar graph depicting significant differences in DNA methylation for the cg13989295 Illumina probe within the *SKA2* 3′UTR for the discovery sample neurons (F= 784, df=3/6, p= 2.76×10^{-8}), discovery sample glia (F= 421, df= 3/6, p= 1.82×10^{-7}), replication neurons (F= 2.2, df= 3/8, p= 0.04) and replication sample glia (F= 3.2, df= 3/8, p= 0.0163). Large error bars derive from unaccounted for variation in rs7208505 genotype. c.) Bar graph depicting significant differences in *SKA2* gene expression for N= 23 suicide and N=35 controls from prefrontal cortical samples from the NICHD cohort (Wilcoxon Rank Sum test; W= 97, df= 49.91, p= 3.8×10^{-06}). d.) Bar graph depicting uncorrected DNA methylation levels at rs7208505 (y axis) across all cohorts included in the study (x axis). Associated significance metrics are located in Table S4. e.) Bar graph depicting genotype corrected DNA methylation levels at rs7208505 (y axis) across all cohorts included in the study (x axis). Associated significance metrics are located in Table 2. All error bars represent standard deviations.

* P≤ 0.05

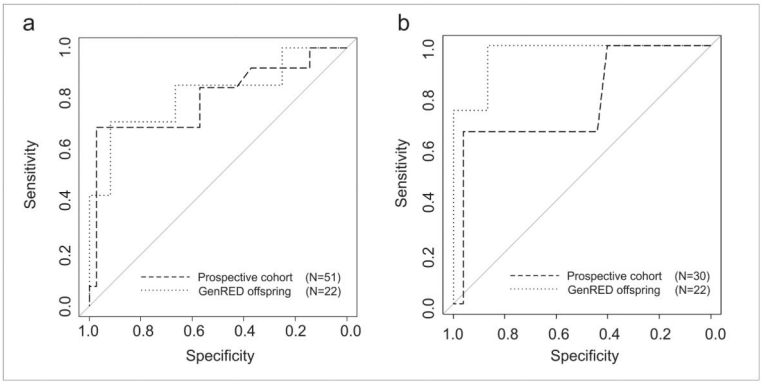

Figure 2. Prediction of suicidal behavior

a.) Receiver operator characteristic curves depicting the sensitivity (y axis) as a function of the specificity (x axis) for suicidal ideation predictions generated for the GenRED offspring and prospective cohorts based on models built on the PRC cohort data b.) ROC curves depicting the sensitivity (y axis) as a function of the specificity (x axis) for N=4 suicide attempt cases from the GenRED offspring cohort and those prospectively predicted third trimester suicidal ideation cases from the prospective cohort where blood was derived from first or second trimester blood.

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Table 1

Sample demographics

Post Mortem Brain Samples

Cohort	Diagnosis	Suicidal Behavior(Y:N)	N	Age	Age (sd)	Sex (M:F)	Substance Use (Y:N:MD)	Psychiatric Medication (Y:N)	PMI	PMI (sd)
NICHD	Major depression	21:8	29	32.00	15.92	14:15	9:20	12:17	18.10	7.09
	Control	2:27	29	32.10	16.05	14:15	2:27	0:29	16.14	4.97
SMRI	Bipolar disorder	13:15	28	46.14	11.09	13:15	24:3:1	27:1	39.21	19.7
	Control	0:29	29	43.76	7.73	22:7	16:13	0:29	29.10	13.7
	Schizophrenia	6:23	29	43.07	6.58	22:7	19:8:1	29:0	31.86	16.1
McL	Bipolar disorder	4:8	12	60.50	20.01	7:5	1:11	8:4	21.07	10.5
	Control	0:12	12	61.67	16.22	8:4	4:8	0:12	21.33	5.67

Peripheral Blood Samples

Cohort	Diagnosis	Suicidal Behavior (Y:N)	N	Age	Age (sd)	Sex (M:F)	Substance Use(Y:N)	Psychiatric Medication (Y:N)
GenRED Offspring	Bipolar disorder	1:0	1	21	NA	0:1	1:0	0:1
Suicidal Ideation	Major depression	3:5	8	18.13	3.04	6:02	2:6	1:7
	Control	3:10	13	15.31	2.32	5:08	0:13	4:9
GenRED Offspring	Bipolar disorder	1:0	1	21	NA	0:1	1:0	0:1
Suicide Attempt	Major depression	2:6	8	18.13	3.04	6:2	2:6	1:7
	Control	1:12	13	15.31	2.32	5:8	0:13	4:9
Prospective	Bipolar disorder	3:11	14	29.29	6.39	0:14	1:13	8:6
Suicidal Ideation	Major depression	10:27	37	31.45	6.38	0:37	1:36	25:12
PRC	Major depression	20:10	30	29.64	1.21	11:19	17:13	NA
	Control	59:236	295	30.46	2.56	117:178	70:225	NA
	Major depression	15:15	30	29.64	1.21	11:19	17:13	NA
	Control	33:262	295	30.46	2.56	117:178	70:225	NA

Y= yes

N=no

MD= missing data

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Table 2

SKA2 rs7208505 epigenetic and genetic effects on suicide risk

Brain									
	NICHD Neurons Suicide (N=58)			**SMRI Brain Suicide (N= 87)**			**McL Brain Suicide (N= 24)**		
Model Terms	**β value**	**Error**	**P value**	**β value**	**Error**	**P value**	**β value**	**Error**	**P value**
DNA methylation	0.015	0.0067	0.026	0.0063	0.0026	0.018	0.085	0.022	0.0014
rs7208505 C/T	−0.58	0.27	0.037	−0.3	0.13	0.027	−3.4	0.96	0.0026
rs7208505 C/C	−1.5	0.57	0.014	−0.47	0.22	0.037	−7.1	1.9	0.0014
Age	−0.01	0.0041	0.019	−0.0039	0.0051	0.44	−0.014	0.003	0.00018
Sex	−0.08	0.13	0.54	0.17	0.094	0.068	0.035	0.11	0.75
PMI	0.019	0.011	0.084	0.0037	0.0027	0.17	−0.0071	0.0075	0.35
F	2.2			2.4			8.3		
DF	8/48			10/75			6/17		
Model R^2	0.27		0.042	0.24		0.017	0.75		2.6×10^{-4}
Blood									
	GenRED offspring Suicidal Ideation (N= 22)			**Prospective Suicidal Ideation (N= 51)**			**PRC Suicidal Ideation (N= 325)**		
Model Terms	**β value**	**Error**	**P value**	**β value**	**Error**	**P value**	**β value**	**Error**	**P value**
DNA methylation	0.17	0.074	0.043	0.071	0.02	0.00075	0.0023	0.0011	0.043
rs7208505 C/T	−6.5	3.1	0.056	−3	1	0.0046	−0.051	0.06	0.4
rs7208505 C/C	−15	6.7	0.047	−6.1	1.8	0.0016	−0.14	0.1	0.16
Age	0.033	0.039	0.41	0.018	0.021	0.41	0.0061	0.0093	0.51
Sex	−0.087	0.21	0.69	na	na	na	−0.0016	0.047	0.97
F	2			2.7			3.9		
DF	6/12			8/42			10/314		
Model R^2	0.5		0.14	0.34		0.018	0.11		6.1×10^{-5}

DNAm= DNA methylation

C/T= rs7208505 heterozygotes

C/C= rs7208505 alternative homozygotes

Table 3

Interactive effects on suicidal behavior

Sample	Prospective Suicidal Ideation (N=31)			GenRED Offspring Suicide Attempt (N= 22)		
Model Terms	**β value**	**Error**	**P value**	**β value**	**Error**	**P value**
DNAm	−0.074	0.048	0.14	0.0054	0.039	0.89
C/T	2.66	2.32	0.27	−0.23	1.6	0.89
C/C	6.30	4.42	0.17	−0.34	3.5	0.92
Anxiety	−0.13	0.13	0.31	−0.32	0.3	0.3
Age	0.010	0.014	0.48	0.039	0.025	0.15
Sex	na	na	na	0.21	0.13	0.14
DNAm X Anxiety	0.049	0.016	0.007	0.21	0.08	0.02
C/T X Anxiety	−1.92	0.78	0.025	−7.8	3.2	0.031
C/C X Anxiety	−4.18	1.46	0.011	−18	7.1	0.024
F	6.8			4.3		
DF	10/17			9/12		
Model R^2	0.8		0.00032	0.8		0.012
Sample	**PRC Suicidal Ideation (N= 325)**			**PRC Suicide Attempt (N= 325)**		
Model Terms	**β value**	**Error**	**P value**	**β value**	**Error**	**P value**
DNAm	0.001	0.0012	0.37	3.7×10^{-4}	9.7×10^{-4}	0.70
C/T	−0.017	0.064	0.79	−0.014	0.053	0.79
C/C	−0.047	0.11	0.66	0.054	0.088	0.54
Anxiety	0.15	0.15	0.32	−0.11	0.12	0.36
Age	0.004	0.01	0.66	0.0017	0.008	0.83
Sex	−0.03	0.05	0.58	−0.01	0.040	0.87
DNAm X Anxiety	0.009	0.004	0.033	0.0106	0.0036	0.0039
C/T X Anxiety	−0.39	0.24	0.10	−0.11	0.20	0.57
C/C X Anxiety	−0.98	0.41	0.02	−0.95	0.34	0.006
F	2.6			1.9		
DF	12/312			12/312		
Model R^2	0.09	0.003	0.003	0.067		0.037

Race was adjusted for in all models but data are not shown due to space

DNAm= DNA methylation

C/T= rs7208505 heterozygotes

C/C= rs7208505 alternative homozygotes

X denotes an interaction