Molecular Psychiatry (2016), 1–18

www.nature.com/mp

IMMEDIATE COMMUNICATION

Towards understanding and predicting suicidality in women: biomarkers and clinical risk assessment

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Women are under-represented in research on suicidality to date. Although women have a lower rate of suicide completion than men, due in part to the less-violent methods used, they have a higher rate of suicide attempts. Our group has previously identified genomic (blood gene expression biomarkers) and clinical information (apps) predictors for suicidality in men. We now describe pilot studies in women. We used a powerful within-participant discovery approach to identify genes that change in expression between no suicidal ideation (no SI) and high suicidal ideation (high SI) states ($n = 12$ participants out of a cohort of 51 women psychiatric participants followed longitudinally, with diagnoses of bipolar disorder, depression, schizoaffective disorder and schizophrenia). We then used a Convergent Functional Genomics (CFG) approach to prioritize the candidate biomarkers identified in the discovery step by using all the prior evidence in the field. Next, we validated for suicidal behavior the top-ranked biomarkers for SI, in a demographically matched cohort of women suicide completers from the coroner's office ($n = 6$), by assessing which markers were stepwise changed from no SI to high SI to suicide completers. We then tested the 50 biomarkers that survived Bonferroni correction in the validation step, as well as top increased and decreased biomarkers from the discovery and prioritization steps, in a completely independent test cohort of women psychiatric disorder participants for prediction of SI ($n = 33$) and in a future follow-up cohort of psychiatric disorder participants for prediction of psychiatric hospitalizations due to suicidality ($n = 24$). Additionally, we examined how two clinical instruments in the form of apps, Convergent Functional Information for Suicidality (CFI-S) and Simplified Affective State Scale (SASS), previously tested in men, perform in women. The top CFI-S item distinguishing high SI from no SI states was the chronic stress of social isolation. We then showed how the clinical information apps combined with the 50 validated biomarkers into a broad predictor (UP-Suicide), our apriori primary end point, predicts suicidality in women. UP-Suicide had a receiver-operating characteristic (ROC) area under the curve (AUC) of 82% for predicting SI and an AUC of 78% for predicting future hospitalizations for suicidality. Some of the individual components of the UP-Suicide showed even better results. SASS had an AUC of 81% for predicting SI, CFI-S had an AUC of 84% and the combination of the two apps had an AUC of 87%. The top biomarker from our sequential discovery, prioritization and validation steps, BCL2, predicted future hospitalizations due to suicidality with an AUC of 89%, and the panel of 50 validated biomarkers (BioM-50) predicted future hospitalizations due to suicidality with an AUC of 94%. The best overall single blood biomarker for predictions was PIK3C3 with an AUC of 65% for SI and an AUC of 90% for future hospitalizations. Finally, we sought to understand the biology of the biomarkers. BCL2 and GSK3B, the top CFG scoring validated biomarkers, as well as PIK3C3, have anti-apoptotic and neurotrophic effects, are decreased in expression in suicidality and are known targets of the anti-suicidal mood stabilizer drug lithium, which increases their expression and/or activity. Circadian clock genes were overrepresented among the top markers. Notably, PER1, increased in expression in suicidality, had an AUC of 84% for predicting future hospitalizations, and CSNK1A1, decreased in expression, had an AUC of 96% for predicting future hospitalizations. Circadian clock abnormalities are related to mood disorder, and sleep abnormalities have been implicated in suicide. Docosahexaenoic acid signaling was one of the top biological pathways overrepresented in validated biomarkers, which is of interest given the potential therapeutic and prophylactic benefits of omega-3 fatty acids. Some of the top biomarkers from the current work in women showed co-directionality of change in expression with our previous work in men, whereas others had changes in opposite directions, underlying the issue of biological context and differences in suicidality between the two genders. With this study, we begin to shed much needed light in the area of female suicidality, identify useful objective predictors and help understand gender commonalities and differences. During the conduct of the study, one participant committed suicide. In retrospect, when the analyses were completed, her UP-Suicide risk prediction score was at the 100 percentile of all participants tested.

Molecular Psychiatry advance online publication, 5 April 2016; doi:10.1038/mp.2016.31

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Received 22 November 2015; revised 27 January 2016; accepted 11 February 2016

INTRODUCTION

'Is there no way out of the mind?'
-Sylvia Plath

Predicting suicidality (suicidal ideation (SI), suicide attempts and suicide completion) in individuals is a difficult task, which is even more challenging in an understudied population like women. Although women have a lower rate of suicide completion than men, due in part to the less-violent methods used, they have a higher rate of suicide attempts.[1] It is reasonable to assume that genetic and biological differences may exist in suicidality between men and women. Studies by gender are a first step toward individualized medicine. We have previously shown in men with psychiatric disorders how blood biomarkers for suicide, alone or in combination with quantitative phenomic data for anxiety and mood, the Simplified Affective State Scale (SASS), and with a risk profile scale we have developed, Convergent Functional Information for Suicide (CFI-S), collected in the form of apps, could have predictive ability for SI, and for future hospitalizations for suicidality.[2] We now present data for discovery, prioritization, validation and testing of blood biomarkers for suicidality in women, across psychiatric diagnoses. We also show the utility of SASS and CFI-S in predicting suicidality in women. Both these type of tools, biomarkers and phenomic data apps, do not directly ask about SI. We demonstrate how our apriori primary end point, a comprehensive universal predictor for suicide (UP-Suicide), composed of the combination of 50 top Bonferroni validated biomarkers, along with SASS, and CFI-S, predicts in independent test cohorts SI and future psychiatric hospitalizations for suicidality. Finally, we uncover biological pathways involved in suicide in women, and potential therapeutics.

MATERIALS AND METHODS

Human participants

We derived our data from four cohorts: one live psychiatric participants discovery cohort; one postmortem 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 that we are continuously collecting. Participants are recruited from the patient population at the Indianapolis Veterans' Affairs (VA) Medical Center and Indiana University School of Medicine 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, per institutional review board-approved protocol. 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 new psychiatric 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 SI rating item (Figure 2a), 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 female population. We have recently described a similar study in males,[2] and data from that study are used for gender comparison purposes in this paper.

Our within-participant discovery cohort, from which the biomarker data were derived, consisted of 12 female participants with psychiatric disorders and 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. There were 7 participants with 3 visits each, and 5 participants with 2 visits each, resulting in a total of 31 blood samples for subsequent microarray studies (Figure 2 and Supplementary Table S1).

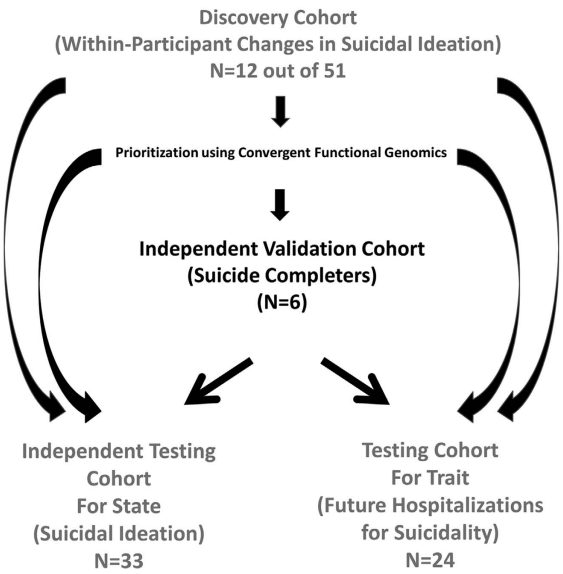


Figure 1. Cohorts used in study depicting, flow of discovery, prioritization, validation and testing of biomarkers from each step.

Our postmortem cohort, in which the top biomarker findings were validated for behavior, consisted of a demographically matched cohort of six female violent suicide completers obtained through the Marion County coroner's office (Table 1 and Supplementary Table S1). We required a last observed alive postmortem interval of 24 h or less, and the cases selected had completed suicide by means other than overdose, which could affect gene expression. Five participants completed suicide by gunshot to head or chest, and one by asphyxiation. 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 33 female 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 74 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 24 female 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, then 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 (ideation or attempt), was tabulated from electronic medical records. 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 documented by us at the time of each testing visit. The participants were on a variety of different psychiatric medications: mood stabilizers, antidepressants, antipsychotics, benzodiazepines and others (data not shown). Medications can have a strong influence on gene expression. However, our discovery of differentially expressed genes was based on within-participant analyses, which factor out not only genetic background effects but also medication effects, as the participants had no major medication changes between visits. Moreover, there was no consistent pattern in any particular type of medication, or between any change in medications and SI, in the rare instances where there were changes in medications between visits.

Human blood gene expression experiments and analyses

RNA extraction. Whole blood (2.5–5 ml) was collected into each PaxGene tube by routine venipuncture. PaxGene tubes contain proprietary reagents for the stabilization of RNA. RNA was extracted and processed as previously described.[3]

Microarrays. Microarray work was carried out using previously described methodology.[4]

Analysis. We have used the participant's SI scores at the time of blood collection (0—no SI compared with 2 and above—high SI). We looked at gene expression differences between the no SI and the high SI visits, using a within-participant design, then an across-participants summation (Figure 2).

Gene expression analyses in the discovery cohort

We analyzed the data in two ways: an Absent-Present (AP) approach, and a differential expression (DE) approach, as in previous work by us on suicide biomarkers.[2,3] The AP approach may capture turning on and off of genes, and the DE approach may capture gradual changes in expression. For the AP approach, we used Affymetrix Microarray Suite Version 5.0 (MAS5) to generate Absent (A), Marginal (M) or Present (P) calls for each probeset on the chip (Affymetrix U133 Plus 2.0 GeneChips) for all participants in the discovery cohort (Affymetrix, Santa Clara, CA, USA). For the DE approach, we imported all Affymetrix microarray data as .cel files into Partek Genomic Suites 6.6 software package (Partek, St Louis, MO, USA). Using only the perfect match values, we ran a robust multi-array analysis (RMA), background corrected with quantile normalization and a median polish probeset summarization, to obtain the normalized expression levels of all probesets for each chip. RMA was performed independently for each of the four diagnoses used in the study, to avoid potential artifacts due to different ranges of gene expression in different diagnoses.[5] Then, the participants normalized data were extracted from these RMAs and assembled for the different cohorts used in the study.

A/P analysis. For the longitudinal within-participant AP analysis, comparisons were made within-participant between sequential visits to identify changes in gene expression from Absent to Present that track changes in phene expression (SI) from no SI to high SI. For a comparison, if there was a change from A to P tracking a change from no SI to high SI, or a change from P to A tracking a change from high SI to no SI, that was given a score of +1 (increased biomarker in High SI). If the change was in opposite direction in the gene vs the phene (SI), that was given a score of − 1 (decreased biomarker in High SI). If there was no change in gene expression between visits despite a change of phene expression (SI), or a change in gene expression between visits despite no change in phene expression (SI), that was given a score of 0 (not tracking as a biomarker). If there was no change in gene expression and no change in SI between visits, that was given a score of +1 if there was concordance (P-P with High SI-High SI or A-A with No SI-No SI), or a score of − 1 if there was the opposite (A-A with High SI-High SI or P-P with No SI-No SI). If the changes were to M (moderate) instead of P, then the values used were 0.5 or –0.5. These values were then summed up across the comparisons in each participant, resulting in an overall score for each gene/probeset in each participant. We also used a perfection bonus. If the gene expression perfectly tracked the SI in a participant that had at least two comparisons (three visits), that probeset was rewarded by a doubling of its overall score. Additionally, we used a non-tracking correction. If there was no change in gene expression in any of the comparisons for a particular participant, that overall score for that probeset in that participant was zero.

DE analysis. For the longitudinal within-participant DE analysis, fold changes (FC) in gene expression were calculated between sequential visits within each participant. Scoring methodology was similar to that used above for AP. Probesets that had a FC $\geqslant$ 1.2 were scored +1 (increased in high SI) or − 1 (decreased in high SI). FC $\geqslant$ 1.1 were scored +0.5 or − 0.5. FC lower than 1.1 were considered no change. The only difference between the DE and the AP analyses was when scoring comparisons where there was no phene expression (SI) change between visits and no change in gene expression between visits (FC lower than 1.1). In that case, the comparison received the same score as the nearest preceding comparison where there was a change in SI from visit to visit. If no preceding comparison with a change in SI was available, then it was given the same score as the nearest subsequent comparison where there was a change in SI. For DE also, we used a perfection bonus and a non-tracking correction. If the gene expression perfectly tracked the SI in a participant that had at least two comparisons (3 visits), that probeset was rewarded by a doubling of its score. If there was no change in gene expression in any of the comparisons for a particular participant, that overall score for that probeset in that participant was zero.

Internal score. Once scores within each participant were calculated, an algebraic sum across all participants was obtained, for each probeset. Probesets were then given internal points based upon these algebraic sum scores. Probesets with scores above the 33.3% of the maximum score (for increased probesets and decreased probesets) received 1 point, those above 50% received 2 points and those above 80% received 4 points. For AP analyses, we have 30 probesets which received 4 points, 647 probesets with 2 points and 2596 probesets with 1 point, for a total of 3273 probesets. For DE analyses, we have 95 probesets which received 4 points, 2215 probesets with 2 points and 7520 probesets with 1 point, for a total of 9829 probesets. The overlap between the two discovery methods for probesets with an internal score of 1 is shown in Figure 2d. Different probesets may be found by the two methods due to differences in scope (DE capturing genes that are present in both visits of a comparison, that is, PP, but are changed in expression), thresholds (what makes the 33.3% change cutoff across participants varies between methods), and technical detection levels (what is considered in the noise range varies between the methods).

Gene names for the probesets were identified using NetAffyx (Affymetrix) for Affymetrix HG-U133 Plus 2.0 GeneChips, followed by GeneCards to confirm the primary gene symbol. In addition, for those probesets that were not assigned a gene name by NetAffyx, we used the UCSC Genome Browser to directly map them to known genes, with the following limitations: (1) in case the

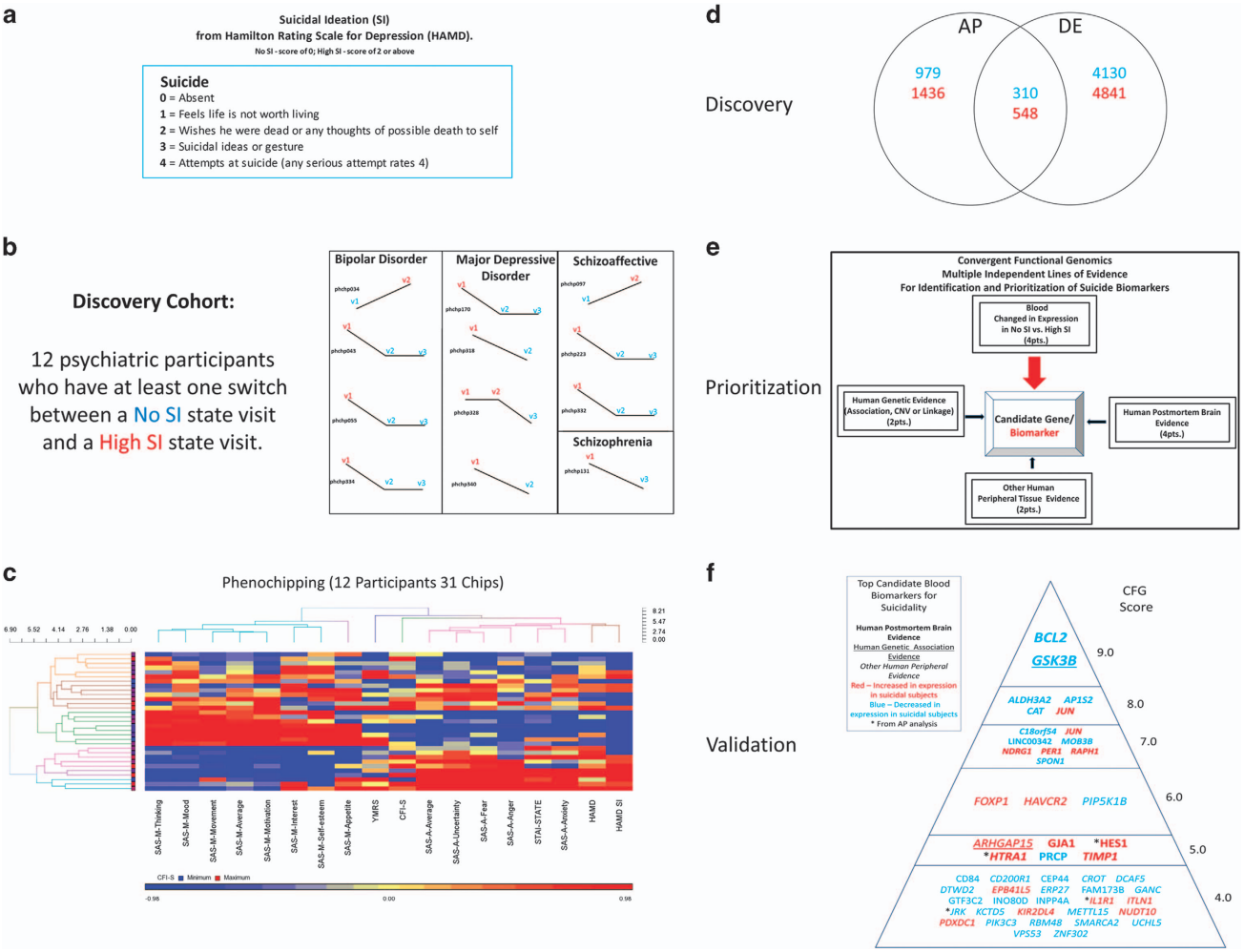


Figure 2. Biomarker discovery, prioritization and validation. Discovery cohort: longitudinal within-participant analysis. Phchp### is study ID for each participant. V# denotes visit number (1, 2 or 3). (**a**) Suicidal ideation (SI) scoring. (**b**) Participants and visits. (**c**) PhenoChipping: two-way unsupervised hierarchical clustering of all participant visits in the discovery cohort vs 18 quantitative phenotypes measuring affective state and suicidality. SASS, Simplified Affective State Scale. A—Anxiety items (Anxiety, Uncertainty, Fear, Anger, Average). M—Mood items (Mood, Motivation, Movement, Thinking, Self-esteem, Interest, Appetite, Average). STAI-STATE is State Trait Anxiety Inventory, State Subscale. YMRS is Young Mania Rating Scale. (**d**) Discovery—number of probesets carried forward from the Absent-Present (AP) and differential expression (DE) analyses, with an internal score of 1 and above. Red—increased in expression in high SI and blue—decreased in expression in high SI; (**e**) Prioritization—CFG integration of multiple lines of evidence to prioritize suicide—relevant genes from the discovery step. (**f**) Validation—Top CFG genes, with a total score of 4 and above, validated in the cohort of suicide completers. All the genes shown were significantly changed and survived Bonferroni correction in ANOVA from no SI to high SI to suicide completers.

probeset fell in an intron, that particular gene was assumed to be implicated; (2) only one gene was assigned to each probeset. Genes were then scored using our manually curated Convergent Functional Genomics (CFG) databases as described below (Figure 2).

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 (postmortem 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 2). For this study, data from 442 papers on suicide were present in the databases at the time of the CFG analyses (genetic studies—164, brain studies—192, peripheral fluids—86).

Human postmortem brain gene expression evidence. Converging evidence was scored for a gene if there were published reports of human postmortem 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

Table 1. Cohorts used in study

	Participants	*Diagnosis*	*Ethnicity*	*Age mean (s.d.)*	*T-test for age*	
Discovery cohort (within-participant changes in suicidal ideation)	12	BP = 4 MDD = 4 SZA = 3 SZ = 1	EA = 9 AA = 2 Asian = 1	All = 44.39 (11.65) No SI = 44.56 High SI = 44.15	*T-test for age between no SI and high SI 0.926*	
Independent validation cohort for gene expression (suicide completers)	6	BP = 1 MDD = 3 PTSD = 1 Non-psychiatric = 1	EA = 5 AA = 1	43.5 (14.24)		*T-test for age with discovery cohort P = 0.890*
Independent testing cohort for state predictions (suicidal ideation)	33	All BP = 17 MDD = 7 SZA = 7 SZ = 2 No SI BP = 13 MDD = 4 SZA = 6 SZ = 2 Intermediate SI BP = 3 SZA = 1 High SI BP = 3 MDD = 3 SZA = 1	EA = 26 AA = 5 Asian = 1 Mixed = 1	All = 44.05 (8.81) No SI = 43.98 High SI = 41.28	*T-test for age between no SI and high SI 0.553*	*T-test for age with discovery cohort P = 0.887*
Combined discovery and testing cohort for state (suicidal ideation) used for CFI-S analysis (Figure 3)	45	All BP = 21 MDD = 11 SZA = 10 SZ = 3 No SI BP = 17 MDD = 8 SZA = 9 SZ = 3 Intermediate SI BP = 3 SZA = 1 High SI BP = 7 MDD = 7 SZA = 4 SZ = 1	EA = 35 AA = 7 Asian = 2 Mixed = 1	All = 44.15 (9.68) No SI = 44.12 High SI = 43.15	*T-test for age between no SI and high SI 0.727*	
Testing cohort for trait predictions (future hospitalizations for suicidality)	24	All BP = 10 MDD = 9 SZA = 3 SZ = 2 No Hosp for SI BP = 8 MDD = 8 SZA = 1 SZ = 2 Hosp for SI BP = 2 MDD = 1 SZA = 2 SZ = 0	EA = 19 AA = 4 Mixed = 1	All = 46.51 (6.66) No Hosp for SI = 47.2 Hosp for SI = 43.4	*T-test for age between no Hosp for SI and Hosp for SI 0.0430*	*T-test for age with discovery cohort P = 0.354*

Abbreviation: BP, bipolar; MDD, major depressive disorder; PTSD, post-traumatic stress disorder; SZ, schizophrenia; SZA, schizoaffective disorder; SI, suicidal ideation.

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

Table 2. Top biomarkers for suicidality in women

Gene symbol/Gene Name	*Probeset*	*Discovery (change) method/score*	*Prior human genetic evidence*	*Prior human brain expression evidence*	*Prior human peripheral expression evidence*	*Prioritization total CFG score for suicide*	*Validation ANOVA P-value*	*Predictions ROC/P-value*	*Clock function*
Best predictive biomarkers out of validated biomarkers (Bonferroni) (49 genes, 50 probesets)									
BCL2 B-cell CLL/Lymphoma 2	203684_s_at	(D) DE/2	Linkage[38]	(D) PFC[39]	(D) Blood[4]	9.00	**3.95E−06**	SI: 0.48/0.56 Hosp: 0.89/0.007	
ALDH3A2 Aldehyde dehydrogenase 3 family, member A2	202053_s_at	(D) DE/2		(I) PFC, Thalamus[40]	(D) Blood[4]	8.00	**1.62E−06**	SI: 0.63/0.14 Hosp: 0.6/0.29	
MOB3B MOB kinase activator 3B	229568_at	(D) DE/1		(I) ACC[13]	(I) Blood[4]	7.00	**4.69E−06**	SI: 0.55/0.35 Hosp: 0.85/0.015	
PER1 Period circadian clock 1	202861_at	(I) DE/1		(D) PFC[13]	(D) Blood[4]	7.00	**5.32E−12**	SI: 0.45/0.66 Hosp: 0.84/0.018	Clock Core
HAVCR2 Hepatitis A virus cellular receptor 2	1555629_at	(I) DE/4			(D) Blood[4]	6.00	**1.69E−12**	SI: 0.62/0.15 Hosp: 0.8/0.022	
ARHGAP15 Rho GTPase activating protein 15	1561489_at	(I) DE/1	Suicide [41]		(I) Blood[4]	5.00	**3.05E−06**	SI: 0.55/0.34 Hosp: 0.79/0.041	
HTRA1 HtrA serine peptidase 1	201185_at	(I) AP/1		(I) NAC [13]		5.00	**3.17E−07**	SI: 0.36/0.89 Hosp: 0.84/0.01	Clock Distant Output
EPB41L5 erythrocyte membrane protein band 4.1 like 5	229292_at	(I) DE/1	Linkage[42]		(I) Blood [4]	4.00	**4.58E−14**	**SI: 0.68/0.062** Hosp: 0.63/0.24	
GTF3C2 general transcription factor IIIC, polypeptide 2, beta 110kDa	210620_s_at	(D) DE/2			(D) Blood[4]	4.00	**1.68E−07**	SI: 0.64/0.12 Hosp: 0.81/0.05	
PDXDC1 Pyridoxal-dependent decarboxylase domain containing 1	1560013_at	(I) DE/2			(I) Blood[4]	4.00	**1.03E−05**	SI: 0.51/0.46 Hosp:0.81/0.018	
PIK3C3 Phosphatidylinositol 3-kinase, catalytic subunit type 3	232086_at	(D) DE/1	Suicide, Antidepressants [43]		(I) Blood [4]	4.00	**3.14E−08**	**SI: 0.65/0.098 Hosp: 0.9/0.011**	
Best predictive biomarkers out of top discovery and prioritization biomarkers (non-Bonferroni validated, 65 genes)									
LRRC8B Leucine-rich repeat containing 8 family, member B	212976_at	(D) DE/4		(I) PFC[44]		8.00	0.231881	SI: 0.60/0.19 Hosp: 0.69/0.14	
ACTR3 ARP3 actin-related protein 3 homolog (yeast)	213102_at	(D) DE/4	Linkage[42]		(I) Blood[4]	7.00	*0.0045239*	SI: 0.62/0.15 Hosp: 0.73/0.12	
ASPH aspartate beta-hydroxylase	242037_at	(I) DE/4			(I) Blood[4]	6.00	*0.01087*	SI: 0.65/.098 Hosp: 0.8/.022	
CSNK1A1 Casein kinase 1, alpha 1	235464_at	(D) DE/4			(D) Blood[4]	6.00	NC	SI: 0.56/0.32 **Hosp: 0.96/0.0007**	Clock Immediate Input
DPCD Deleted in primary ciliary dyskinesia homolog (mouse)	226009_at	(I) DE/4			(D) Blood [4]	6.00	NC	SI: 0.67/0.067 Hosp: 0.76/0.044	
GTF3C3 General transcription factor IIIC, polypeptide 3, 102kDa	1555439_at	(I) AP/4			(I) Blood[4]	6.00	NC	SI: 0.67/0.075 Hosp: 0.75/.067	
KLHL28 Kelch-like family member 28	220374_at	(I) AP/4			(I) Blood [4]	6.00	NC	SI: 0.64/0.11 Hosp: 0.91/0.002	
LARP4 La ribonucleoprotein domain family, member 4	214155_s_at	(D) DE/4			(D) Blood[4]	6.00	*0.014911*	SI: 0.49/0.55 Hosp: 0.9/0.005	
NUDT6 Nudix (nucleoside diphosphate linked moiety X)-type motif 6	220183_s_at	(D) AP/4			(D) Blood[4]	6.00	NC	SI: 0.62/0.16 Hosp: 0.71/0.14	
SNX27 Sorting nexin family member 27	244349_at	(I) AP/4			(I) Blood[4]	6.00	NC	SI: 0.63/0.13 Hosp: 0.85/0.015	
UIMC1 Ubiquitin interaction motif containing 1	233596_at	(I) DE/4			(I) Blood[4]	6.00	NC	SI: 0.41/0.76 Hosp: 0.86/0.006	
ZNF548 Zinc finger protein 548	1553718_at	(D) DE/4			(D) Blood[4]	6.00	*0.000461*	SI: 0.40/0.82 Hosp: 0.83/0.012	

Abbreviations: ACC, anterior cingulate cortex; AP, Absent-Present; CFG, Convergent Functional Genomics; DE, differential expression; NAC, nucleus accumbens; PFC, pre-frontal cortex; ROC, receiver-operating characteristic. The 3 best predictive markers, increased and decreased, for suicidal ideation (SI) and for hospitalizations (Hosp), from Validated group and from Top Discovery and Prioritization groups, are shown, in order of Total CFG score ($3\times2\times2\times2=24$ possible; in fact 23 as one (PIK3C3) is shared between SI and Hosp. Underlined means changed in the same direction as in prior studies in men:[2] 16 out of the 23 biomarkers listed here (70%) are co-directional in men for the exact same probeset. Evidence in human peripheral expression evidence from column from Niculescu *et al*.[4] shows direction of change of best probeset in men, not necessarily the same one as in women. Two have no prior blood evidence in the field. In validation column, bold means Bonferroni significant, italic means nominally significant. NC means non concordant stepwise. In predictions column, bold are best predictor for SI, and best predictor for future hospitalizations for suicidality (Hosp), and best overall predictor for SI and Hosp.

Table 3. Biological pathways and diseases

A.

		Ingenuity pathways			*KEGG pathways*			*GeneGO pathways*		
	#	*Top canonical pathways*	P-*value*	*Ratio*	*Pathway name*	*Ratio*	*Enrichment* P-*value*	*Process networks*	*Ratio*	P-*value*
Prioritization CFG score ≥4 ($n=1471$ genes)	1	B-cell receptor signaling-	2.88E−13	22.9% 41/179	Morphine addiction	19/239	9.27E−06	Immune response_BCR pathway	42/137	4.332E-11
	2	Protein kinase A signaling	3.61E−13	16.6% 66/398	Phosphatidylinositol signaling system	18/245	4.20E−05	Apoptosis_Anti-Apoptosis mediated by external signals via MAPK and JAK/STAT	45/179	1.070E-08
	3	PI3K signaling in B lymphocytes	5.80E−12	24.8% 33/133	Neurotrophin signaling pathway	29/545	7.23E−05	Reproduction_Gonadotropin regulation	48/199	1.452E-08
	4	IGF-1 signaling	7.76E−12	28.3% 28/99	Amoebiasis	22/363	9.46E−05	Cell cycle_G1-S Growth factor regulation	47/195	2.115E-08
	5	Glucocorticoid receptor signaling	1.96E−11	17.8% 50/281	Insulin signaling pathway	27/520	0.0001855	Development_Hemopoiesis, Erythropoietin pathway	37/136	2.393E-08
Validation Stepwise in Suicide Completers ($n=589$ genes)	1	Glucocorticoid receptor signaling	2.86E−06	7.8% 22/281	Morphine addiction	9/249	0.0006493	Reproduction_Gonadotropin regulation	24/199	9.843E-07
	2	IGF-1 signaling	7.18E−06	12.1% 12/99	Colorectal cancer	9/287	0.0016932	Reproduction_GnRH signaling pathway	20/166	8.256E-06
	3	Renin-angiotensin signaling	8.72E−06	11.0% 13/118	Cocaine addiction	6/155	0.0037291	Reproduction_Progesterone signaling	23/214	1.194E-05
	4	Protein kinase A signaling	1.02E−05	6.5% 26/398	Insulin signaling pathway	12/535	0.0047284	Signal transduction_NOTCH signaling	24/236	1.962E-05
	5	Melanocyte development and pigmentation signaling	1.02E−05	12.8% 11/86	Inositol phosphate metabolism	6/193	0.0101986	Signal transduction_Androgen receptor signaling cross-talk	12/72	2.241E-05
Validation Nominally significant In Suicide Completers ($n=396$ genes)	1	Neurotrophin/TRK signaling	3.48E−06	12.5% 9/72	Cocaine addiction	6/155	0.000454	Reproduction_Gonadotropin regulation	16/199	7.748E-05
	2	Glucocorticoid receptor signaling	2.68E−05	5.7% 16/281	Colorectal cancer	7/289	0.002226	Reproduction_GnRH signaling pathway	14/166	1.323E-04
	3	Melanocyte development and pigmentation signaling	1.06E−04	9.3% 8/86	Wnt signaling pathway	9/495	0.003675	Reproduction_Progesterone signaling	16/214	1.822E-04
	4	G-Protein coupled receptor signaling	1.79E−04	5.3% 14/264	Notch signaling pathway	5/170	0.004315	Signal transduction_NOTCH signaling	16/236	5.495E-04
	5	Corticotropin releasing hormone signaling	2.23E−04	7.4% 9/121	Adherens junction	7/340	0.005315	Signal transduction_WNT signaling	13/177	8.738E-04
Validation Bonferroni significant in Suicide Completers ($n=49$ genes)	1	**IL-17 signaling**	1.34E−05	5.6% 4/72	**Inositol phosphate metabolism**	3/196	0.000383	Cell cycle_G1-S **Interleukin regulation**	6/128	6.400E-06
	2	p53 signaling	4.52E−05	4.1% 4/98	**Phosphatidylinositol signaling system**	3/260	0.000863	Immune response_BCR pathway	5/137	1.387E-04
	3	Role of osteoblasts, osteoclasts and chondrocytes in rheumatoid arthritis	8.71E−05	2.2% 5/225	Colorectal cancer	3/293	0.001214	Immune response_Th17-derived cytokines	4/98	4.589E-04
	4	**Docosahexaenoic acid (DHA) signaling**	1.02E−04	6.7 % 3/45	Tryptophan metabolism	2/132	0.004229	Inflammation_IL-2 signaling	4/104	5.752E-04
	5	Ovarian cancer signaling	1.48E−04	3.0 % 4/133	**Neurotrophin signaling pathway**	3/571	0.007844	Cell cycle_G1-S Growth factor regulation	5/195	7.127E-04

B.

		Ingenuity			*GeneGO*		
	#	*Diseases and Disorders*	P-*value*	# *Molecules*	*Diseases*	*Ratio*	P-*value*
Prioritization CFG score ≥4 ($n=1471$ genes)	1	Cancer	2.25E−06–2.21E−45	1242	Mental Disorders	256/1610	1.890E−35
	2	Organismal Injury and Abnormalities	2.25E−06–2.21E−45	1242	Psychiatry and Psychology	284/1904	2.194E−34
	3	Gastrointestinal Disease	1.02E−06–5.07E−31	905	Depressive Disorder, Major	120/543	2.660E−29
	4	Reproductive System Disease	1.43E−06–2.31E−24	617	Central Nervous System Diseases	379/3060	8.770E−29
	5	Infectious Diseases	8.30E−07–1.15E−17	246	Depressive Disorder	120/557	3.125E−28
Validation Stepwise in Suicide Completers ($n=589$ genes)	1	Cancer	6.57E−04–6.34E−17	487	Breast Neoplasms	356/8894	3.727E−15

Table 3. (Continued)

B.	*Ingenuity*				*GeneGO*		
	#	*Diseases and Disorders*	*P-value*	*# Molecules*	*Diseases*	*Ratio*	*P-value*
	2	Organismal Injury and Abnormalities	6.57E−04–6.34E−17	492	Breast Diseases	356/8895	3.798E−15
	3	Gastrointestinal Disease	6.23E−04–2.76E−10	355	Psychiatry and Psychology	115/1904	2.268E−14
	4	Reproductive System Disease	6.50E−04–8.34E−09	240	Pathological Conditions, Signs and Symptoms	207/4433	1.078E−13
	5	Infectious Diseases	6.57E−04–6.95E−08	104	Mental Disorders	101/1610	1.146E−13
Validation	1	Cancer	2.36E−03–1.87E−10	325	Depressive Disorder, Major	40/543	1.045E−12
Nominally significant In Suicide	2	Organismal Injury and Abnormalities	2.47E−03–1.87E−10	330	Pathological Conditions, Signs and Symptoms	150/4433	2.002E−12
Completers ($n = 396$ genes)	3	Tumor Morphology	2.29E−03–1.17E−07	36	Depressive Disorder	40/557	2.333E−12
	4	Developmental Disorder	2.47E−03–1.40E−06	69	Breast Neoplasms	245/8894	2.770E−11
	5	Gastrointestinal Disease	2.44E−03–2.43E−06	230	Breast Diseases	245/8895	2.806E−11
Validation	1	Immunological Disease	4.03E−03–1.27E−06	14	Lymphoma, Mantle-Cell	8/196	3.430E−08
Bonferroni significant in Suicide	2	Cancer	4.15E−03–3.97E−06	42	Psychiatry and Psychology	19/1904	1.209E−07
Completers ($n = 49$ genes)	3	Dermatological Diseases and Conditions	4.03E−03–3.97E−06	10	Lymphoma, Non-Hodgkin	12/726	2.323E−07
	4	Hematological Disease	4.03E−03–3.97E−06	5	Mental Disorders	17/1610	3.079E−07
	5	Organismal Injury and Abnormalities	4.15E−03–3.97E−06	42	Leukemia, Myeloid	16/1436	3.667E−07

Abbreviations: CFG, Convergent Functional Genomics; KEGG, Kyoto Encyclopedia of Genes and Genomes. Bold values signify pathways of interest.

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 2e), the external cross-validating lines of evidence were weighted such that findings in human postmortem 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 4 points, whereas human peripheral evidence was given 2 points and human genetic evidence was given a maximum of 2 points for association, and 1 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 4 points. Thus, the maximum possible total CFG score for each gene was 12 points (4 points for the internal score and 8 points for the external CFG score) (Table 2 and Supplementary Table S2). 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.[6] 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 2). In the future, with multiple large data sets, machine learning approaches could be used and validated to assign weights to CFG.

Clock gene database

We compiled a database of genes associated with circadian function, by using a combination of review papers[7,8] and searches of existing databases CircaDB (http://circadb.hogeneschlab.org), GeneCards (http://www.genecards.org) and GenAtlas (http://genatlas.medecine.univ-paris5.fr). Using the data we compiled from these sources we identified a total of 1468 genes that show circadian functioning. We further classified genes into 'core' clock genes, that is, those genes that are the main engine driving circadian function ($n = 18$), 'immediate' clock genes, that is, the genes that directly input or output to the core clock ($n = 331$) and 'distant' clock genes, that is, genes that directly input or output to the immediate clock genes ($n = 1119$).

Pathway analyses

IPA (Ingenuity Pathway Analyses, version 24390178, Qiagen, Hilden, Germany), GeneGO MetaCore (Thompson Reuters, New York, NY, USA) and KEGG (Kyoto Encyclopedia of Genes and Genomes) (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 Tables S4 and S5). We ran the pathway analyses together for all the AP and DE probesets with a total CFG score of $\geqslant 4$, then for those of them who showed stepwise change in the suicide completers validation cohort, then for those of them who were nominally significant and finally for those of them who survived Bonferroni correction (Table 3).

Validation analyses

For the AP analyses, we imported the Affymetrix microarray .chp data files from the participants in the validation cohort of suicide

completers into MAS5 Affymetrix Expression Console, alongside the data files from the participants in the discovery cohort, to compare expression levels of biomarkers in the validation cohort with those in the no SI and high SI groups in the discovery cohort. We then transferred the AP data to an Excel sheet and transformed A into 0, M into 0.5 and P into 1.

For the DE analyses, we imported Affymetrix microarray .cel files from the participants in the validation cohort of suicide completers into Partek Genomic Suites. We then ran an 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-logtransformed expression data by taking 2 to the power of the transformed expression value. We then used the non-logtransformed expression data to compare expression levels of biomarkers in the validation cohort with those in the no SI and high SI groups in the discovery cohort. We then transferred the expression data to an Excel sheet.

For validation analyses of our candidate biomarker genes, we examined which of the top candidate genes (Total CFG score of 4 or above), separately from AP and from DE, were stepwise changed in expression from the no SI group to the high SI group to the suicide completers group. We used an empirical cutoff of 33.3% of the maximum possible CFG score of 12, which also permits the inclusion of potentially novel genes with maximal internal score but no external evidence score. We imported the Excel sheets with the raw expression data from AP and DE into Partek, and statistical analyses were performed using a one-way ANOVA for the stepwise changed probesets, and stringent Bonferroni corrections for all the probesets tested (stepwise and non-stepwise).

Clinical measures

The SASS is an 11-item scale for measuring mood and anxiety, previously developed and described by us.[4,9] The SASS has a set of 11 visual analog scales (7 for mood and 4 for anxiety) that ends up providing a number ranging from 0 to 100 for mood state, and the same for anxiety state. We have developed an Android app version (Supplementary Figure S2).

CFI-S (Figure 3 and Supplementary Figure S2) is a 22-item scale and Android app for suicide risk,[4] which integrates, in a simple binary manner (Yes—1 and No—0), similar to a polygenic risk score, information about known life events, mental health, physical health, stress, addictions and cultural factors that can influence suicide risk.[10,11] The scale was administered at participant testing visits ($n = 39$), or scored based on retrospective electronic medical record information and Diagnostic Interview for Genetic Testing information ($n = 48$). When information was not available for an item, it was not scored (NA).

Combining gene expression biomarkers and clinical measures

The Universal Predictor for Suicide (UP-Suicide) construct, our primary end point, was decided upon as part of our apriori study design to be broad spectrum, and combine our top Bonferroni validated biomarkers with the phenomic (clinical) markers (SASS and CFI-S). It is calculated as the average of three increased markers (BioM-18 averaged increased Bonferroni biomarkers, Anxiety, CFI-S) minus the average of two decreased markers (BioM-32 averaged decreased Bonferroni biomarkers, Mood). All individual markers are Z-scored by diagnosis, to account for different ranges and be able to combine them into a composite predictor.

Testing analyses

The test cohort for SI and the test cohort for future hospitalizations analyses were assembled out of data that was RMA normalized by diagnosis. Phenomic (clinical) and gene expression markers used for predictions were z-scored by diagnosis, to be able to combine different markers into panels and to avoid potential artifacts due to different ranges of phene expression and gene expression in different diagnoses. Markers were combined by computing the average of the increased risk markers minus the average of the decreased risk markers. Predictions were performed using R-studio.

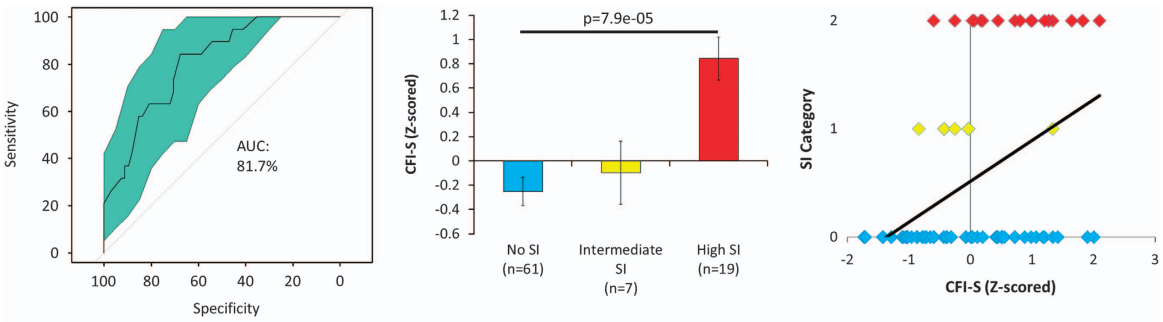
Predicting SI. Receiver-operating characteristic (ROC) analyses between genomic and phenomic marker levels and SI were performed by assigning participants with an HAMD-SI score of 2 and greater into the high SI category. We used the pROC function of the R-studio. We used the z-scored biomarker and app scores, running them in this ROC generating program against the 'diagnostic' groups in the independent test cohort (high SI vs the rest of subjects). Additionally, ANOVA was performed between no SI (HAMD-SI 0), intermediate (HAMD-SI 1) and high SI participants (HAMD-SI 2 and above) and Pearson *R* (one-tail) was calculated between HAMD-SI scores and marker levels (Table 4 and Figure 4).

Predicting future hospitalizations for suicidality. We conducted analyses for hospitalizations in the years following testing (on average 2.75 years, range 0.3–7.5 years; see Supplementary Table S1). For each participant in the test cohort for future hospitalizations, the study visit with highest levels for the marker or combination of markers was selected as index visit (or with the lowest levels, in the case of decreased markers). ROC analyses between genomic and phenomic marker levels and future hospitalizations were performed as described above, based on assigning if participants had been hospitalized for suicidality (ideation, attempts) or not following the index testing visit. Additionally, a one tailed *t*-test with unequal variance was performed between groups of participants with and without hospitalizations for suicidality. Pearson *R* (one-tail) correlation was performed between hospitalization frequency (number of hospitalizations for suicidality divided by duration of follow-up) and marker scores. We conducted correlation analyses for hospitalizations frequency for all future hospitalizations due to suicidality as this calculation, unlike the ROC and *t*-test, accounts for the actual length of follow-up at our VA, which varied from participant to participant. The ROC and *t*-test might in fact, if anything, underrepresent the power of the markers to predict, as the more severe psychiatric patients are more likely to move geographically and/or be lost to follow-up.

RESULTS

Discovery of biomarkers for SI

We conducted whole-genome gene expression profiling in the blood samples from a longitudinally followed cohort of female participants with psychiatric disorders that predispose to suicidality. The samples were collected at repeated visits, 3–6 months apart. State information about SI was collected from a questionnaire (HAMD) administered at the time of each blood draw (Supplementary Table S1). Out of 51 female psychiatric participants (with a total of 123 visits) followed longitudinally in our study, with a diagnosis of BP, MDD, schizophrenia and schizoaffective disorder, there were 12 participants that switched from a no SI (SI score of 0) to a high SI state (SI score of 2 and above) at different visits, which was our intended discovery group (Figure 2). We used a powerful within-participant design to analyze data from these 12 participants and their 31 visits. A within-participant design factors out genetic variability, as well as



Predictor	ROC AUC	AUC p-value	ANOVA	Correlation R	Correlation p-value
CFI-S	0.817	1.29e-05	7.9e-5	0.441	9.35e-06

CFI-S Item	Description	Correct direction	T-test (one tailed) High SI vs. No SI p-value
16	**Chronic stress: lack of positive relationships, social isolation**	**Y**	**0.0040**
12	**Current substance abuse**	**Y**	**0.0071**
17	**History of excessive extroversion and impulsive behaviors (including rage, anger, physical fights, seeking revenge)**	**Y**	**0.0147**
14	**Lack of religious beliefs**	**Y**	**0.0175**
13	**Past history of suicidal acts/gestures**	**Y**	**0.0253**
15	**Acute stress: Rejection (within last 3 months)**	**Y**	**0.0294**
20	**History of command hallucinations of self-directed violence**	**Y**	**0.0453**
10	Dissatisfaction with life at this moment in time	Y	*0.0583*
8	Chronic stress: perceived uselessness, not feeling needed, burden to extended kin.	Y	*0.0635*
4	Personally knowing somebody who committed suicide	Y	*0.0733*
7	Acute stress: Losses, grief (within last 3 months)	Y	*0.0748*
3	Family history of suicide in blood relatives	Y	0.1422
21	Age: Older >60 or Younger <25	Y	0.2374
2	With poor treatment compliance	Y	0.2477
6	Acute/severe medical illness, including acute pain ("I just can't stand this pain anymore.") (within last 3 months)	Y	0.2714
5	History of abuse: physical, sexual, emotional, neglect	Y	0.3348
9	History of excessive introversion, conscientiousness (including planned suicide attempts)	Y	0.3388
18	Lack of coping skills when faced with stress (cracks under pressure)	Y	0.3723
19	Lack of children. If has children, not in touch /not helping take care of them.	N	0.0714
1	Psychiatric illness diagnosed and treated	All have dx	All have dx
11	Lack of hope for the future	No difference	1
22	Gender: Male	All females	All females

Figure 3. Convergent Functional Information for Suicide (CFI-S) scale testing in women. Prediction of high suicidal ideation in women in a larger cohort that combines the discovery and test cohorts used for biomarker work. CFI-S was developed independently of any data from this study, by compiling known socio-demographic 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. Table depicts individual items and their ability to differentiate between no SI and high SI.

some medications, lifestyle and demographic effects on gene expression, permitting identification of relevant signal with Ns as small as 1.[12] Another benefit of a within-participant design may be accuracy/consistency of self-report of psychiatric symptoms ('phene expression'), similar in rationale to the signal detection benefits it provides in gene expression.

For discovery, we used two methodologies: Absent/Present (reflecting on/off of transcription) and Differential Expression (reflecting more subtle gradual changes in expression levels). The genes that tracked SI in each participant were identified in our analyses. We used three thresholds for increase in expression genes and for decrease in expression genes: $\geqslant$33.3% (low), $\geqslant$50% (medium) and $\geqslant$80% (high) of the maximum scoring increased and decreased gene across participants. Such a restrictive approach was used as a way of minimizing false positives, even at the risk of having false negatives. For example, there were genes on each of the two lists, from AP and DE analyses, that had clear prior evidence for involvement in suicidality, such as AKAP10 (ref. 13) (31.7%) and MED28 (ref. 13) (31.8%) from AP, and S100B[13,14] (31.7%) and SKA2 (ref. 15) (31.4%) for DE, but were not included in our subsequent analyses because they did not meet our apriori set 33.3% threshold. Notably, SKA2 reproduces our results in males,[2] as well as the work from Kaminsky and colleagues.[15,16]

Prioritization of biomarkers based on prior evidence in the field

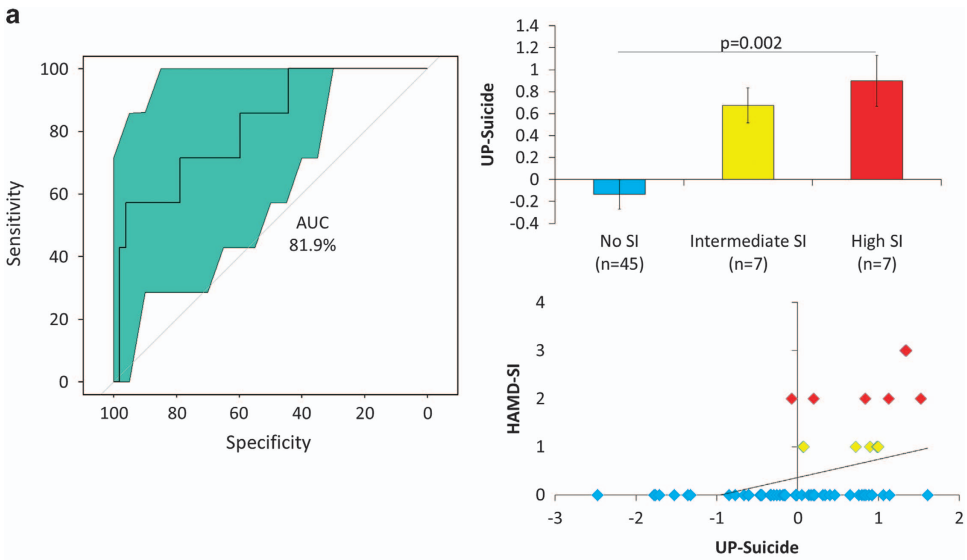
These differentially expressed genes were then prioritized using a Bayesian-like CFG approach (Figure 2) integrating all the

Table 4. Prediction of suicidal ideation and future hospitalizations for suicidality

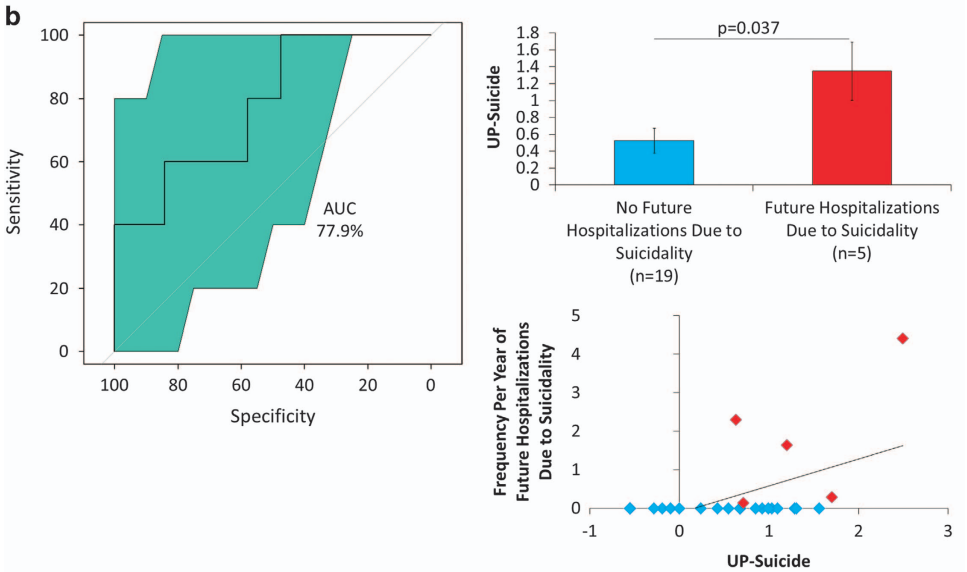
	Marker	*Participants with high SI/Participants total*	*ROC AUC/P-value*	*Pearson's Correlation R/P-value*	*Student's t-test P-value*
Suicidal ideation independent cohort, n = 33					
Best blood biomarker predictors	Out of validated biomarkers (Bonferroni) (49 genes, 50 probesets)				
	EPB41L5	7|33	*0.68/0.06*	**0.22/0.03**	*0.09*
	HAVCR2	7|33	0.62/0.15	*0.17/0.07*	0.18
	ARHGAP15	7|33	0.55/0.34	0.12/0.15	0.22
	PIK3C3	7|33	0.65/0.1	**−0.21/0.037**	*0.08368*
	GTF3C2	7|33	0.64/0.115	−0.11/0.179	*0.07208*
	ALDH3A2	7|33	0.62/0.142	**−0.21/0.036**	0.1421
	Out of top discovery and prioritization biomarkers (Non-Bonferroni Validated, 65 genes)				
	DPCD	7|33	*0.67/0.07*	**0.21/0.04**	0.12
	GTF3C3	7|33	*0.67/0.07*	**0.23/0.02**	0.11
	ASPH	7|33	0.65/0.1	0.07/0.27	0.13
	ACTR3	7|33	0.62/0.15	**−0.19/0.05**	0.13
	NUDT6	7|33	0.62/0.15	−0.07/0.27	0.19
	LRRC8B	7|33	0.60/0.19	**−0.15/0.11**	0.13
	Panels of Validated Biomarkers (Increased, Decreased, Combined)				
	BioM-18	7|33	0.37/0.87	0.032/0.39	0.59
	BioM-32	7|33	0.43/0.72	−0.0031/0.49	0.68
	BioM-50	7|33	0.5/0.515	0.02/0.429	0.465
Clinical measures	**Anxiety**	7|33	**0.72/0.029**	**0.26/0.011**	**0.0083**
	Mood	7|33	**0.78/0.0078**	**−0.37/0.0006**	**0.002**
	SASS	7|33	**0.81/0.0035**	**0.38/0.0005**	**5.04E-05**
	CFI-S	7|29	**0.84/0.002**	**0.39/0.0013**	**0.003**
	CFI-S + SASS	7|29	**0.87/0.00088**	**0.48/0.0001**	**0.00027**
Combined	**UP-Suicide**	7|29	**0.82/0.003**	**0.43/0.0003**	**0.001467**

	Marker	*Participants hospitalized for suicidality/ participants hospitalized total*	*ROC AUC/P-value*	*Pearson's correlation R/ P-value*	*Student's t-test P-value*	*Cox regression hazard ratio*	*Cox regression P-value*
Future hospitalizations for suicidality cohort, n = 24 participants							
Best Blood Biomarker Predictors	Out of Validated Biomarkers (Bonferroni) (49 genes, 50 probesets)						
	HTRA1	5|24	**0.84/0.01**	**0.62/0.00058**	**0.02**	**4.55**	**0.01**
	PER1	4|24	**0.84/0.018**	**0.39/0.029**	**0.1314**	1.535	0.1615
	PDXDC1	5|24	**0.81/0.018**	**0.64/0.0004**	**0.04187**	**2.4436**	**0.01503**
	PIK3C3	3|24	**0.9/0.011**	−0.25/0.115	**0.02583**	5.995	0.12
	BCL2	4|24	**0.89/0.007**	**−0.35/0.047**	**0.05385**	**3.0848**	**0.01**
	MOB3B	4|24	**0.85/0.015**	**−0.34/0.053**	**0.000462**	*9.572*	*0.09*
	Out of top discovery and prioritization biomarkers (Non-Bonferroni Validated, 65 genes)						
	KLHL28	5|24	**0.91/0.002**	**0.50/0.007**	**0.003**	**7.36**	**0.04**
	UIMC1	5|24	**0.86/0.006**	0.40/0.08	**0.04**	**2.26**	**0.03**
	SNX27	4|24	**0.85/0.02**	0.73/2.5E-05	**0.05**	**4.27**	**0.01**
	CSNK1A1	4|24	**0.96/0.0007**	−0.27/0.10	**0.0007**	**620.5**	**0.02**
	LARP4	4|24	**0.9/0.005**	−0.3/0.08	**6.30E-05**	37.01	0.11
	ZNF548	5|24	**0.83/0.012**	−0.31/0.07	**0.008**	**15.94**	**0.02**
	Panels of validated biomarkers (increased, decreased, combined)						
	BioM-18	4|24	**0.88/0.0088**	**0.46/0.011**	**0.033**	**27.6**	**0.021**
	BioM-32	4|24	0.71/11	−0.34/0.053	0.16	10.57	0.23
	BioM-50	5|24	**0.94/0.002**	**0.54/0.003**	**0.005813**	**89.459**	**0.02**
Clinical measures	**Anxiety**	4|24	**0.86/0.01**	**0.44/0.01**	**0.0039**	*14.4*	*0.061*
	Mood	3|24	0.68/0.18	−0.22/0.16	0.22	33.4	0.1
	SASS	4|24	**0.83/0.02**	**0.39/0.03**	**0.034**	*3.72*	*0.066*
	CFI-S	3|24	0.5/0.52	0.24/0.12	0.38	1.17	0.79
	CFI-S + SASS	4|24	*0.74/0.08*	**0.40/0.03**	*0.083*	*4.68*	*0.06*
Combined	**UP-Suicide**	5|24	**0.78/0.032**	**0.51/0.006**	**0.03691**	**9.6068**	**0.01**

Abbreviations: AUC, area under the curve; CFI-S, Convergent Functional Information for Suicidality; ROC, receiver-operating characteristic; SASS, Simplified Affective State Scale; SI, suicidal ideation. Top predictive biomarkers ($n = 23$) from Table 2 are shown, out of the total tested ($n = 115$) from Supplementary Table S2. Also shown are predictions by the panels of Bonferroni validated biomarkers, by the clinical measures/apps and by the combined genomic and clinical predictor, UP-Suicide, for a total of $n = 124$ predictors tested. UP-Suicide is composed of the panels of increased and decreased validated biomarkers (BioM-18 and BioM-32), along with clinical measures app scores from CFI-S and from SASS (Mood and Anxiety). Red—increased marker and blue—decreased marker. Bold—nominally significant. Italic—trend towards significance. *T*-tests are between high SI and no SI, and between hospitalized for suicidality vs not hospitalized for suicidality.



Predictor	Type	ROC AUC	AUC p-value	ANOVA	Correlation R	Correlation p-value
UP-Suicide	State	0.82	0.003	0.002	0.43	0.0003



Predictor	Type	ROC AUC	AUC p-value	T-test	Correlation R	Correlation p-value
UP-Suicide	Trait	0.78	0.032	0.037	0.51	0.006

Figure 4. UP-Suicide predicting suicidal ideation in the independent test cohort, and predicting future hospitalizations due to suicidality. UP-Suicide is composed of the 50 Bonferroni validated biomarkers along with CFI-S scores and SASS (Mood and Anxiety scores). *n* = number of testing visits. (**a**) *Top left:* Receiver operating curve identifying participants with suicidal ideation against participants with no SI or intermediate SI. *Top right*: Y axis contains the average UP-Suicide scores with standard error of mean for no SI, intermediate SI and high SI. *Bottom right:* Scatter plot depicting HAMD-SI score on the Y axis and UP-Suicide score on the X axis with linear trend line. *Bottom*: Table summarizing descriptive statistics. (**b**) *Top left:* Receiver operating curve identifying participants with future hospitalizations due to suicidality against participants without future hospitalizations due to suicidality. *Top right*: Y axis contains the average UP-Suicide scores with standard error of mean for no future hospitalizations due to suicidality and participants with future hospitalizations due to suicidality. *Bottom right:* Scatter plot depicting frequency of future hospitalizations due to suicidality on the Y axis and UP-Suicide score on the X axis with linear trend line. *Bottom*: Table summarizing descriptive statistics.

previously published human genetic evidence, postmortem brain gene expression evidence and peripheral fluids evidence for suicide in the field available at the time of our analyses (September 2015). This is a way of identifying and prioritizing disease relevant genomic biomarkers, extracting generalizable signal out of potential cohort-specific noise and genetic heterogeneity. We have built in our laboratory manually curated databases of the psychiatric genomic and proteomic literature to date, for use in CFG analyses. The CFG approach is thus a *de facto* field-wide collaboration. We use in essence, in a Bayesian manner, the whole body of knowledge in the field to leverage findings from our discovery data sets.

Validation of biomarkers for behavior in suicide completers

For validation in suicide completers, we used 1471 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, 882 did not show any stepwise change in suicide completers (NC, non-concordant). As such, they may be involved primarily in ideation and not in behavior (Supplementary Table S5). The remaining 589 genes (40.0%) had levels of expression that were changed stepwise from no SI to high SI to suicide completion. In all, 396 of these genes (26.9%) were nominally significant, and 49 genes (50 probesets—two for JUN) (3.33%) survived Bonferroni correction for multiple comparisons (Figure 2f). These genes are likely involved in SI and suicidal behavior. (A person can have SI without suicidal behavior, but cannot have suicidal behavior without SI.)

Selection of biomarkers for testing of predictive ability

For testing, we decided apriori to focus on the Bonferroni validated biomarkers (49 genes, 50 probesets). We also examined in a secondary analysis the top scoring biomarkers from both discovery and prioritization (65 genes), so as to avoid potential false negatives in the validation step due to possible postmortem artifacts or extreme stringency of statistical cutoff (Supplementary Figure S1). The top CFG scoring genes after the Bonferroni validation step were BCL2 and GSK3B. The top CFG scoring genes from the discovery and prioritization steps were FAM214A, CLTA, HSPD1 and ZMYND8. Notably, all have co-directional gene expression changes evidence in brains of suicide completers in studies from other groups (Figure 2, Table 2 and Supplementary Table S2).

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 clozapine (Table 3 and Supplementary Tables S3 and S4). Administration of omega-3s in particular may be a mass-deployable therapeutic and preventive strategy.[18,19]

The sets of biomarkers identified have biological roles in inflammation, neurotrophins, inositol signaling, stress response, and perhaps overall the switch between cell survival and proliferation vs apoptosis (Table 3 and Supplementary Table S5).

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). FAM214A, MOB3B, ZNF548 and ARHGAP35 seem to be relatively specific for suicide, based on the evidence to date in the field. BCL2, GSK3B, HSPD1 and PER1 are less specific for suicide, having equally high evidence for involvement in suicide and in other psychiatric disorders.

These boundaries and understanding will likely change as additional evidence in the field accumulates. For example, HSPD1, discovered in this work as a top biomarker increased in expression in suicidality, is also increased in expression in the blood following anti-depressant treatment,[20,21] and thus might be a useful biomarker for treatment-emergent suicidal ideation.

A number of the genes are changed in expression in opposite direction in suicide in this study vs high mood in our previous mood biomarker study[22]—SSBP2, ZNF596 (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[23]—HERC4, PIP5K1B, SLC35B3, SNX27, KIR2DL4 and NUDT10 (Supplementary Table S3), suggesting that suicidal participants may be in a psychosis-like state. Taken together, the data indicate that suicidality could be viewed as a psychotic dysphoric state. This molecularly informed view is consistent with the emerging clinical evidence in the field.[24]

A number of top biomarkers identified by us have biological roles that are related to the core circadian clock (such as PER1), or modulate the circadian clock (such as CSNK1A1), or show at least some circadian pattern (such as HTRA1). To be able to ascertain all the genes in our data set that were circadian and do estimates for enrichment, we compiled from the literature a database of all the known genes that fall into these three categories, numbering a total of 1468 genes. Using an estimate of about 21 000 genes in the human genome, that gives about 7% of genes having some circadian pattern. Out of our 49 Bonferroni validated biomarker genes, 7 had circadian evidence (14.3%) (Supplementary Table S3), suggesting a two-fold enrichment for circadian genes. Circadian clock abnormalities are related to mood disorders,[8,25] and sleep abnormalities have been implicated in suicide.[26]

Finally, we conducted biological pathway analyses on the genes that, after discovery and prioritization, were stepwise changed in suicide completers ($n = 882$) and may be involved in ideation *and* behavior, vs those that were not stepwise changed ($n = 589$), and that may only be involved in ideation (Supplementary Table S5). The genes involved in ideation map to pathways related to PI3K signaling. The genes involved in behavior map to pathways related to glucocorticoid receptor signaling. This is consistent with ideation *without* behavior being related to neurotrophic factors, and ideation *with* behavior being related to stress.

Clinical information

We used a simple new 22-item scale and app for suicide risk, CFI-S, which scores in a simple binary manner and integrates information about known life events, mental health, physical health, stress, addictions and cultural factors that can influence suicide risk.[10,4,11] Clinical risk predictors and scales are of high interest in the military[27] and in the general population at large.[28] Our scale aims for comprehensiveness, simplicity and quantification similar to a polygenic risk score, and may provide context to the blood biomarker signals. We analyzed which items of the CFI-S scale were the most significantly different between no and high SI live participants (Figure 3). We identified seven items that were significantly different: lack of positive relationships/social isolation ($P = 0.004$), substance abuse ($P = 0.0071$), history of impulsive behaviors ($P = 0.015$), lack of religious beliefs ($P = 0.018$), past history of suicidal acts/gestures ($P = 0.025$), rejection ($P = 0.029$) and history of command auditory hallucinations ($P = 0.045$). Social isolation increases vulnerability to stress, which is independently consistent with our biological marker results.

We also used an 11-item scale for measuring mood and anxiety, the SASS.[4] The SASS is a set of 11 visual analog scales (7 for mood and 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 increased (risk) biomarker predictor for SI state is EPB41L5 (ROC area under the curve (AUC) 0.68, *P*-value 0.06; Pearson Correlation 0.22, *P*-value 0.03), an increase in expression, Bonferroni validated biomarker (Tables 2 and 4). This biomarker was also identified co-directionally in our previous male work,[4] and has no evidence for involvement in other psychiatric disorders. The best single decreased (protective) biomarker predictor for SI is PIK3C3 (ROC AUC 0.65, *P*-value 0.1; Pearson Correlation −0.21, *P*-value 0.037), a decrease in expression,

Bonferroni validated biomarker (Tables 2 and 4). PIK3C3 is also decreased in expression in postmortem brains in depression.[29]

The best single increased (risk) biomarker predictor for future hospitalizations for suicidality is HTRA1 (ROC AUC 0.84, *P*-value 0.01; Cox regression hazard ratio 4.55, *P*-value 0.01), an increase in expression, Bonferroni validated biomarker (Tables 2 and 4). HTRA1 is also increased in expression in the blood of schizophrenics.[30] The best single decreased (protective) biomarker predictor for future hospitalizations for suicidality is CSNK1A1 (ROC AUC 0.96, *P*-value 0.0007; Cox Regression Hazard Ratio 620.5, *P*-value 0.02), a top discovery and prioritization, non-Bonferroni validated biomarker (Tables 2 and 4). This biomarker was also identified co-directionally in our previous male work.[4] CSNK1A1 (casein kinase 1, alpha 1) is a circadian clock gene, part of the input into the core clock. It is decreased in expression in suicidality in our work, and decreased in postmortem brains of alcoholics.[31] Interestingly, it is increased in expression by mood stabilizers[32] and by omega-3 fatty acids.[33] PIK3C3 is also a good predictor for future hospitalizations for suicidality (ROC AUC 0.9, *P*-value 0.011).

BCL2, the top CFG scoring biomarker from validation, has good accuracy at predicting future hospitalizations for suicidality (ROC AUC 0.89, *P*-value 0.007; Cox regression hazard ratio 3.08, *P*-value 0.01). The panel of 50 validated biomarkers, BioM-50, had even better accuracy at predicting future hospitalizations for suicidality (ROC AUC 0.94, *P*-value 0.002; Cox regression hazard ratio 89.46, *P*-value 0.02). Overall, in women, blood biomarkers seemed to perform better for predicting future hospitalizations for suicidality (trait) than for predicting SI (state). This is different from the trend we saw in men,[4] where blood biomarkers were somewhat better predictors of state than of trait. These gender differences are interesting, and merit exploration in additional future comparative studies.

CFI-S has very good accuracy (ROC AUC 0.84, *P*-value 0.002; Pearson Correlation 0.39, *P*-value 0.001) at predicting SI in psychiatric participants across diagnostic groups. The other app, SASS, also has very good accuracy (ROC AUC 0.81, *P*-value 0.003; Pearson Correlation 0.38, *P*-value 0.0005) at predicting SI in women psychiatric participants. The combination of the apps is synergistic (ROC AUC 0.87, *P*-value 0.0009; Pearson Correlation 0.48, *P*-value 0.0001). Thus, even without the benefit of potentially more costly, invasive and labor intensive blood biomarker testing, clinically useful predictions could be made with the apps.

Our apriori primary end point was a combined universal predictor for suicide (UP-Suicide), composed of the scores in CFI-S and in SASS (Mood, Anxiety), along with the Bonferroni validated biomarkers ($n = 50$) resulting from the sequential discovery for ideation, prioritization with CFG, and validation for behavior in suicide completers steps. UP-Suicide is a good predictor of SI (ROC AUC 0.82, *P*-value 0.003; Pearson Correlation 0.43, *P*-value 0.0003) (Table 4 and Figure 4). UP-Suicide also has good predictive ability for future psychiatric hospitalizations for suicidality (ROC AUC 0.78, *P*-value 0.032; Cox Regression Hazard Ratio 9.61, *P*-value 0.01). Overall, while there may *post hoc* appear to be better individual predictors for SI and for future hospitalizations (Table 4), our apriori primary broad-spectrum end point (UP-Suicide) has been successful, may be more robust to effects of fit to cohort, and might be more generalizable to other populations.

DISCUSSION

We carried out systematic studies to identify clinically useful predictors for suicide in women, an understudied population to date. Our work focuses on identifying markers involved in SI *and* suicidal behavior, including suicide completion. Markers involved in behavior may be on a continuum with some of the markers involved in ideation, varying in the degree of expression changes from less severe (ideation) to more severe (behavior). One cannot have suicidal behavior without SI, but it may be possible to have SI without suicidal behavior.

As a first step, we sought to use a powerful but difficult to conduct within-participant design for discovery of blood biomarkers. Such a design is more informative than case–control, case–case or even identical twins designs. The power of a within-participants longitudinal design for multi-omic discovery was first illustrated by Snyder and colleagues[12] in a landmark paper with an $n = 1$. We also have previously demonstrated its power in an initial pilot study in male bipolar participants ($n = 9$ out of 75 showed a switch from a no suicidal ideation to a high suicidal ideation state),[3] and then a larger studies in males with major psychiatric disorders ($n = 37$ out of 217).[4] In this small ($n = 12$ out of 51) but very valuable pilot study in women, we followed a similar path.

Second, we conducted whole-genome gene expression discovery studies in the participants that exhibited the switches, using a longitudinal within-participant design, that factors out genetic variability and reduces environmental variability as well. We have demonstrated the power of such a design in our earlier successful pilot work on suicide biomarkers in men with an $n = 9$.[3] Our current $n = 12$ is comparable (Figure 2). Genes whose levels of expression tracked SI within each participant were identified.

Third, the lists of top candidate biomarkers for SI from the discovery and prioritization step (genes with a CFG score of 4 and above, reflecting genes that have maximal experimental internal evidence from this study and/or additional external literature cross-validating evidence) were additionally validated for involvement in suicidal behavior in a cohort of demographically matched suicide completers from the coroner's office ($n = 6$) (Figure 2).

We ended up with 50 biomarkers that survived Bonferroni correction (49 genes; one gene, JUN, had two different probesets that validated). Additionally, we tested 65 other biomarkers that were non-Bonferroni validated but had maximum internal score of 4 in discovery *and* a CFG score of 6 and above, which means that in addition to strong evidence in this study they also had prior independent evidence of involvement in suicide from other studies. These additional biomarkers are likely involved in suicide but did not make our Bonferroni validation cutoff due to its stringency or potential technical/postmortem artifact reasons (Table 2 and Supplementary Table S2).

Fourth, we describe the use in a female population of the simple and comprehensive phenomic (clinical) risk assessment scale, CFI-S scale,[4] as well as of the companion app to it for use by clinicians and individuals (Supplementary Figure S2). CFI-S was developed independently of any data from this study, by integrating known risk factors for suicide from the clinical literature. It has a total of 20 items (scored in a binary manner—1 for present, 0 for absent, NA for information not available) that assess the influence of mental health factors, as well as of life satisfaction, physical health, environmental stress, addictions and cultural factors known to influence suicidal behavior. It also has two demographics risk factors items: age and gender. The result is a simple polyphenic risk score with an absolute range of 0–22, normalized by the number of items on which we had available information, resulting in a score in the range from 0 to 1 (Figure 3 and Supplementary Figure S2). We present data validating the CFI-S in women, in the combined discovery and test cohort of live psychiatric participants (Figure 3). We identified the chronic stress of lack of positive relationships/social isolation as the top differential item between no and high SI in women, which is consistent with biological data from the biomarker side of our study.

Fifth, we also assessed anxiety and mood, using a visual analog SASS, previously described by us,[4,9] for which we now have developed an app version (Supplementary Figure S2). Using a PhenoChipping approach[9] in our discovery cohort of psychiatric participants, we show that anxiety measures cluster with SI and CFI-S, and mood measures are in the opposite cluster, suggesting that our participants have high SI when they have high anxiety

Table 5. Cross-prediction in the other gender

Gene symbol/Gene Name	*Probesets*	*Males Discovery (direction of change) method/internal score (%)*	*Males Participants tested with suicidality/total*	*Males Predictions ROC/P-value*	*Females Discovery (direction of change) method/internal score (%)*	*Females Participants tested with suicidality/total*	*Females Predictions ROC/P-value*
Top biomarkers from males that were co-directional in females							
SLC4A4 Solute carrier family 4 (sodium bicarbonate cotransporter), member 4	210739_x_at	(I) AP/2 (71%)	SI: 33 | 108 Hosp: 32 | 157	**SI: 0.72/2.41E-05** Hosp: 0.44/0.87	(I) DE/0 (20%)	SI: 7 | 33 Hosp: 3 | 24	SI: 0.62/0.15 **Hosp: 0.86/0.03**
SKA2 Spindle and kinetochore associated complex subunit 2	225686_at	(D) DE/1 (34%) AP/1 (42%)	SI: 33 | 108 Hosp: 32 | 157	**SI: 0.69/0.0002** Hosp: 0.46/0.75	(D) DE/0 (6%)	SI: 7 | 33 Hosp: 3 | 24	SI: 0.50/0.51 *Hosp: 0.78/0.07*
Top biomarkers from females that were co-directional in males							
PIK3C3 Phosphatidylinositol 3-kinase, catalytic subunit type 3	232086_at	(D) DE/0 (14%)	SI: 33 | 108 Hosp: 32 | 157	**SI: 0.62/0.01** Hosp: 0.5/0.49	(D) DE/1 (49%)	SI: 7 | 33 Hosp: 3 | 24	*SI: 0.65/0.098* **Hosp: 0.9/0.011**
CSNK1A1 Casein kinase 1, alpha 1	235464_at	(D) AP/0 (21%)	SI: 33 | 108 Hosp: 31 | 157	**SI: 0.63/0.007** Hosp: 0.5/0.53	(D) DE/4 (86%) AP/1 (36%)	SI: 7 | 33 Hosp: 3 | 24	SI: 0.56/0.316 **Hosp: 0.96/0.0007**

Abbreviations: AP, Absent-Present; DE, differential expression; ROC, receiver-operating characteristic; SI, suicidal ideation. Examples of top predictive biomarkers of interest from men[2] and from women (current study) that were changed in expression in the same direction in both genders. These biomarkers were discovered in just one gender, as they were in the other gender below the apriori set threshold for discovery (33.3%). However, they display ability to predict in the other gender as well. SI—predicting suicidal ideation. Hosp—predicting future hospitalizations for suicidality. Bold—*P*-value is significant. Italic—trend towards significance.

and low mood (Figure 2). We would also like to include in the future measures of psychosis, and of stress, to be more comprehensive.

Sixth, we examined how the biomarkers identified by us are able to predict *state* (SI) in a larger independent cohort of women psychiatric participants ($n = 33$ participants).

Seventh, we examined whether the biomarkers are able to predict *trait* (future hospitalizations for suicidal behavior) in women psychiatric participants ($n = 24$).

Last but not least, we demonstrate how our apriori primary end point, a comprehensive universal predictor for suicide (UP-Suicide), composed of the combination of the Bonferroni validated biomarkers ($n = 50$), along with the scores from CFI-S and SASS, predicts state (SI) and trait (future psychiatric hospitalizations for suicidality).

The rationale for identifying blood biomarkers as opposed to brain biomarkers is a pragmatic one—the brain cannot be readily accessed in live individuals. Other peripheral fluids, such as cerebrospinal fluid, require more invasive and painful procedures. Nevertheless, it is likely that many of the peripheral blood transcriptomic changes are not necessarily mirroring what is happening in the brain, and vice-versa. The keys to find peripheral biomarkers[5] are, first, to have a powerful discovery approach, such as our within-participant design, that closely tracks the phenotype you are trying to measure and reduces noise. Second, cross-validating and prioritizing the results with other lines of evidence, such as brain gene expression and genetic data, are important in order to establish relevance to disease and generalizability of findings. Third, it is important to validate for behavior in an independent cohort with a robust and relevant phenotype, in these case suicide completers. Fourth, testing for predictive ability in independent/prospective cohorts is a must (Supplementary Figure S1).

Biomarkers that survive such a rigorous stepwise discovery, prioritization, validation and testing process are likely directly relevant to the disorder studied. As such, we endeavored to study their biology, whether they are involved in other psychiatric disorders or are relatively specific for suicide, and whether they are modulated by existing drugs in general, and drugs known to treat suicidality in particular.

We have identified a series of biomarkers that seem to be changed in opposite direction in suicide vs in treatments with omega-3 fatty acids, lithium and clozapine (Supplementary Table S4). These biomarkers could potentially be used to stratify patients to different treatment approaches, and monitor their response. BCL2, JUN, GHA1, ENTPD1, ITIH5, MBNL1 and SSBP2 are changed in expression by two of these three treatments, suggesting that they may be core to the anti-suicidal mechanism of these drugs. Interestingly, MBNL1, which is decreased in expression in suicidality, was identified as increased in expression in longevity/healthy aging.[34] BCL2, CAT and JUN may be useful blood pharmacogenomic markers of response to lithium. CD84, MBNL1 and RAB22A may be useful blood pharmacogenomic markers of response to clozapine. NDRG1, FOXP1, AFF3, ATXN1, CSNK1A1, ENTPD1, ITIH5, PRDX3 and SSBP2 may be useful blood pharmacogenomic markers of response to omega-3 fatty acids. Three existing drugs used for other indications have been identified as targeting the top suicide biomarkers identified by us (Supplementary Table S4), and could potentially be re-purposed for testing in treatment of acute suicidality: anakinra (inhibiting ILR1), enzastaurin (inhibiting AKT3) and tesevatinib (inhibiting EPHB4). Additionally, Connectivity Map[35] analyses (Supplementary Table S6) identified novel compounds that induce gene expression signatures that are the opposite of those present in suicide, and might generate leads and/or be tested for use to treat/prevent suicidality, including mifepristone, LY294002, acetylsalicylic acid, estradiol, buspirone, corticosterone, metformin, diphenhydramine, haloperidol and fluoxetine (Supplementary Table S6).

a

Predictors	Visit 1 High SI HAMD-SI 3 (T- 14 months) %	+ 1 H	Visit 2 High SI HAMD-SI 2 (T- 10 months) %	+ 1 H	Visit 3 No SI HAMD-SI 0 (T- 5 months) %	+ 4 H	T Suicide
EPB41L5	*39.03*		*19.68*		*100*		
HAVCR2	62.85		34.2		17.67		
ARHGAP15	29.17		33.26		25.67		
HTRA1	**100**		40.46		20.6		
PER1	69.72		26.62		20.98		
PDXDC1	*17.75*		*62.26*		*100*		
PIK3C3	81.07		65.73		56.85		
GTF3C2	*42.63*		*51.89*		*81.08*		
ALDH3A2	81.47		66.03		55.81		
BCL2	71.02		40.43		61.61		
MOB3B	86.13		65.50		23.66		
DPCD	*33.51*		*46.59*		*44.6*		
GTF3C3	23.14		62.60		17.21		
ASPH	57.87		16.57		7.95		
KLHL28	59.17		86.01		37.19		
UIMC1	46.25		60.38		13.84		
SNX27	40.27		100		20.41		
ACTR3	78.33		80.32		61.09		
NUDT6	*65.18*		*80.59*		*73.34*		
LRRC8B	86.34		89.48		70.00		
CSNK1A1	93.4		93.83		78.25		
LARP4	90.63		91.63		71.60		
ZNF548	83.18		69.06		34.55		
BioM18	82.99		32.54		17.85		
BioM32	95.40		85.90		29.51		
BioM50	**100**		62.49		21.16		
Anxiety	96.00		84.18		59.80		
CFI-S	87.15		79.83		65.19		
Mood	83.61		50.86		21.43		
SASS	97.13		72.27		42.75		
CFI-S +SASS	**100**		72.24		40.62		
UP-Suicide	**100**		72.14		36.80		

b

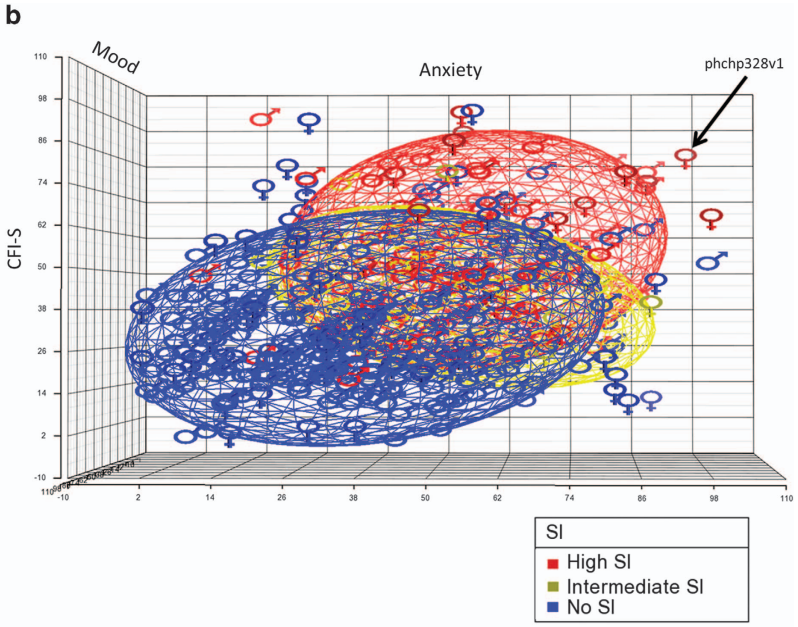


Figure 5. Study participant who committed suicide. Subject phchp328 was a 38-year-old divorced Caucasian female with a long history of MDD, PTSD, BP and polysubstance abuse/dependence. She had multiple psychiatric hospitalizations due to suicidal ideation ($n = 21$) and due to suicidal attempts ($n = 3$), in the 5 years before her suicide. She committed suicide by overdose with pills, leaving behind a suicide note addressed to her mother. (**a**) Percentile for scores on top predictors in all the female subjects in this study ($n = 105$ for biomarkers and $n = 88$ for apps and UP-Suicide). Her panel of Bonferroni validated biomarkers (BioM50) score, apps score (CFI-S+SASS), and UP-Suicide predictor score at a study visit (Visit 1) were at the 100% of the scores of all the psychiatric participant visits tested in this current study. Of note, that testing was conducted during an inpatient hospitalization due to suicidal ideation. While her scores did improve at subsequent outpatient testing visits (Visits 2 and 3), this high watermark score indicated her high risk. After the last testing visit in our study, she had four subsequent psychiatric hospitalizations: three due to suicidal ideation, one for opioid withdrawal/detox (the last one), ending 2 weeks before date of committing suicide (T). For decreased biomarkers, a higher percentile corresponds to lower expression values. Only 5 of the 32 predictors (biomarkers, clinical, combined) were discordant between the highest and lowest SI visit (italicized). In all, 17 of the 32 predictors (bold) were stepwise decreased corresponding to her SI scores. One of the biomarkers (HTRA1) was in the 100% of the subjects tested, as was the panel of 50 validated markers (BioM-50), the combination of the clinical measures/apps (CFI-S+SASS), and the combined biomarker panels and clinical/apps predictor (UP-Suicide). (**b**) Tri-dimensional representation of the percentilized scores of the combination of the two apps, CFI-S and SASS (Anxiety and Mood) of all the female participant visits tested in the current study ($n = 87$) and all the male participant visits in our previous work ($n = 317$). A tri-dimensional scatter plot was created using Partek. Tri-dimensional 95% confidence intervals were inserted as ellipsoids, color coded blue, yellow and red for No SI, Intermediate SI and High SI, respectively. Subject phchp328visit1 had the highest Euclidian D (distance from origin), as indicated by the arrow. This is the only subject that completed suicide as far as we know, as of the end of this study in November 2015. BP, bipolar disorder; MDD, major depressive disorder; PTSD, post-traumatic stress disorder.

Of note, a number of biomarkers from the current study in women reproduce and are co-directional with our previous findings in men (Table 5, Table 2 and Supplementary Table S2), whereas others had changes in opposite directions (Table 2 and Supplementary Table S2), underlying the issue of biological context and differences in suicidality between the two genders. This avenue merits attention in the field, and detailed future comparative studies, as do studies by diagnostic groups.

Before any testing, we planned to use a comprehensive combination of genomic data (specifically, the top validated biomarkers) and phenomic data (specifically, the CFI-S and the SASS) as the primary end point measure, a broad-spectrum universal predictor (UP-Suicide) for state SI and trait future hospitalizations. It has not escaped our attention that certain single biomarkers, particular phenotypic items, or combinations thereof seem to perform better than the UP-Suicide in one or another type of prediction (see Table 4). However, since such markers and combinations were not chosen by us apriori and such insights derive from testing, we cannot exclude a fit to cohort effect for them and reserve judgement as to their robustness as predictors until further testing in additional independent cohorts, by us and others. What we can put forward for now based on the current work is the UP-Suicide, which seems to be a robust predictor across different scenarios and diagnostic groups.

Our study has a number of limitations. All this work was carried out in psychiatric patients, a high-risk group, and it remains to be seen how such predictors apply to non-psychiatric participants. For the UP-Suicide testing, the prevalence rate for suicidality in our test cohorts was 21% (7 out of 33 for SI and 5 out of 24 for future hospitalizations) (Table 4). Of note, this rate was remarkably similar to our previous work in men.[4] It is to be noted that the incidence of suicidality in the general population is lower, for example at 1.5% in adolescents in an European cohort[36] and estimates of 0.2–2% in the United States,[37] which underlines the rationale

of using a very high-risk group like we did for magnifying and enabling signal detection with a relatively small *N*. Over 40% of the live participants from the discovery cohort (5 out of 12) and independent test cohort (14 out of 33) are non-VA, and all the suicide completers used for validation are from the general population, not VA, so we believe our results have broader relevance. Studies with larger numbers and longer follow-up, currently ongoing, as well as studies in different clinical settings, may provide more generalizability.

The current studies were carried out exclusively in females. Similar work is needed in larger meta-analyses across gender, in participants with and without psychiatric disorders, to find generalizable predictors. Conversely, a narrow focus by gender, diagnosis (or lack of), and perhaps age, may be needed to find more individualized predictors. Such work is ongoing in our group.

In conclusion, we have advanced the biological understanding of suicidality in women, highlighting behavioral and biological mechanisms related to inflammation, neurotrophic factors, circadian clock, stress response and apoptosis. Biomarkers that may track treatment response to lithium and intriguingly, omega-3 fatty acids, have been identified. Of equal importance, we developed instruments (biomarkers and apps) for predicting suicidality, that do not require asking the person assessed if they have suicidal thoughts, as individuals who are truly suicidal often do not share that information with people close to them or with clinicians. We propose that the widespread use of such risk prediction tests as part of routine or targeted health-care assessments will lead to early disease interception followed by preventive lifestyle modifications or treatment. Given the magnitude and urgency of the problem, the importance of efforts to implement such tools cannot be overstated. We note that we have sadly lost one study participant to suicide (Figure 5), that in retrospect was highlighted by UP-Suicide as being the highest risk participant in our study.

Note

Supplementary information is also available from the Niculescu Laboratory website (www.neurophenomics.info).

CONFLICT OF INTEREST

ABN is listed as an inventor on a patent application being filed by Indiana University.

ACKNOWLEDGMENTS

This work is, in essence, a field-wide collaboration. We acknowledge our debt of gratitude for the efforts and results of the many other groups, cited in our paper, who have conducted and published studies (clinical, genetic and biological) in suicidality. With their arduous and careful work, a convergent approach such as ours is possible. We thank David Welsh for advice on clock genes, Joseph Niezer and Tammy Jones for helpful clinical discussions, as well as Meghan Carpenter and Jay Natarajan for help with building literature databases. We also would particularly like to thank the participants who participated in these studies, their families and their caregivers. Without their contribution, such work to advance the understanding of suicide would not be possible. This work was supported by an NIH Directors' New Innovator Award (1DP2OD007363) and a VA Merit Award (2I01CX000139) to ABN.

AUTHOR CONTRIBUTIONS

ABN designed the study and wrote the manuscript. DFL, EN, HLN, HD, PLP, TL and ECS analyzed the data. NV and FNK performed database work. HW, EB and DLG organized, conducted and scored testing in psychiatric participants. AB, MY, AS, GES and ABN organized and carried out postmortem samples collection. TG, NJS, SMK and DRS conducted microarray experiments and provided input on data analyses. All authors discussed the results and commented on the manuscript.

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