

IMMEDIATE COMMUNICATION

Understanding and predicting suicidality using a combined genomic and clinical risk assessment approach

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Worldwide, one person dies every 40 seconds by suicide, a potentially preventable tragedy. A limiting step in our ability to intervene is the lack of objective, reliable predictors. We have previously provided proof of principle for the use of blood gene expression biomarkers to predict future hospitalizations due to suicidality, in male bipolar disorder participants. We now generalize the discovery, prioritization, validation, and testing of such markers across major psychiatric disorders (bipolar disorder, major depressive disorder, schizoaffective disorder, and schizophrenia) in male participants, to understand commonalities and differences. We used a powerful within-participant discovery approach to identify genes that change in expression between no suicidal ideation and high suicidal ideation states ($n = 37$ participants out of a cohort of 217 psychiatric participants followed longitudinally). We then used a convergent functional genomics (CFG) approach with existing prior evidence in the field to prioritize the candidate biomarkers identified in the discovery step. Next, we validated the top biomarkers from the prioritization step for relevance to suicidal behavior, in a demographically matched cohort of suicide completers from the coroner's office ($n = 26$). The biomarkers for suicidal ideation only are enriched for genes involved in neuronal connectivity and schizophrenia, the biomarkers also validated for suicidal behavior are enriched for genes involved in neuronal activity and mood. The 76 biomarkers that survived Bonferroni correction after validation for suicidal behavior map to biological pathways involved in immune and inflammatory response, mTOR signaling and growth factor regulation. mTOR signaling is necessary for the effects of the rapid-acting antidepressant agent ketamine, providing a novel biological rationale for its possible use in treating acute suicidality. Similarly, MAOB, a target of antidepressant inhibitors, was one of the increased biomarkers for suicidality. We also identified other potential therapeutic targets or biomarkers for drugs known to mitigate suicidality, such as omega-3 fatty acids, lithium and clozapine. Overall, 14% of the top candidate biomarkers also had evidence for involvement in psychological stress response, and 19% for involvement in programmed cell death/cellular suicide (apoptosis). It may be that in the face of adversity (stress), death mechanisms are turned on at a cellular (apoptosis) and organismal level. Finally, we tested the top increased and decreased biomarkers from the discovery for suicidal ideation (CADM1, CLIP4, DTNA, KIF2C), prioritization with CFG for prior evidence (SAT1, SKA2, SLC4A4), and validation for behavior in suicide completers (IL6, MBP, JUN, KLHDC3) steps in a completely independent test cohort of psychiatric participants for prediction of suicidal ideation ($n = 108$), and in a future follow-up cohort of psychiatric participants ($n = 157$) for prediction of psychiatric hospitalizations due to suicidality. The best individual biomarker across psychiatric diagnoses for predicting suicidal ideation was SLC4A4, with a receiver operating characteristic (ROC) area under the curve (AUC) of 72%. For bipolar disorder in particular, SLC4A4 predicted suicidal ideation with an AUC of 93%, and future hospitalizations with an AUC of 70%. SLC4A4 is involved in brain extracellular space pH regulation. Brain pH has been implicated in the pathophysiology of acute panic attacks. We also describe two new clinical information apps, one for affective state (simplified affective state scale, SASS) and one for suicide risk factors (Convergent Functional Information for Suicide, CFI-S), and how well they predict suicidal ideation across psychiatric diagnoses (AUC of 85% for SASS, AUC of 89% for CFI-S). We hypothesized *a priori*, based on our previous work, that the integration of the top biomarkers and the clinical information into a universal predictive measure (UP-Suicide) would show broad-spectrum predictive ability across psychiatric diagnoses. Indeed, the UP-Suicide was able to predict suicidal ideation across psychiatric diagnoses with an AUC of 92%. For bipolar disorder, it predicted suicidal ideation with an AUC of 98%, and future hospitalizations with an AUC of 94%. Of note, both types of tests we developed (blood biomarkers and clinical information apps) do not require asking the individual assessed if they have thoughts of suicide, as individuals who are truly suicidal often do not share that information with clinicians. We propose that the widespread use of such risk prediction tests as part of routine or targeted healthcare assessments will lead to early disease interception followed by preventive lifestyle modifications and proactive treatment.

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INTRODUCTION

'Do the difficult things while they are easy and do the great things while they are small'.
- Lao Tzu

Predicting suicidal behavior in individuals is one of the hard problems in psychiatry, and in society at large. Improved, objective, and quantitative ways to do it are needed. One cannot always ask individuals if they are suicidal, as desire not to be stopped or future impulsive changes of mind may make their self-report of feelings, thoughts and plans to be unreliable. We had previously provided proof of principle of how first generation blood biomarkers for suicide discovered in male bipolar participants, alone or in combination with clinical symptoms data for anxiety and mood, could have predictive ability for future hospitalizations for suicidality. We now present comprehensive new data for discovery, prioritization, validation, and testing of next-generation broad-spectrum blood biomarkers for suicidal ideation (SI) and behavior, across psychiatric diagnoses. We also describe two clinical information questionnaires in the form of apps, one for affective state (Simplified Affective State Scale, SASS) and one for suicide risk factors (Convergent Functional Information for Suicide, CFI-S), and show their utility in predicting suicidality. Both these instruments do not directly ask about SI. Lastly, we demonstrate how our *a priori* primary end point, a comprehensive universal predictor for suicide (UP-Suicide), composed of the combination of top biomarkers (from discovery, prioritization and validation), along with CFI-S, and SASS, predicts in independent test cohorts SI and future psychiatric hospitalizations for suicidality.

MATERIALS AND METHODS

Human participants

We present data from four cohorts: one live psychiatric participants discovery cohort; one post-mortem coroner's office validation cohort; and two live psychiatric participants test cohorts—one for predicting SI and one for predicting future hospitalizations for suicidality (Figure 1).

The live psychiatric participants are part of a larger longitudinal cohort being collected and studied by us. Participants are recruited from the patient population at the Indianapolis VA Medical Center. The participants are recruited largely through referrals from care providers, the use of brochures left in plain sight in public places and mental health clinics, and through word of mouth. All participants understood and signed informed consent forms detailing the research goals, procedure, caveats and safeguards. Participants completed diagnostic assessments by an extensive structured clinical interview—Diagnostic Interview for Genetic Studies—at a baseline visit, followed by up to six testing visits, 3–6 months apart or whenever a hospitalization occurred. At each testing visit, they received a series of psychiatric rating scales, including the Hamilton Rating Scale for Depression-17, which includes a suicidal ideation (SI) rating item (Figure 2), and the blood was drawn. Whole blood (10 ml) was collected in two RNA-stabilizing PAXgene tubes, labeled with an anonymized ID number, and stored at -80°C in a locked freezer until the time of future processing. Whole-blood (predominantly lymphocyte) RNA was extracted for microarray gene expression studies from the PAXgene tubes, as detailed below. We focused this study on a male population because of the demographics of our catchment area (primarily male in a VA Medical Center), and to minimize any potential gender-related effects on gene expression, which would have decreased the discriminative power of our analysis given our relatively small sample size.

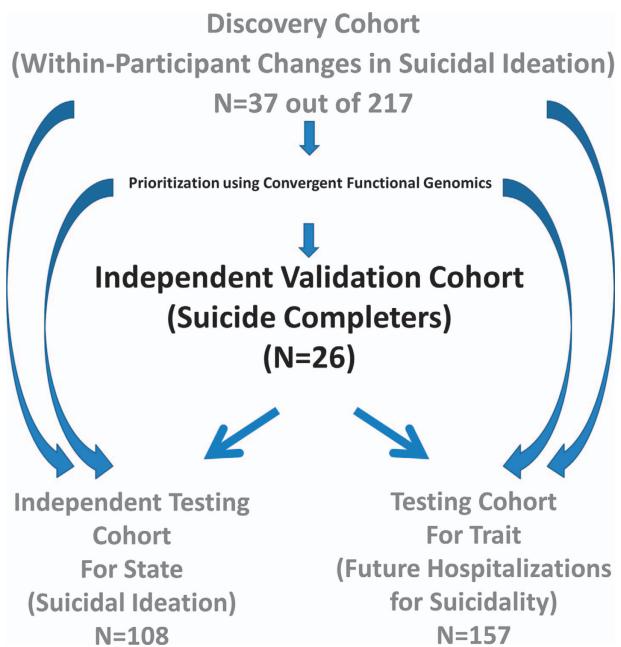


Figure 1. Cohorts used in study depicting flow of discovery, prioritization, validation and testing of biomarkers from each step.

Our within-participant discovery cohort, from which the biomarker data were derived, consisted of 37 male participants with psychiatric disorders, with multiple visits in our laboratory, who each had at least one diametric change in SI scores from no SI to high SI from one testing visit to another testing visit. There was one participant with six visits, one participant with five visits, one participant with four visits, 23 participants with three visits each, and 11 participants with two visits each, resulting in a total of 106 blood samples for subsequent microarray studies (Figure 2 and Table 1).

Our post-mortem cohort, in which the top biomarker findings were validated, consisted of a demographically matched cohort of 26 male violent suicide completers obtained through the Marion County coroner's office (Table 1 and Supplementary Table S2). We required a last observed alive post-mortem interval of 24 h or less, and the cases selected had completed suicide by means other than overdose, which could affect gene expression. Fifteen participants completed suicide by gunshot to head or chest, nine by hanging, one by electrocution and one by slit wrist. Next of kin signed informed consent at the coroner's office for donation of blood for research. The samples were collected as part of our INBRAIN initiative (Indiana Center for Biomarker Research in Neuropsychiatry).

Our independent test cohort for predicting SI (Table 1) consisted of 108 male participants with psychiatric disorders, demographically matched with the discovery cohort, with one or multiple testing visits in our laboratory, with either no SI, intermediate SI, or high SI, resulting in a total of 223 blood samples in whom whole-genome blood gene expression data were obtained (Table 1 and Supplementary Table S1).

Our test cohort for predicting future hospitalizations (Table 1 and Supplementary Table S1) consisted of male participants in whom whole-genome blood gene expression data were obtained by us at testing visits over the years as part of our longitudinal study. If the participants had multiple testing visits, the visit with the highest marker (or combination of markers) levels was selected for the analyses (so called "high watermark" or index visit). The participants' subsequent number of psychiatric hospitalizations, with or without suicidality, was tabulated from electronic medical records. All participants had at least 1 year of

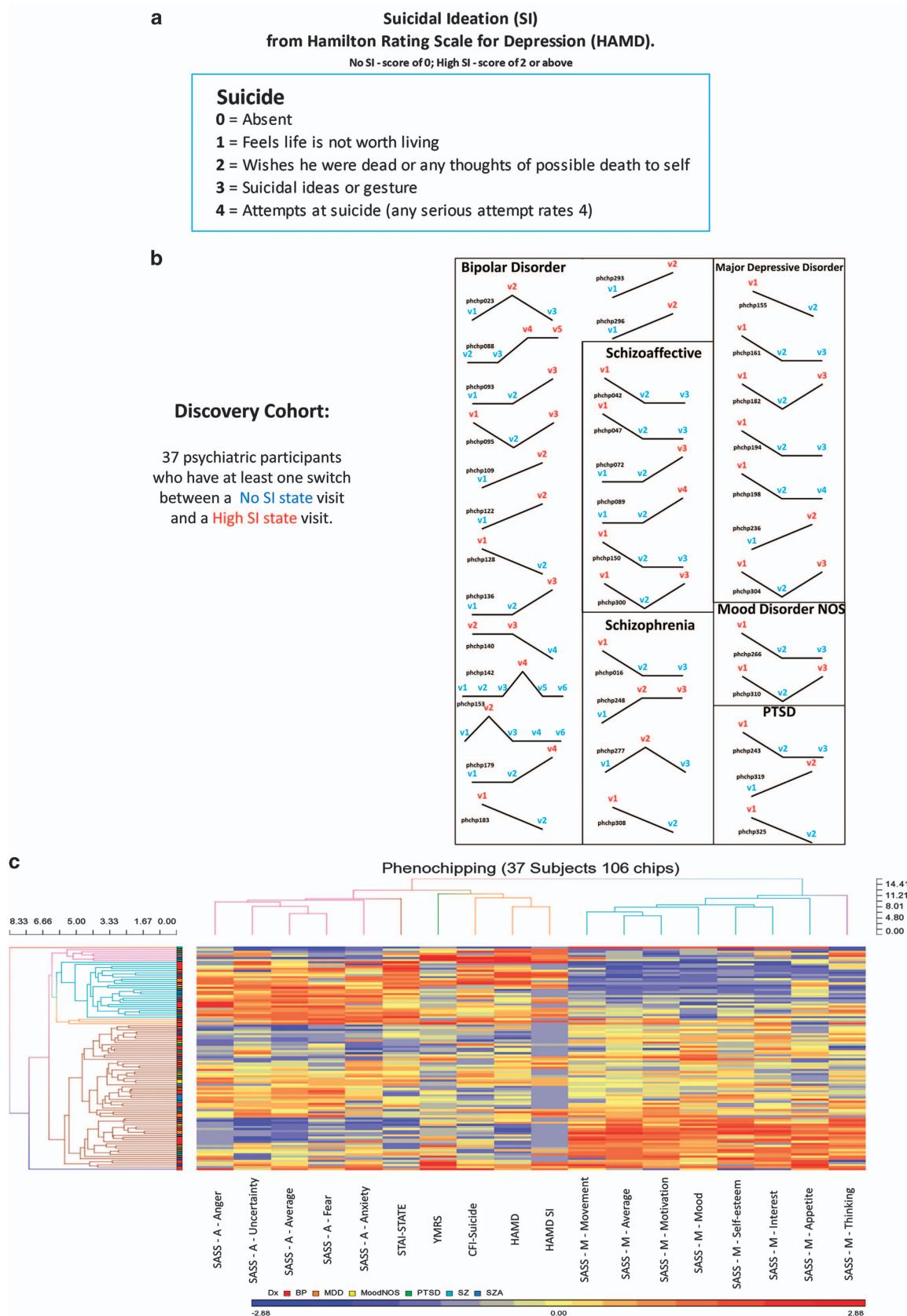


Figure 2. Discovery cohort: longitudinal within-participant analysis. Phchp### is study ID for each participant. V# denotes visit number (1, 2, 3, 4, 5 or 6). (a) Suicidal ideation (SI) scoring. (b) Participants and visits. (c) PhenoChipping: two-way unsupervised hierarchical clustering of all participant visits in the discovery cohort vs 18 quantitative phenotypes measuring affective state and suicidality. A—anxiety items (anxiety, uncertainty, fear, anger, average). M—mood items (mood, motivation, movement, thinking, self-esteem, interest, appetite, average). SASS, simplified affective state scale; STAI-STATE, state trait anxiety inventory, state subscale; YMRS, Young Mania Rating Scale.

Table 1. Cohorts used in study

	Subjects	Diagnosis	Ethnicity	Age mean s.d.	T-test for age	
Discovery cohort (within-participant changes in suicidal ideation)	37	BP = 15 MDD = 7 SZA = 6 SZ = 4 PTSD = 3 Mood NOS = 2	EA = 29 AA = 8 Other = 0	47.25 8.59		
Independent validation cohort-gene expression (suicide completers)	26	NP = 13 MDD = 8 BP = 2 SZ = 1 AX = 1 Alcoholism = 1	EA = 21 AA = 4 Other = 1	40.81 17.47	T-test for age with discovery cohort 0.114	
Independent validation cohort-CFI-S (suicide completers)	35	NP = 14 MDD = 16 BP = 2 SZ = 1 AX = 1 Alcoholism = 1	EA = 29 AA = 4 Other = 2	42.46 17.82	T-test for age with discovery cohort 0.156	
Independent testing cohort for state (suicidal ideation)	108	No SI BP = 17 MDD = 17 SZA = 19 SZ = 20 Intermediate SI BP = 5 MDD = 0 SZA = 3 SZ = 4 High SI BP = 7 MDD = 8 SZA = 6 SZ = 2	EA = 71 AA = 36 Other = 1	47.1 9.6 No SI = 47.8 High SI = 45.7	T-test for age between no and high SI 0.554	T-test for age with discovery cohort P = 0.919
Testing cohort for trait (first year hospitalizations for suicidality)	157 (No Hosp for Suicidality = 139 Hosp for Suicidality = 18)	No Hosp for Suicidality BP = 43 MDD = 20 SZA = 41 SZ = 35 Hosp for Suicidality BP = 7 MDD = 3 SZA = 3 SZ = 5	No hosp for SI EA = 90 AA = 47 Other = 2	49.6 9.5 No hosp for SI = 49.56 Hosp for SI = 49.92	T-test for age between no Hosp for suicidality and Hosp for suicidality 0.886	T-test for age with discovery cohort 0.149
Testing cohort for trait (all future hospitalizations for suicidality)	157 (No Hosp for Suicidality = 122 Hosp for Suicidality = 35)	No Hosp for Suicidality BP = 41 MDD = 20 SZA = 29 SZ = 32 Hosp for Suicidality BP = 9 MDD = 3 SZA = 15 SZ = 8	No hosp for Suicidality EA = 78 AA = 43 Other = 1	49.6 9.5 No Hosp for suicidality = 49.9 Hosp for suicidality = 48.4	T-test for age between no Hosp for suicidality and Hosp for suicidality 0.436	T-test for age with discovery cohort 0.149

Abbreviations: AX, anxiety disorder nos; BP, bipolar; CFI-S, Convergent Function Information for Suicide; MDD, major depressive disorder; NP, non-psychiatric; PTSD, post-traumatic stress disorder; SZA, schizoaffective; SZ, schizophrenia; SI, suicidal ideation.

follow-up or more at our VA Medical Center since the time of the testing visits in the laboratory. Participants were evaluated for the presence of future hospitalizations for suicidality, and for the frequency of such hospitalizations. A hospitalization was deemed to be without suicidality if suicidality was not listed as a reason for admission, and no SI was described in the admission and discharge medical notes. Conversely, a hospitalization was deemed to be because of suicidality if suicidal acts or intent

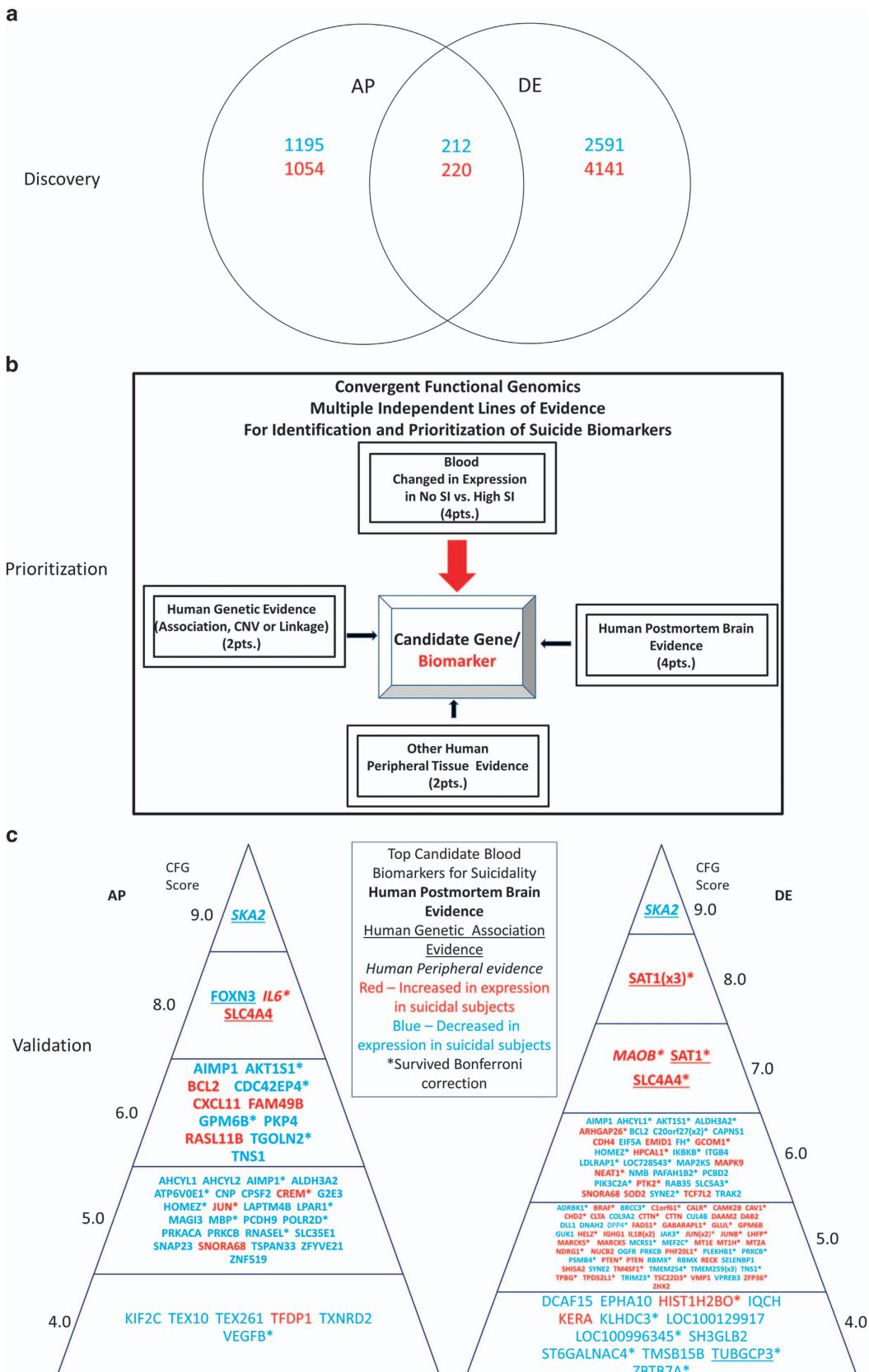
was listed as a reason for admission, and/or SI was described in the admission and discharge medical notes.

Medications

The participants in the discovery cohort were all diagnosed with various psychiatric disorders (Table 1). Their psychiatric medications were listed in their electronic medical records, and

GeneCards to confirm the primary gene symbol. In addition, for those probesets that were not assigned a gene name by NetAffyx or Partek, we used the UCSC Genome Browser to directly map

them to known genes, with the following limitations: (1) in case the probeset fell in an intron, that particular gene was assumed to be implicated; and (2) only one gene was assigned to each



probeset. Genes were then scored using our manually curated CFG databases as described below (Figure 3).

Convergent functional genomics

Databases. We have established in our laboratory (Laboratory of Neurophenomics, Indiana University School of Medicine, www.neurophenomics.info) manually curated databases of all the human gene expression (post-mortem brain, blood and cell cultures), human genetics (association, copy number variations and linkage) and animal model gene expression and genetic studies published to date on psychiatric disorders. Only the findings deemed significant in the primary publication, by the study authors, using their particular experimental design and thresholds, are included in our databases. Our databases include only primary literature data and do not include review papers or other secondary data integration analyses to avoid redundancy and circularity. These large and constantly updated databases have been used in our CFG cross validation and prioritization (Figure 3). For this study, data from 437 papers on suicide were present in the databases at the time of the CFG analyses.

Human post-mortem brain gene expression evidence. Converging evidence was scored for a gene if there were published reports of human post-mortem data showing changes in expression of that gene or changes in protein levels in brains from participants who died from suicide.

Human blood and other peripheral tissue gene expression data. Converging evidence was scored for a gene if there were published reports of human blood, lymphoblastoid cell lines, cerebrospinal fluid or other peripheral tissue data showing changes in expression of that gene or changes in protein levels in participants who had a history of suicidality or who died from suicide.

Human genetic evidence (association and linkage). To designate convergence for a particular gene, the gene had to have independent published evidence of association or linkage for suicide. For linkage, the location of each gene was obtained through GeneCards (<http://www.genecards.org>), and the sex averaged cM location of the start of the gene was then obtained through <http://compgen.rutgers.edu/mapinterpolator>. For linkage convergence, the start of the gene had to map within 5 cM of the location of a marker linked to the disorder.

CFG scoring. For CFG analysis (Figure 3), the external cross-validating lines of evidence were weighted such that findings in human post-mortem brain tissue, the target organ, were prioritized over peripheral tissue findings and genetic findings, by giving them twice as many points. Human brain expression evidence was given four points, whereas human peripheral evidence was given two points, and human genetic evidence was given a maximum of two points for association, and one point for linkage. Each line of evidence was capped in such a way that any positive findings within that line of evidence result in maximum points, regardless of how many different studies support that single line of evidence, to avoid potential popularity

biases. In addition to our external CFG score, we also prioritized genes based upon the initial gene expression analyses used to identify them. Probesets identified by gene expression analyses could receive a maximum of four points. Thus, the maximum possible total CFG score for each gene was 12 points (four points for the internal score and eight points for the external CFG score) (Table 2). The scoring system was decided upon before the analyses were carried out. We sought to give twice as much weight to external score as to internal in order to increase generalizability and avoid fit to cohort of the prioritized genes.⁵ It has not escaped our attention that other ways of scoring the lines of evidence may give slightly different results in terms of prioritization, if not in terms of the list of genes *per se*. Nevertheless, we feel this simple scoring system provides a good separation of genes based on gene expression evidence and on independent cross-validating evidence in the field (Figure 3). In the future, with multiple large data sets, machine learning approaches could be used and validated to assign weights to CFG.

Pathway analyses

IPA 9.0 (Ingenuity Systems, www.ingenuity.com, Redwood City, CA, USA), GeneGO MetaCore (Encinitas, CA, USA), and Kyoto Encyclopedia of Genes and Genomes (KEGG) (through the Partek Genomics Suite 6.6 software package) were used to analyze the biological roles, including top canonical pathways, and diseases, of the candidate genes resulting from our work, as well as to identify genes in our data set that are the target of existing drugs (Table 3 and Supplementary Table S3). We ran the analyses together for all the AP and DE probesets with a total CFG score ≥ 4 , then for those of them that showed stepwise change in the suicide completers validation cohort, then for those of them that were nominally significant, and finally for those of them that survived Bonferroni correction.

Validation analyses

For validation of our candidate biomarker genes, we examined which of the top candidate genes (CFG score of 4 or above) were stepwise changed in expression from the no SI group to the high SI group to the suicide completers group. We used an empirical cut-off of 33.3% of the maximum possible CFG score of 12, which also permits the inclusion of potentially novel genes with maximal internal CFG score but no external CFG score. Statistical analyses were performed in SPSS using one-way analysis of variance and Bonferroni corrections.

For the AP analyses, we imported the Affymetrix microarray data files from the participants in the validation cohort of suicide completers into MASS, alongside the data files from the participants in the discovery cohort.

For the DE analyses, we imported .cel files into Partek Genomic Suites. We then ran a RMA, background corrected with quantile normalization, and a median polish probeset summarization of all the chips from the validation cohort to obtain the normalized expression levels of all probesets for each chip. Partek normalizes expression data into a log base of 2 for visualization purposes. We non-log-transformed expression data by taking 2 to the power of the transformed expression value. We then used the non-log-



Figure 3. Biomarker discovery, prioritization and validation. (a) Discovery—number of probesets carried forward from the absent–present and differential expression analyses, with an internal score of 1 and above. Red-increased in expression in high suicidal ideation, blue-decreased in expression in high suicidal ideation. (b) Prioritization—convergent functional genomics integration of multiple lines of evidence to prioritize suicide-relevant genes from the discovery step. (c) Validation—top convergent functional genomics genes, with a total score of 4 and above, validated in the cohort of suicide completers. All the genes shown were significantly changed in analysis of variance from no suicidal ideation to high suicidal ideation to suicide completers. *Survived Bonferroni correction. SAT1 (x3) had three different probesets with the same total score of 8.

Table 2. Top biomarkers for suicidality from discovery, prioritization and validation

Gene symbol/gene name	Probesets	Discovery (change) method/ score	Prior human genetic evidence	Prior human brain expression evidence	Prior human peripheral expression evidence	Total CFG score For suicide	Prioritization CFG score For suicide	Validation ANOVA P-value	Comment
SKA2 spindle and kinetochore associated complex subunit 2	225686_at	(D) DE/1 AP/1	Suicide ¹⁵	(D) PFC ¹⁵	(D) Methylation ¹⁵ in blood ¹⁵	9	0.006	Top Decreased BioM In prioritization from AP and DE	
IL6 interleukin 6 (interferon, beta 2)	205207_at	(I) AP/2		(I) PFC ²⁷ Hippocampus ²⁸	(I) CSF ^{29,30} (D) Blood ³¹	8	1.44e-08	Top Increased BioM in Validation from AP	
SAT1 spermidine/spermine N1-acetyltransferase 1	213988_s_at 210592_s_at 230333_at 203455_s_at	(I) DE/2 DE/1	Suicide ^{32,33}	(I) PFC BA46 ¹²	(I) Blood ¹	8	1.08e-44 1.24e-40 6.93e-12	Top Increased BioM in Prioritization from DE Top biomarker in our previous work	
SLC4A4 solute carrier family 4 (sodium bicarbonate cotransporter), member 4	211494_s_at 210739_x_at	(I) AP/2 DE/1	Suicide ³⁴	(D) PFC BA46/10 ³⁵		8	5.84e-05 0.002	Top Increased BioM in Prioritization from AP	
JUN jun proto-oncogene	201464_x_at 213281_at 201166_s_at	(I) DE/1 AP/1		(D) Hipp ³⁶		5	2.63e-51 1.02e-41 2.21e-08	Top Increased BioM in Validation from DE	
MBP myelin basic protein	225408_at	(D) AP/1		(I) NAC ¹¹		5	6.74e-10	Top Decreased BioM in Validation from AP	
CADM1 cell adhesion molecule 1	237759_at	(I) DE/4				4	NC	Top Increased BioM in Discovery from DE	
CLIP4 CAP-GLY domain containing linker protein family, member 4	2119944_at	(D) DE/4				4	NC	Top Decreased BioM in Discovery from DE	
DTN4 dystrobrevin, alpha	211493_x_at		(I) AP/4			4	NC	Top Increased BioM in Discovery from AP	
KIF2C kinesin family member 2C	211519_s_at	(D) AP/4				4	0.00056	Top Decreased BioM in Discovery from AP	
KLHD3 kelch domain containing 3	214383_x_at	(D) DE/4		(D) Blood ¹		4	1.57e-17	Top Decreased BioM in Validation from DE A top biomarker in our previous study	
MAOB monoamine oxidase B	204041_at	(I) DE/1		(I) PFC ³⁷	(D) Blood ³⁸	7	8.11e-08	Top Pharmacological Target	
MARC/S myristoylated alanine-rich protein kinase C substrate homolog	213002_at 201670_s_at	(I) DE/1		(I) Hipp_PFC ³⁹ PFC ⁴⁰	(I) Blood ¹	5	1.51e-06; 0.0004	A top biomarker in our previous study	
PTEN phosphatase and tensin homolog	204053_x_at 222176_at	(I) DE/1		(I) PFC, Hipp ^{41,42}	(I) Blood ¹	5	7.66e-17; 0.0003	A top biomarker in our previous study	

Abbreviations: ANOVA, analysis of variance; AP, absent-present; CFG, convergent functional genomics; CSF, cerebrospinal fluid; DE, differential expression; SI, suicidal ideation. Bolded P-values are Bonferroni significant. NC—Non-concordant-not stepwise from no SI to high SI to suicide completers.

facto field-wide collaboration. We use in essence, in a Bayesian fashion, the whole body of knowledge in the field to leverage findings from our discovery data sets. Unlike our use of CFG in many previous studies, for the current one we did not use any animal model evidence, as there are to date no clear animal models of self-harm or suicidality published to date.

Validation of biomarkers for behavior in suicide completers

For validation in suicide completers, we used 412 genes that had a CFG score of 4 and above, from AP and DE, reflecting either maximum internal score from discovery or additional external literature cross-validating evidence. Out of these, 208 did not show any stepwise change in suicide completers (non-concordant, NC). As such, they may be involved primarily in ideation and not in behavior (Supplementary Table S6). The remaining 204 genes (49.5%) had levels of expression that were changed stepwise from no SI to high SI to suicide completion. 143 of these genes (34.7%) were nominally significant, and 76 genes (18.4%) survived Bonferroni correction for multiple comparisons (Figure 3 and Supplementary Figure S1). These genes are likely involved in SI and suicidal behavior. (You can have SI without suicidal behavior, but you cannot have suicidal behavior without SI).

Selection of biomarkers for testing of predictive ability

For testing, we decided *a priori* to select the top scoring increased and decreased biomarkers from each step (discovery, prioritization, validation), so as to avoid potential false negatives in the prioritization step due to lack of prior evidence in the literature, or false negatives in validation step due to possible post-mortem artifacts. The top scoring genes after the discovery step were DTNA and KIF2C from AP, CADM1 and CLIP4 from DE. The top genes after the prioritization with CFG step were SLC4A4 and SKA2 from AP, SAT1 and SKA2 from DE. The top genes after the validation in suicide completers step were IL6 and MBP from AP, JUN and KLHDC3 from DE (Figure 3). Notably, our SAT1 finding is a replication and expansion of our previously reported results identifying SAT1 as a blood biomarker for suicidality in bipolars (Le-Niculescu et al. 2013), and our SKA2 finding is an independent replication of a previous report identifying SKA2 as a blood biomarker for suicidality by Kaminsky and colleagues.¹⁵ We also replicated in this larger cohort other top biomarkers from our previous work in bipolar disorder, notably MARCKS and PTEN (Table 2, Supplementary Figure S4). A number of other genes we identified (CADM1, KIF2C, DTNA, CLIP4) are completely novel in terms of their involvement in suicidality.

Biological understanding

We also sought to understand the biology represented by the biomarkers identified by us, and derive some mechanistic and practical insights. We conducted: 1. unbiased biological pathway analyses and hypothesis driven mechanistic queries, 2. overall disease involvement and specific neuropsychiatric disorders queries, and 3. overall drug modulation along with targeted queries for omega-3, lithium and clozapine¹⁶ (Table 3, Supplementary Tables S3). Administration of omega-3s in particular may be a mass-deployable therapeutic and preventive strategy.¹⁷

The sets of biomarkers identified have biological roles in immune and inflammatory response, growth factor regulation, mTOR signaling, stress, and perhaps overall the switch between cell survival and proliferation vs apoptosis (Table 3 and Supplementary Table S3). 14% of the candidate biomarkers in Supplementary Table S3 have evidence for involvement in psychological stress response, and 19% for involvement in programmed cell death/cellular suicide (apoptosis). An extrapolation can be made and model proposed whereas suicide is a whole

body apoptosis (or 'self-poptosis') in response to perceived stressful life events.

We also examined evidence for the involvement of these biomarkers for suicidality in other psychiatric disorders, permitting us to address issues of context and specificity (Supplementary Table S3). SKA2, HADHA, SNORA68, RASL11B, CXCL11, HOMEZ, LOC728543, AHCYL1, LDLRAP1, NEAT1 and PAFAH1B2 seem to be relatively specific for suicide, based on the evidence to date in the field. SAT1, IL6, FOXN3 and FKBP5 are less specific for suicide, having equally high evidence for involvement in suicide and in other psychiatric disorders, possibly mediating stress response as a common denominator.^{11,18} These boundaries and understanding will likely change as additional evidence in the field accumulates. For example, CADM1, discovered in this work as a top biomarker for suicide, had previous evidence for involvement in other psychiatric disorders, such as autism and bipolar disorder. Interestingly, it was identified in a previous study by us as a blood biomarker increased in expression in low mood states in bipolar participants, and it is increased in expression in the current study in high SI states. Increased expression of CADM1 is associated with decreased cellular proliferation and with apoptosis, and this gene is decreased in expression or silenced in certain types of cancers.

A number of other genes besides CADM1 are changed in opposite direction in suicide in this study vs high mood in our previous mood biomarker study-CHD2, MBP, LPAR1, IGHG1, TEX261 (Supplementary Table S3), suggesting that suicidal participants are in a low mood state. Also, some of the top suicide biomarkers are changed in expression in the same direction as in high psychosis participants in a previous psychosis biomarker study of ours -PK3C2A, GPM6B, PCBD2, DAB2, IQCH, LAMB1, TEX261 (Supplementary Table S3), suggesting that suicidal participants may be in a psychosis-like state. TEX261 in particular appears in all three studies, decreased in expression in suicide and high hallucinations, and increased in expression in high mood. This protective marker may be an interesting target for future biological studies and drug development. Taken together, the data indicates that suicidality could be viewed as a psychotic dysphoric state, and that TEX261 may be a key biomarker reflecting that state. This molecularly informed view is consistent with the emerging clinical evidence in the field.¹⁹

Lastly, we conducted biological pathway analyses on the genes that, after discovery and prioritization, were stepwise changed in suicide completers ($n=204$) and may be involved in ideation and behavior, vs those that were not stepwise changed ($n=208$), and that may only be involved in ideation (Supplementary Table S6). The genes involved in ideation map to pathways related to neuronal connectivity (cytoskeleton rearrangement, axonal guidance) and schizophrenia. The genes involved in behavior map to pathways related to neuronal activity (WNT, growth factors) and mood disorders. This is consistent with ideation being related to psychosis, and behavior being related to mood. Of note, clinically, the risk for suicide behavior/completion is higher in mood disorders than in psychotic disorders.

Clinical information

We also developed a simple new 22 item scale and app for suicide risk, Convergent Functional Information for Suicidality (CFI-S), which scores in a simple binary fashion and integrates, information about known life events, mental health, physical health, stress, addictions, and cultural factors that can influence suicide risk.^{7,8} Clinical risk predictors and scales are of high interest in the military²⁰ and in the general population at large.²¹ Our scale builds on those excellent prior achievements, while aiming for comprehensiveness, simplicity and quantification similar to a polygenic risk score. CFI-S is able to distinguish between individuals who committed suicide (coroner's cases $n=35$, information obtained from the next-of-kin) and those high-risk participants who did not

but had experienced changes in SI (our discovery cohort of psychiatric participants) (Figure 4). We analyzed which items of the CFI-S scale were the most significantly different between high SI live participants and suicide completers. We identified 7 items that were significantly different, 5 of which survived Bonferroni correction: lack of coping skills when faced with stress ($P=3.35e-11$), dissatisfaction with current life ($P=2.77e-06$), lack of hope for the future ($4.58e-05$), current substance abuse

($P=1.25e-04$), and acute loss/grief ($P=9.45e-4$). It is highly interesting that the top item was inability to cope with stress, which is independently consistent with our biological marker results.

We also simplified the wording (and developed a new app for) an 11 item scale for measuring mood and anxiety, the SASS, previously developed and described by us as TASS (Total Affective State Scale).⁶ The SASS is a set of 11 visual analog scales (7 for

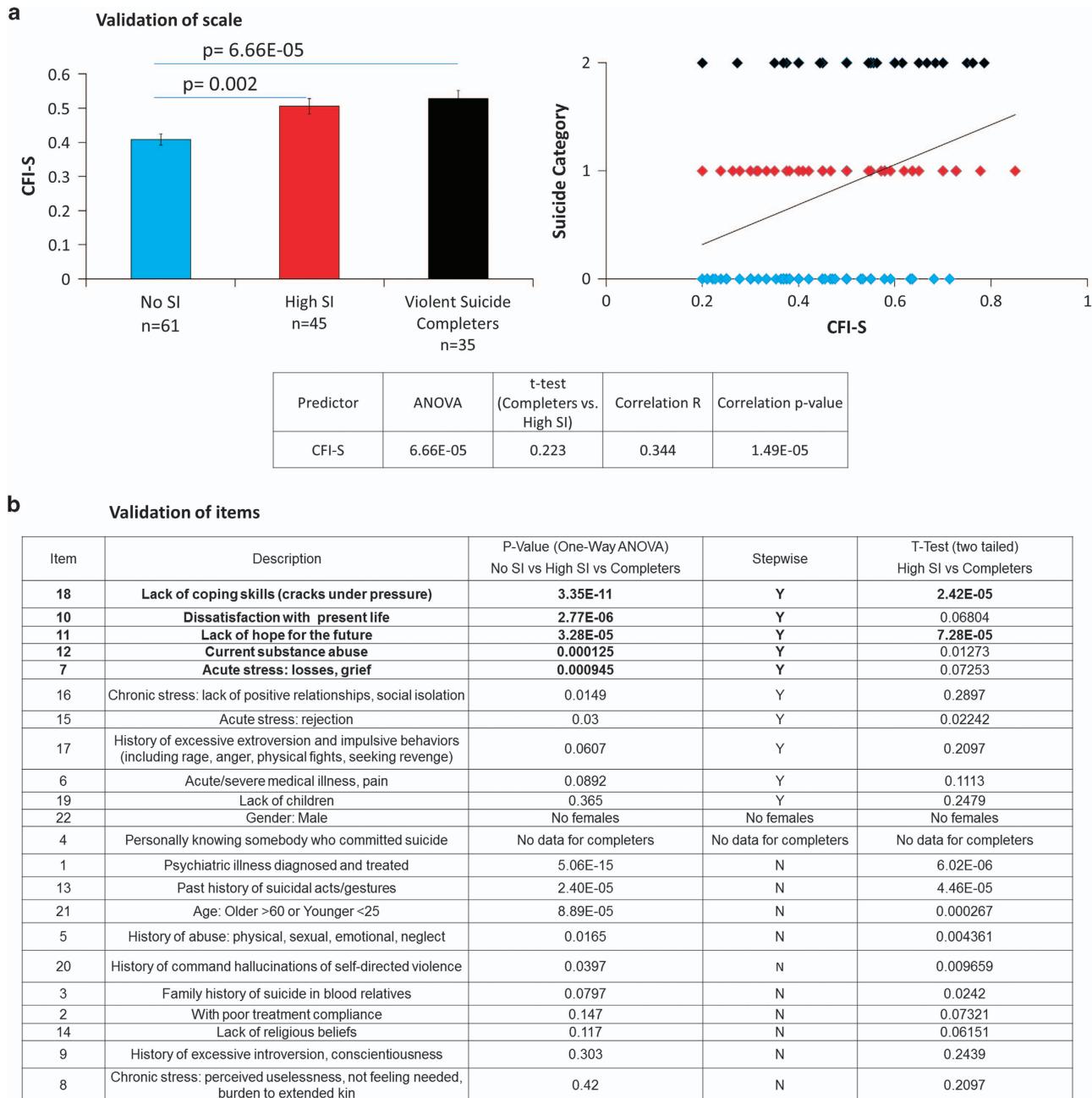
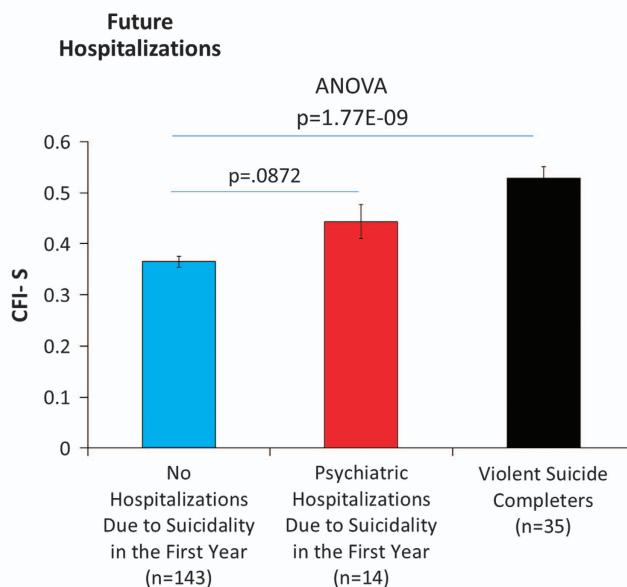
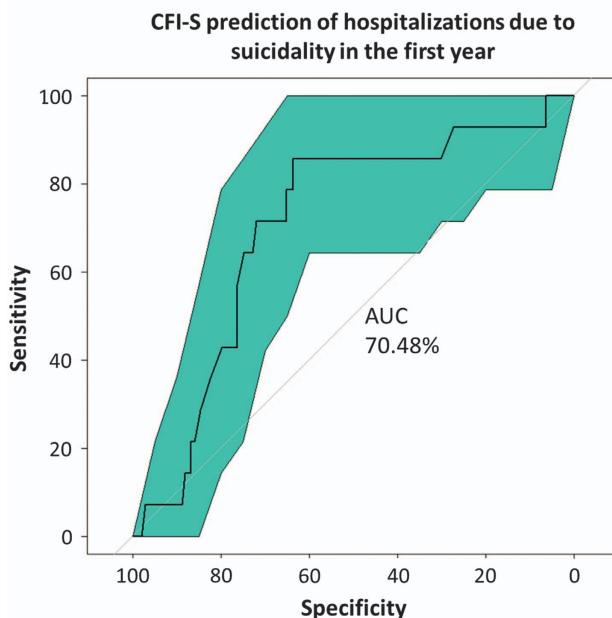
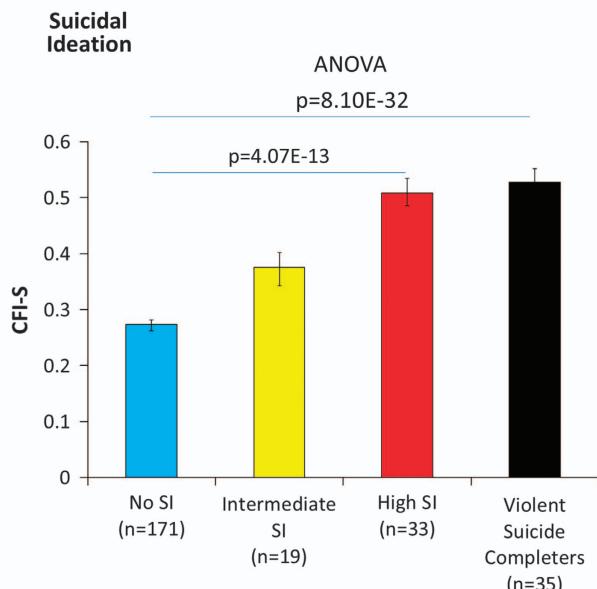
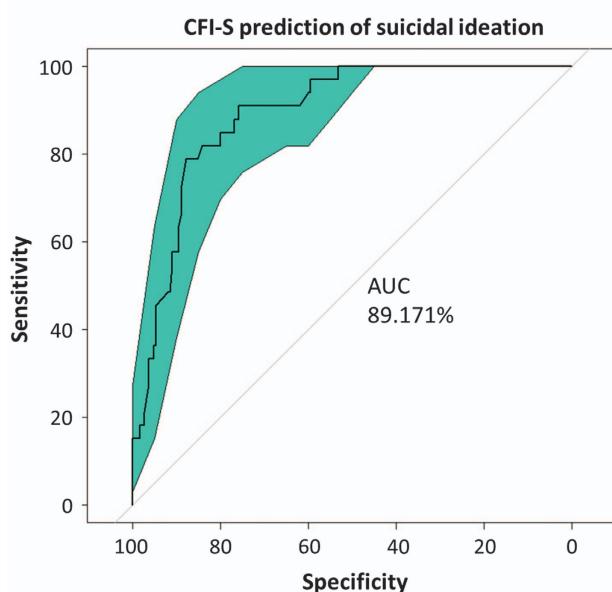


Figure 4. Convergent Functional Information for Suicide (CFI-S) Scale. **(a)** Validation of scale. Convergent Functional Information for Suicide levels in the discovery cohort and suicide completers. **(b)** Validation of items. Convergent Functional Information for Suicide was developed independently of any data from this study, by compiling known sociodemographic and clinical risk factors for suicide. It is composed of 22 items that assess the influence of mental health factors, as well as of life satisfaction, physical health, environmental stress, addictions, cultural factors known to influence suicidal behavior, and two demographic factors, age and gender. These 22 items are shown here validated in the discovery cohort and suicide completers in a manner similar to that for biomarkers. Additionally, a student's t-test was used to evaluate items that were increased in suicide completers when compared to living participants with high suicidal ideation. **(c)** Predictions. Convergent Functional Information for Suicide predicting SI in the independent test cohort, and predicting future hospitalizations due to suicidality.

C Predictions by CFI-S**Figure 4.** Continued.

mood, 4 for anxiety) that ends up providing a number ranging from 0 to 100 for mood state, and the same for anxiety state.

Testing for predictive ability

The best single biomarker predictor for SI state across all diagnostic groups is SLC4A4 (ROC AUC 0.72, P -value 2.41e-05), the top increased biomarker from our prioritization with CFG of discovery data from AP (Table 5). Within diagnostic groups, the accuracy is even higher. SLC4A4 has very good accuracy at predicting future high SI in bipolar participants (AUC 0.93, P -value 9.45e-06) and good accuracy in schizophrenia participants (AUC 0.76, P -value 0.030). SLC4A4 is a sodium-bicarbonate co-transporter that regulates intracellular pH, and possibly apoptosis. Very little is known to date about its roles in the brain, thus

representing a completely novel finding. Brain pH has been reported by Wemmie et al.²² to have a role in pain, fear and panic attacks, which clinically share features with acute SI states.

SKA2, the top decreased biomarker from prioritization with CFG of discovery data from AP and DE, has good accuracy at predicting SI across all diagnostic groups (AUC 0.69, P -value 0.00018), and even better accuracy in bipolar participants (AUC 0.76, P -value 0.0045) and schizophrenia participants (AUC 0.82, P -value 0.011).

The best single biomarker predictor for future hospitalizations for suicidal behavior in the first year across all diagnostic groups was SAT1, the top increased biomarker from the prioritization with CFG of discovery data from DE (AUC 0.55, P -value 0.28). The results across all diagnoses are modest, likely due to the significant variation of markers by diagnostic group (Table 5 and Supplementary Figure S4). This seems to be even more of an

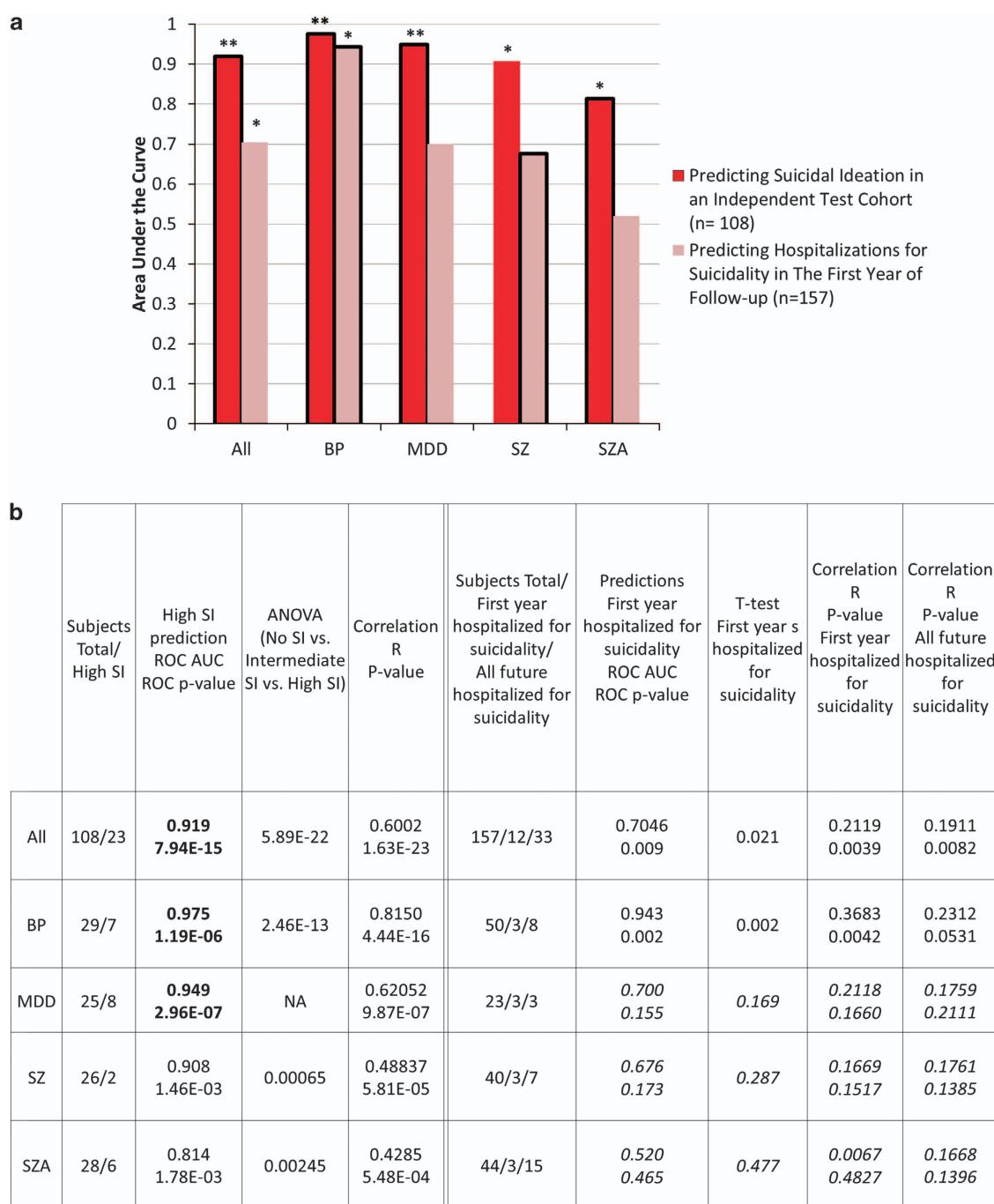


Figure 5. Testing of universal predictor for suicide (UP-Suicide). UP-Suicide is a combination of our best gene expression biomarkers (top increased and decreased biomarkers from discovery, prioritization by CFG, and validation in suicide completers steps), and phenomic data (CFI-S and SASS). **(a)** Area Under the Curve (AUC) for the UP-Suicide predicting suicidal ideation and hospitalizations within the first year in all participants, as well as separately in bipolar (BP), major depressive disorder (MDD), schizophrenia (SZ), and schizoaffective (SZA) participants. **Indicates the comparison survived Bonferroni correction for multiple comparisons. *Indicates nominal significance of $P < 0.05$. Bold outline indicates that the UP-Suicide was synergistic to its components, i.e., performed better than the gene expression biomarkers or phenomic data individually. **(b)** Table containing descriptive statistics for all participants together, as well as separately in BP, MDD, SZ, and SZA. Bold indicates the measure survived Bonferroni correction for 200 comparisons (20 genomic and phenomic markers/combinations \times 2 testing cohorts for SI and future hospitalizations in the first year \times 5 diagnostic categories—all, BP, MDD, SZA, SZ). We also show Pearson correlation data in the suicidal ideation test cohort for HAMD-SI vs. UP-Suicide, as well as Pearson correlation data in the hospitalization test cohort for frequency of hospitalizations for suicidality in the first year, and for frequency of hospitalizations for suicidality in all future available follow-up interval (which varies among participants, from 1 year to 8.5 years).

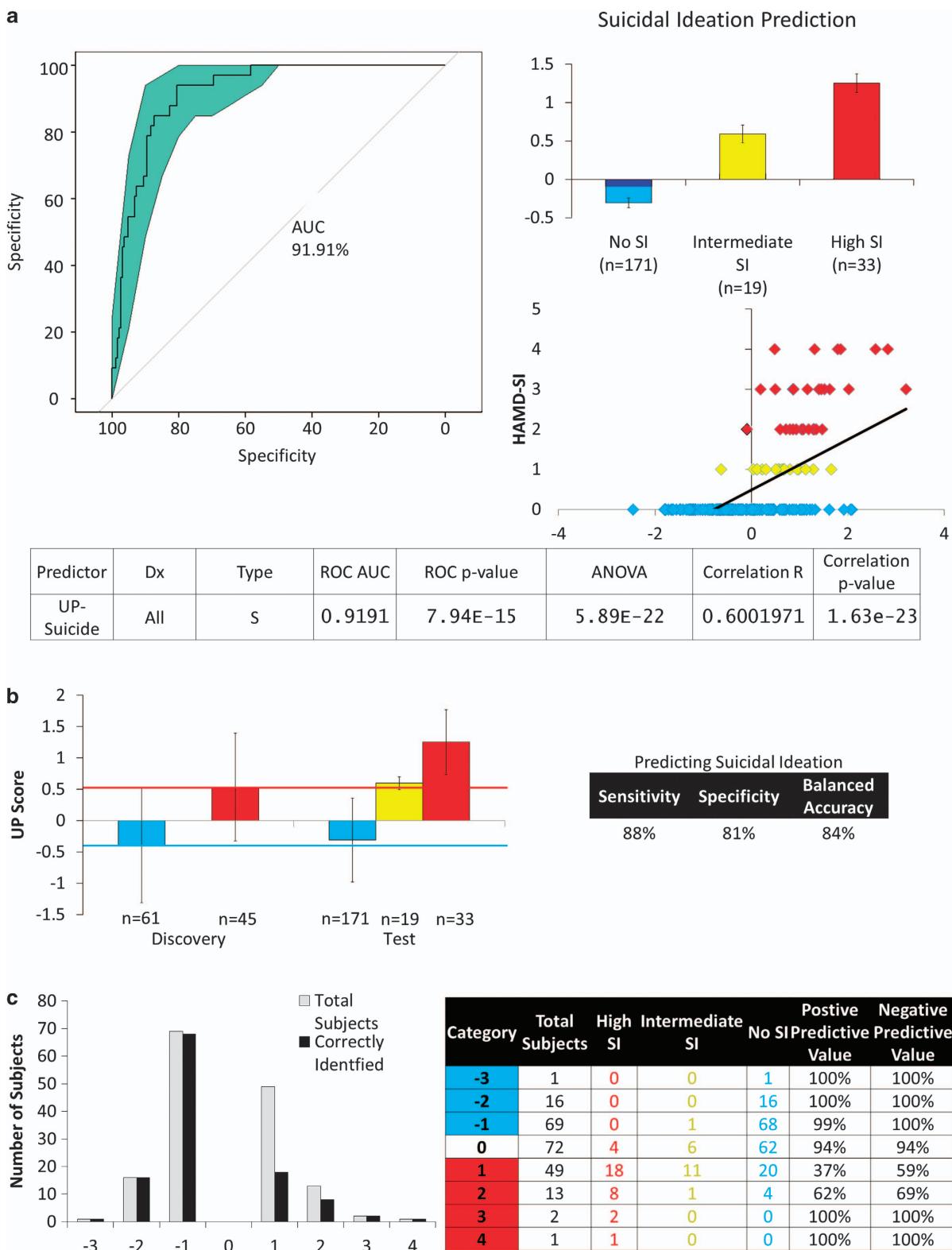


Figure 6. Prediction of suicidal ideation by universal predictive measure-suicide. (a) (top left) Receiver-operating curve identifying participants with suicidal ideation against participants with no suicidal ideation or intermediate SI. (top right) Y axis contains the average UP-Suicide scores with standard error of mean for no suicidal ideation, intermediate suicidal ideation and high suicidal ideation. (bottom right) Scatter plot depicting HAMD-SI score on the Y axis and universal predictive measure-suicide score on the X axis with linear trend line. (bottom) Table summarizing descriptive statistics. Analysis of variance was performed between groups with no suicidal ideation, intermediate suicidal ideation and high suicidal ideation. (b) Predictions in test cohort based on thresholds in the discovery cohort - average UP-Suicide scores with standard deviation. (c) Number of participants correctly identified in the test cohort by categories based on thresholds in the discovery cohort. Category 1 means within 1 s.d. above the average of high suicidal ideation participants in the discovery cohort, category 2 means between 1 and 2 s.d. above, and so on. Category 1 means within 1 s.d. below the average of the no suicidal ideation participants in the discovery cohort, category 2 means between 1 and 2 s.d. below and so on.

