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IMMEDIATE COMMUNICATION

Precision medicine for suicidality: from universality to subtypes and personalization

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Suicide remains a clear, present and increasing public health problem, despite being a potentially preventable tragedy. Its incidence is particularly high in people with overt or un(der)diagnosed psychiatric disorders. Objective and precise identification of individuals at risk, ways of monitoring response to treatments and novel preventive therapeutics need to be discovered, employed and widely deployed. We sought to investigate whether blood gene expression biomarkers for suicide (that is, a 'liquid biopsy' approach) can be identified that are more universal in nature, working across psychiatric diagnoses and genders, using larger cohorts than in previous studies. Such markers may reflect and/or be a proxy for the core biology of suicide. We were successful in this endeavor, using a comprehensive stepwise approach, leading to a wealth of findings. Steps 1, 2 and 3 were discovery, prioritization and validation for tracking suicidality, resulting in a Top Dozen list of candidate biomarkers comprising the top biomarkers from each step, as well as a larger list of 148 candidate biomarkers that survived Bonferroni correction in the validation step. Step 4 was testing the Top Dozen list and Bonferroni biomarker list for predictive ability for suicidal ideation (SI) and for future hospitalizations for suicidality in independent cohorts, leading to the identification of completely novel predictive biomarkers (such as CLN5 and AK2), as well as reinforcement of ours and others previous findings in the field (such as SLC4A4 and SKA2). Additionally, we examined whether subtypes of suicidality can be identified based on mental state at the time of high SI and identified four potential subtypes: high anxiety, low mood, combined and non-affective (psychotic). Such subtypes may delineate groups of individuals that are more homogenous in terms of suicidality biology and behavior. We also studied a more personalized approach, by psychiatric diagnosis and gender, with a focus on bipolar males, the highest risk group. Such a personalized approach may be more sensitive to gender differences and to the impact of psychiatric co-morbidities and medications. We compared testing the universal biomarkers in everybody versus testing by subtypes versus personalized by gender and diagnosis, and show that the subtype and personalized approaches permit enhanced precision of predictions for different universal biomarkers. In particular, LHFP appears to be a strong predictor for suicidality in males with depression. We also directly examined whether biomarkers discovered using male bipolars only are better predictors in a male bipolar independent cohort than universal biomarkers and show evidence for a possible advantage of personalization. We identified completely novel biomarkers (such as SPTBN1 and C7orf73), and reinforced previously known biomarkers (such as PTEN and SAT1). For diagnostic ability testing purposes, we also examined as predictors phenotypic measures as apps (for suicide risk (CFI-S, Convergent Functional Information for Suicidality) and for anxiety and mood (SASS, Simplified Affective State Scale)) by themselves, as well as in combination with the top biomarkers (the combination being our a priori primary endpoint), to provide context and enhance precision of predictions. We obtained area under the curves of 90% for SI and 77% for future hospitalizations in independent cohorts. Step 5 was to look for mechanistic understanding, starting with examining evidence for the Top Dozen and Bonferroni biomarkers for involvement in other psychiatric and non-psychiatric disorders, as a mechanism for biological predisposition and vulnerability. The biomarkers we identified also provide a window towards understanding the biology of suicide, implicating biological pathways related to neurogenesis, programmed cell death and insulin signaling from the universal biomarkers, as well as mTOR signaling from the male bipolar biomarkers. In particular, HTR2A increase coupled with ARRB1 and GSK3B decreases in expression in suicidality may provide a synergistic mechanistical corrective target, as do SLC4A4 increase coupled with AHCYL1 and AHCYL2 decrease. Step 6 was to move beyond diagnostics and mechanistical risk assessment, towards providing a foundation for personalized therapeutics. Items scored positive in the CFI-S and subtypes identified by SASS in different individuals provide targets for personalized (psycho)therapy. Some individual biomarkers are targets of existing drugs used to treat mood disorders and suicidality (lithium, clozapine and omega-3 fatty acids), providing a means toward pharmacogenomics stratification of patients and monitoring of response to treatment. Such biomarkers merit evaluation in clinical trials. Bioinformatics drug repurposing analyses with the gene expression biosignatures of the Top Dozen and Bonferroni-validated universal biomarkers identified novel potential therapeutics for suicidality, such as ebselen

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(a lithium mimetic), piracetam (a nootropic), chlorogenic acid (a polyphenol) and metformin (an antidiabetic and possible longevity promoting drug). Finally, based on the totality of our data and of the evidence in the field to date, a convergent functional evidence score prioritizing biomarkers that have all around evidence (track suicidality, predict it, are reflective of biological predisposition and are potential drug targets) brought to the fore APOE and IL6 from among the universal biomarkers, suggesting an inflammatory/accelerated aging component that may be a targetable common denominator.

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INTRODUCTION

'Individuality in universality is the plan of creation'.
-Swami Vivekananda

Objective and quantitative markers would permit better and more precise assessment, tracking and prediction of suicidal risk, which would enable preventive therapeutic interventions.[1–3] Previous work by our group has identified blood biomarkers and phenotypic predictors for suicide risk in men[4] and separately in women,[5] showing some gender similarities as well as differences. An essential question remained to be answered, of high relevance for developing this area of research and carrying it to full clinical applicability: would a quest for more universal predictors or a quest for more personalized predictors be more productive? We endeavored to answer this question through our current work. First, we sought to investigate whether blood gene expression biomarkers can be identified that are more universal in nature, working across psychiatric diagnoses and genders, starting with a powerful longitudinal within-participant[6] design and using larger cohorts than in previous studies[7,4,5] (Figure 1). Second, we identified subtypes of suicidality based on mental state (anxiety, mood and psychosis) at the time of high suicidal ideation.[8–10] Third, we used a more personalized approach, by gender and diagnosis, with a focus on the highest clinical risk group, male bipolars. We examined the ability of the universal candidate biomarkers to predict suicidal ideation (SI) and future hospitalizations for suicidality, in completely independent cohorts, in everybody, as well as divided by subtypes, and personalized by gender and diagnosis. We also directly compared the universal biomarkers with biomarkers discovered using male bipolars only, for ability to predict SI and future hospitalizations for suicidality in independent cohorts of male bipolars.

Per our *a priori* design described in previous studies,[4,5] we also combined the top biomarkers with scores obtained with a clinical information measure of suicide risk (Convergent Functional Information for Suicidality, CFI-S), as well as with scores for anxiety and mood obtained with an 11-item visual analog scale (Simplified Affective State Scale, SASS),[4,5,8] to obtain a broader spectrum predictor (UP-Suicide) that puts the biomarkers in the context of the person's life and his/hers mental state. The triggers for suicide may be the individual's perceptions of being unsuccessful, with no future, suffering, damaged, along with mental frailty, addictions and cultural exposure to suicide. Our CFI-S scale captures such information about who a person is, and along with the SASS scale that captures mental state information and permits classification in subtypes, they add a broader context to the objective blood biomarker predictors we have uncovered (Figure 5). Of note, these scales do not ask about SI, as individuals who want to commit suicide may not always share that information, for fear of being stopped.

We also used the lists of top biomarkers we identified as a window into the biology of suicidality, by conducting biological pathways and network analyses, and by looking at co-morbidity with other disorders that may predispose or create a vulnerability to suicidality.

In addition, we leveraged these lists for therapeutics and drug discovery purposes, to see whether some of the biomarkers we identified are modulated by existing compounds used to treat suicidality and also to conduct bioinformatics drug repurposing analyses to discover new drugs and natural compounds that may be useful for treating suicidality.

Finally, we integrated the totality of evidence we have generated in this study and available in the literature to date, to prioritize biomarkers for future clinical studies in the field.

MATERIALS AND METHODS

Cohorts

We used three independent cohorts: discovery (a live psychiatric participants cohort), validation (a postmortem coroner's office suicide completers cohort) and testing (an independent live psychiatric participants test cohort for predicting SI and for predicting future hospitalizations for suicidality) (Figure 1a).

Similar to our previous studies,[7,4,5] the live psychiatric participants are part of a larger longitudinal cohort of adults that we are continuously collecting. Participants are recruited from the patient population at the Indianapolis VA Medical Center and Indiana University School of Medicine. 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 (Supplementary Figure S1), and their blood was drawn. We collected whole blood (10 ml) 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 RNA was extracted for microarray gene expression studies from the PAXgene tubes, as detailed below.

For this study, our within-participant discovery cohort, from which the biomarker data were derived, consisted of 66 participants (49 males and 17 females) with psychiatric disorders and multiple testing visits, who each had at least one diametric change in SI scores from no SI to high SI, or vice versa, from one testing visit to another. There were 2 participants with 6 visits each, 3 participants with 5 visits each, 5 participants with 4 visits each, 34 participants with 3 visits each and 22 participants with 2 visits each, resulting in a total of 193 blood samples for subsequent gene expression microarray studies (Figure 1 and Table 1 and S1).

Our postmortem cohort, in which the top biomarker findings were validated for behavior, consisted of 38 male and 7 female violent suicide completers obtained through the Marion County coroner's office (Table 1). We required a last observed alive postmortem interval of 24 h or less and the cases selected had to have completed suicide by means other than overdose, which could affect gene expression. Thirty-one participants completed suicide by gunshot to head or chest, 12 by asphyxiation, 1 by slit

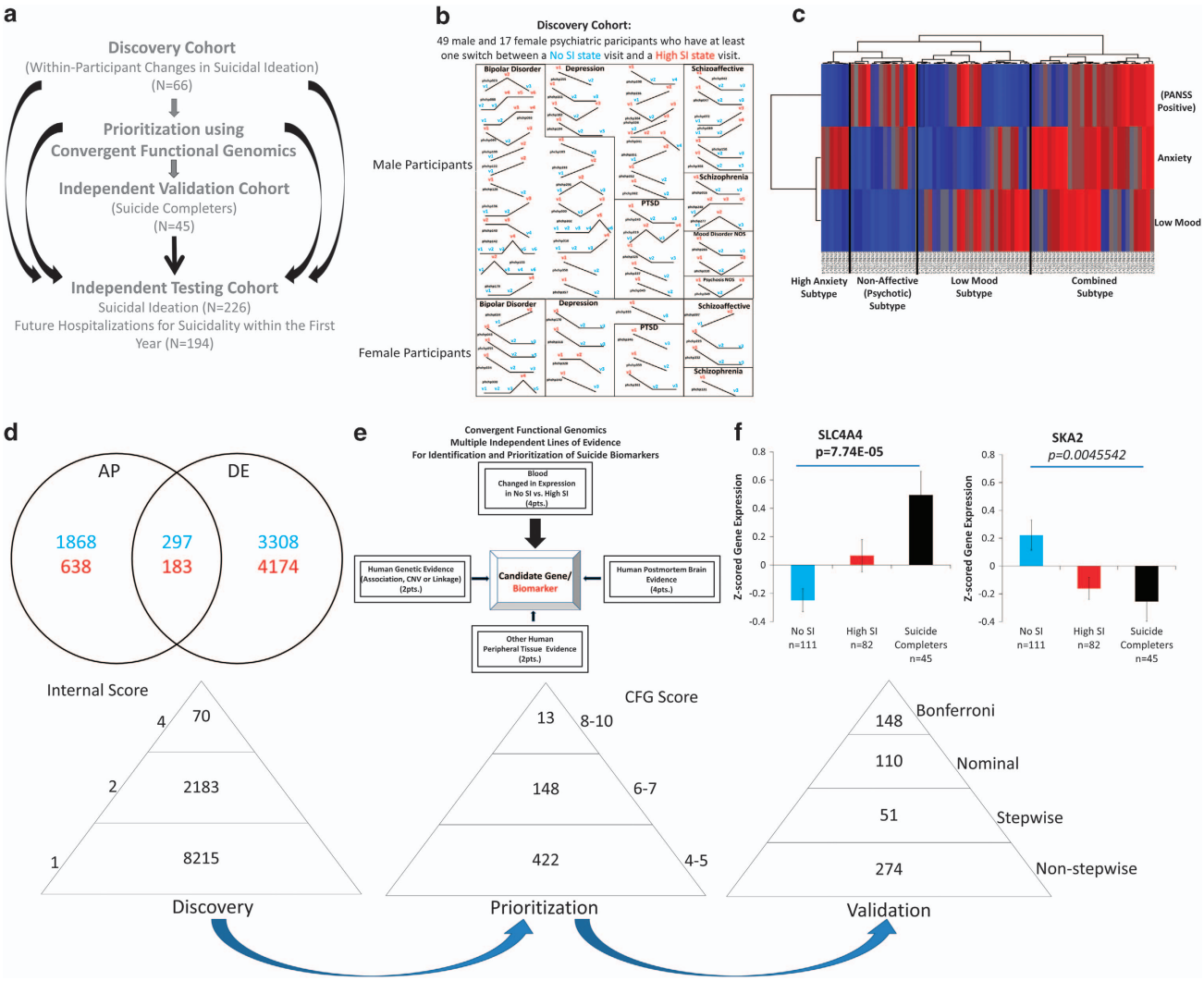


Figure 1. Steps 1–3: discovery, prioritization and validation. (**a**) Cohorts used in study, depicting flow of discovery, prioritization and validation of biomarkers from each step. (**b**) Discovery cohort longitudinal within-participant analysis. Phchp### is study ID for each participant. V# denotes visit number (1, 2, 3, 4, 5 or 6). (**c**) Discovery of possible subtypes of suicidality based on high suicidal ideation (SI) visits in the discovery cohort. Participants were clustered using measures of mood and anxiety (Simplified Affective State Scale (SASS)), as well as psychosis (PANSS Positive) (**d**) Differential gene expression in the Discovery cohort, number of genes identified with DE and AP methods with an internal score of 1 and above. Red, increased in expression in high SI. Blue, decreased in expression in high SI. At the discovery step probesets are identified based on their score for tracking SI with a maximum of internal points of 4 (33% (1 pt), 50% (2 pt) and 80% (4 pt)). (**e**) Prioritization with Convergent Functional Genomics (CFG) for prior evidence of involvement in suicide. In the prioritization step probesets are converted to their associated genes using Affymetrix annotation and GeneCards. Genes are prioritized and scored using CFG for Suicide evidence with a maximum of eight external points. Genes scoring at least 4 points out of a maximum possible of 12 points total internal and external score are carried to the validation step. (**f**) Validation in an independent suicide completers cohort from the coroner's office. In the validation step biomarkers are assessed for stepwise change from the discovery groups of participants with no SI, to high SI, to suicide completion, using analysis of variance. Stringent Bonferroni correction is calculated for the total number of probesets analyzed.

wrist and 1 by electrocution. Next of kin signed informed consent at the coroner's office for donation of blood for research.

Our independent test cohort for predicting SI (Table 1) consisted of 184 male and 42 female participants with psychiatric disorders, demographically matched with the discovery cohort, with one or multiple testing visits in our lab, with either no SI, intermediate SI or high SI, resulting in a total of 226 blood samples in which whole-genome blood gene expression data were obtained (Figure 1 and Table 1 and S1).

Our test cohort for predicting future hospitalizations (Figure 1 and Table 1 and S1) is a subset (170 males and 24 females) of the independent test cohort for which we had longitudinal follow-up with electronic medical records. 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 due to suicidality if suicidal acts or intent were listed as a reason for admission and/or SI was described in the admission and discharge medical notes.

Complete Materials and Methods are available in the Supplementary Information. Of note, all genomic and phenomic data was normalized (z-scored) by gender and psychiatric diagnosis before being combined and analyzed.

Table 1. Demographics

A. Universal	*Participants*	*Gender*	*Diagnosis*	*Ethnicity*	*Age mean (s.d.)*
Discovery					
Discovery cohort (longitudinal within-participant changes in SI)	66	Male = 49 Female = 17	BP = 25 MDD = 17 SZA = 9 SZ = 4 PTSD = 8 MOOD = 2 PSYCH = 1	EA = 51 AA = 14 Asian = 1	47.94 (9.47)
Validation					
Independent validation cohort for gene expression (suicide completers)	45	Male = 38 Female = 7	NP = 19 MDD = 19 BP = 2 SZ = 1 AX = 1 Alcoholism = 1 ADHD = 1 PTSD = 1	EA = 37 AA = 7 Hispanic = 1	40.69 (16.93)
Testing					
All					
Independent testing cohort for predicting state (SI at time of assessment)	226	Male = 184 Female = 42	BP = 68 MDD = 32 SZA = 53 SZ = 45 PTSD = 19 MOOD = 5 PSYCH = 4	EA = 148 AA = 73 Asian = 1 Hispanic = 3 Mixed = 1	All = 50.26 (9.47) No SI = 51.1 Intermediate SI = 49 High SI = 44.3
Independent testing cohort for predicting trait (hospitalizations for suicidality in the year following assessment)	194	Male = 170 Female = 24	BP = 52 MDD = 30 SZA = 45 SZ = 43 PTSD = 16 MOOD = 5 PSYCH = 3	EA = 126 AA = 64 Hispanic = 3 Mixed = 1	All = 50.65 (9.14) No Hosp for SI = 50.91 Hosp for SI = 47.66
Subtypes					
High anxiety subtype	46	Male = 40 Female = 6	BP = 13 MDD = 10 SZA = 9 SZ = 11 PTSD = 2 MOOD = 1	EA = 27 AA = 19	All = 50.96 (7.63) No SI = 52.1 Intermediate SI = 52.5 High SI = 39.4
Low mood subtype	76	Male = 57 Female = 19	BP = 21 MDD = 17 SZA = 15 SZ = 15 PTSD = 6 MOOD = 1 PSYCH = 1	EA = 53 AA = 20 Hispanic = 2 Asian = 1	All = 51.53 (10.04) No SI = 51.44 Intermediate SI = 51.81 High SI = 51.9
Combined subtype	86	Male = 61 Female = 25	BP = 30 MDD = 11 SZA = 21 SZ = 11 PTSD = 11 MOOD = 2	EA = 63 AA = 21 Hispanic = 1 Mixed = 1	All = 47.95 (9.36) No SI = 50.79 Intermediate SI = 45.43 High SI = 43.06
Non-affective (psychotic) subtype	141	Male = 121 Female = 20	BP = 40 MDD = 17 SZA = 35 SZ = 32 PTSD = 10 MOOD = 4 PSYCH = 3	EA = 86 AA = 52 Hispanic = 2 Mixed = 1	All = 50.71 (9.49) No SI = 50.89 Intermediate SI = 51.67 High SI = 42.33

B. Male bipolar	*Participants*	*Gender*	*Diagnosis*	*Ethnicity*	*Age mean (s.d.)*
Discovery					
Male bipolar discovery cohort (within-participant changes in SI)	20	Male = 20	BP = 20	EA = 20	48.12 (9.10)
Validation					
Male independent validation cohort for gene expression (suicide completers)	38	Male = 38	NP = 18 MDD = 16 BP = 1 SZ = 1 AX = 1 Alcoholism = 1	EA = 31 AA = 6 Hispanic = 1	40.82 (17.31)
Testing					
Male bipolar independent testing cohort for predicting state (SI at time of assessment)	49	Male = 49	BP = 49	EA = 43 AA = 5 Hispanic = 1	All = 49.16 (10.01) No SI = 50.19 Intermediate SI = 48.73 High SI = 40.42
Male bipolar independent testing cohort for predicting trait (hospitalizations for suicidality in the year following assessment)	44	Male = 44	BP = 44	EA = 39 AA = 4 Hispanic = 1	All = 48.88 (10.23) No Hosp for SI = 48.76 Hosp for SI = 52.25

Abbreviations: AA, African Amercian; ADHD, attention deficit disorder; AX, anxiety disorder; BP, bipolar; EA, European-American; MDD, major depressive disorder; MOOD, mood disorder not otherwise specified; NP, non-psychiatric; PSYCH, psychosis not otherwise specified; PTSD, post-traumatic stress disorder; SZA, schizoaffective disorder; SZ, schizophrenia.

RESULTS

Step 1: Discovery

We used a powerful within-participant longitudinal discovery approach to identify genes that: (1) change in expression in blood between no SI and high SI states; (2) track the SI state across visits in a participant; and (3) track the SI state in multiple participants. We used a longitudinally followed cohort of participants that showed diametric changes in SI between at least two testing visits ($n = 66$ participants out of a cohort of 293 men and women psychiatric disorder participants followed longitudinally, with diagnoses of bipolar disorder, depression, mood disorder nos, schizophrenia, schizoaffective disorder, psychosis nos and post-traumatic stress disorder). Using our 33% of maximum raw score threshold (internal score of 1 pt),[4,5] we had 10 468 unique probesets from Affymetrix Absent/Present (AP) analyses and Differential Expression (DE) analyses (Figure 1d). These were carried forward to the prioritization step. This represents approximately a fivefold enrichment of the 54 625 probesets on the Affymetrix array.

We also examined in the discovery cohort whether subtypes of suicidality can be identified based on mental state at the time of high SI visits, using two-way hierarchical clustering with anxiety, mood and psychosis measures. The SI state self-report may be more reliable in this cohort, as the participants demonstrated the aptitude and willingness to report different and diametric SI states. We uncovered four potential subtypes of suicidality: high anxiety, low mood, co-morbid and non-affective (psychotic) (Figure 1c). These subtypes need to be tested in independent cohorts for practical utility, diagnostic and therapeutic.

Step 2: Prioritization

We used a Convergent Functional Genomics approach to prioritize the candidate biomarkers identified in the discovery step (internal score of $\geqslant 1$ pt) by using all the published prior independent evidence in the field (Figure 1e). There were 583 probesets that had a Convergent Functional Genomics score (combined internal and external score) of 4 and above. These were carried forward to the validation step. This represents approximately a 100-fold enrichment of the probesets on the Affymetrix array.

Step 3: Validation

Next, we validated for suicidal behavior these prioritized biomarkers, in a demographically matched cohort of men and women suicide completers from the coroner's office ($n = 45$), by assessing which markers were stepwise changed in expression from no SI to high SI to suicide completers (Figure 1). Two hundred and seventy-four probesets were non-stepwise changed and 309 were stepwise changed. Of these, 148 survived Bonferroni correction for all the 583 probesets validated. This represents approximately a 500-fold enrichment of the probesets on the Affymetrix array.

Step 4: Testing for diagnostics

We tested for diagnostic ability in independent cohorts two overlapping sets of biomarkers: the universal Top Dozen biomarkers (composed of the top increased and decreased biomarkers from AP and from DE from each step: discovery, prioritization and validation) (Table 2), as well as all the universal Bonferroni biomarkers, that survived correction for multiple comparisons after the validation step (Supplementary Table S2). The biomarkers were tested individually (the 12 biomarkers from the Top Dozen list and 148 biomarkers from the Bonferroni list), as well as in panels (BioM12 and BioM 148), in a completely independent test cohort of men and women psychiatric disorder participants ($n = 226$), for prediction of SI state, as well as for prediction of future psychiatric hospitalizations due to suicidality (Figures 2 and 3, Table 3 and Supplementary Table S4). We were successful in this endeavor, leading to identification of universal biomarkers that work across gender and diagnoses, such as CLN5 and AK2.

We also studied their predictive ability in participants in the independent cohort grouped by the subtypes described above, as well as grouped by a more personalized approach, by psychiatric diagnosis and gender. We then compared the universal approach to the subtypes approach and the personalized approach, and show that the subtype and personalized approaches permit enhanced precision of predictions for different biomarkers (Figures 2 and 3, Supplementary Figure S2, Table 3 and Supplementary Table S4).

For SI prediction in the independent test cohort, CLN5, from the Bonferroni biomarkers list, a decreased in expression biomarker, had an area under the curve (AUC) of 65% ($P = 1.86E-04$) across all subjects, and 75% (4.43E − 03) in the low mood subtype. It also survived correction for multiple comparisons for the Step 4 testing of the 154 universal biomarkers (from the combined Top Dozen and Bonferroni lists). CLN5 also had an AUC of 87% ($P = 1.16E-02$) for predicting future hospitalizations for suicidality in the first year of follow-up in male post-traumatic stress disorder. CLN5 (ceroid-lipofuscinosis, neuronal 5) is a completely novel finding. It has no prior evidence as a biomarker for suicide or for involvement in other psychiatric disorders. CLN5 is a lysosomal regulatory protein, known to date to be involved in neurodegenerative, lysosomal storage diseases.

For prediction of future hospitalizations for suicidality in the first year of follow-up in the independent test cohort, AK2, from the Bonferroni biomarkers list, a decreased in expression biomarker, had an AUC of 60% ($P = 2.31E-02$) across all subjects, 68% ($P = 6.72E-03$) in the combined subtype and 78% ($P = 2.70E-03$) for male schizoaffectives. AK2 also had an AUC of 64% ($P = 5.39E-04$) for predicting SI state across all subjects in the independent test cohort, as well as an AUC of 75% ($P = 2.93E-02$) in male schizophrenia. AK2 (adenylate kinase 2) is also a completely novel finding. It has no prior evidence as a biomarker for suicide, but does have evidence for a decrease in expression in brains of schizophrenics.[11] AK2 is a mitochondrial gene, known to have an important role in cellular energy homeostasis and in adenine nucleotide metabolism, as well as may have a role in apoptosis.

As for examples of previously known biomarkers reproduced in this study, for SI prediction in the independent test cohort, SLC4A4, a top increased in expression biomarker, had an AUC of 64% ($P = 3.83E-04$) across all subjects, 69% (6.13E − 04) in the combined subtype and 77% (9.72E − 04) in male bipolars. SKA2, a top decreased in expression biomarker, had an AUC of 61% ($P = 3.35E-03$) across all subjects, 74% (5.91E − 03) in the low mood subtype and 79% (1.35E − 02) in male schizophrenics.

In addition, we used two previously described clinical instruments in the form of apps, the SASS that measures anxiety and mood, and the CFI-S that measures risk for suicide indirectly, without asking about SI.[4,5] The scores from these apps showed good predictive ability for both state (SI) and trait (future hospitalizations), in all participants (Figure 4, Table 3). There are interesting variations in different subtypes and personalized by gender and diagnosis (Supplementary Table S4), suggesting the distinctness and homogeneity of those subgroups.

We also combined a panel of the Top Dozen biomarkers with measures of anxiety and mood (SASS), and with the suicide risk scale (CFI-S), into a broad spectrum universal predictor algorithm (UP Suicide), our *a priori* endpoint as in our previous studies. The UP Suicide provides the biomarkers with mental state (SASS) and personal history context (CFI-S), enhancing precision of predictions (Figures 5 and 6). Across all subjects in the independent test cohort, UP Suicide 12 had an AUC of 90% ($P = 3.87E-21$) for state (SI) prediction, as well as an AUC of 77% ($P = 2.87E-08$) for trait (future hospitalizations for suicidality) predictions. The results for predicting SI were even stronger in the low mood subtype (AUC of

Table 2. The Top Dozen list of candidate biomarker genes - evidence for involvement in suicidality

A. Top Dozen biomarkers from universal approach

Gene symbol *Gene name*	*Probesets*	*Discovery (direction of change) method/score*	*Prior human genetic evidence for suicide*	*Prior human brain expression evidence for suicide*	*Prior human peripheral expression evidence for suicide*	*Prioritization total CFG score for suicide*	*Validation ANOVA P-value*	*Descriptor*
Top biomarkers from discovery—step 1								
HIST1H2BO Histone cluster 1, H2bo	214540_at	(I) DE/4			Our previous work in males (I)(214540_at) Blood[4]	4	5.37E – 14	Top discovery DE increased gene
ERG V-ets avian erythroblastosis virus E26 oncogene homolog	213541_s_at	(D) DE/4			Our previous work in males (D) (213541_s_at) Blood[4] Our previous work in females (D) (213541_s_at) Blood[5]	4	NS	Top discovery DE decreased gene
GAB1 GRB2-associated binding protein 1	242572_at	(I) AP/4			Our previous work in males (I) (242572_at) Blood[4]	4	NS	Top discovery AP increased gene
CCL28 Chemokine (C–C motif) ligand 28	224240_s_at	(D) AP/4			Our previous work in males (D) (224240_s_at) Blood[4] Our previous work in females (D) (224240_s_at) Blood[5]	4	NS	Top discovery AP Decreased gene
Top biomarkers from prioritization—step 2								
HTR2A 5-Hydroxytryptamine (serotonin) receptor 2A, G protein-coupled	244130_at	(I) DE/2	Association[28–33]	(I) Prefrontal cortex[34] (I) Frontal cortex[35,36]	(D) Platelets[37] Our previous work in females (D) (244130_at) Blood[5]	10	NS	Top prioritization DE increased gene
SKA2 Spindle and kinetochore-associated complex subunit 2	225686_at	(D)DE/1 (D)AP/1	Association[38]	(D) Prefrontal cortex[38]	(D)Blood[38] (D)Blood, saliva[39] Our previous work in males (D)(225686_at) Blood[4]	9	*0.0045542*	Top prioritization DE decreased gene
SLC4A4 Solute carrier family 4 (sodium bicarbonate cotransporter), member 4	210739_x_at	(I) AP/1	Association[40]	(D) Prefrontal cortex(BA 46/10)[41]	Our previous work in males (I)(210739_x_at) Blood[4]	7	7.74E – 05	Top prioritization AP increased gene
IFNG Interferon, gamma	210354_at	(D) AP/1	Association[42]	(D) Hippocampus[43]	(D) Blood[44] Our previous work in females (D) (210354_at) Blood[5]	9	NS	Top prioritization AP decreased gene
Top biomarkers from validation—step 3								
PSME4 Proteasome activator subunit 4	237180_at	(I) DE/1		(I) Anterior cingulate cortex[20]		5	2.64E – 36	Top validation DE increased gene
LDLRAP1 Low-density lipoprotein receptor adaptor protein 1	57082_at	(D) DE/1		(I) Anterior cingulate cortex[20]	Our previous work in males (D)(221790_s_at) Blood[4]	5	1.48E – 38	Top validation DE decreased gene
PPAP2B Phosphatidic acid phosphatase type 2B	212226_s_at	(I)AP/1		(D) Prefrontal cortex (BA 46/10)[41]	Our previous work in females (I)(212226_s_at) Blood[5]	5	2.76E – 17	Top validation AP increased gene
ARRB1 Arrestin, beta 1	218832_x_at	(D) AP/1		(D) Prefrontal cortex (BA8/9)[45]		5	5.26E – 17	Top validation AP decreased gene

B. Top Dozen biomarkers from male bipolars

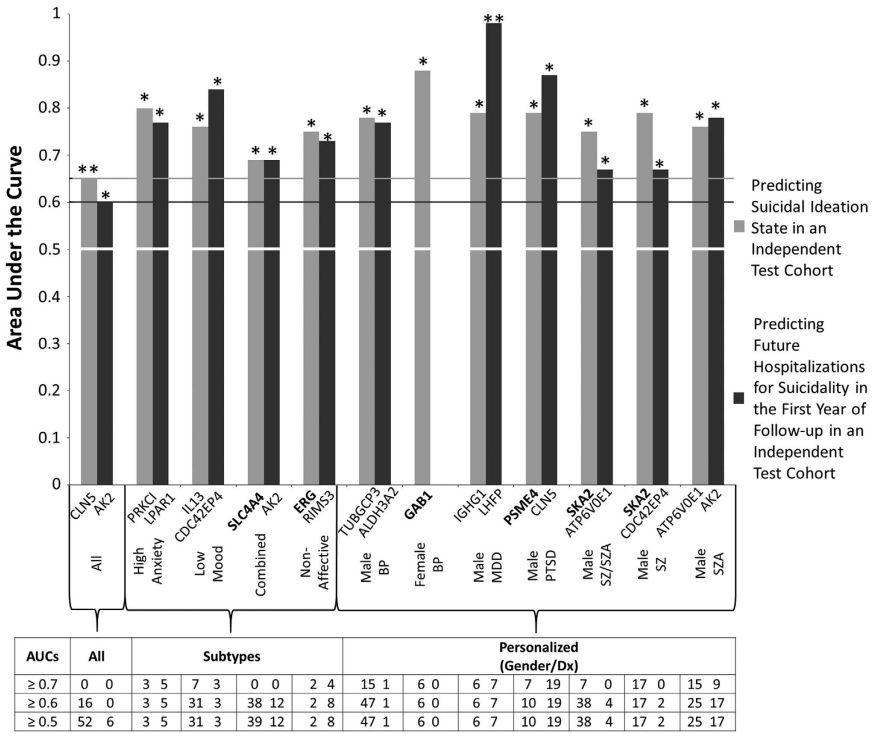
Gene symbol *Gene name*	*Probesets*	*Discovery (direction of change) method/score*	*Prior human genetic evidence for suicide*	*Prior human brain expression evidence for suicide*	*Prior human peripheral expression evidence for suicide*	*Prioritization Total CFG score for suicide*	*Validation ANOVA P-value*	*Descriptor*
Top biomarkers from discovery—step 1								
BE674182	237259_at	(I) DE/4				4.00	NS	Top discovery DE increased gene
ICAM4 Intercellular adhesion molecule 4 (Landsteiner–Wiener blood group)	207194_s_at	(D) DE/4			Our previous work in males (D) (207194_s_at) Blood[4] Our previous work in females (I) (207194_s_at) Blood[5]	4.00	3.81E – 08	Top discovery DE decreased gene
	208299_at	(I) AP/4				4.00	NS	Top discovery AP increased gene

Table 2. (Continued)

B. Top Dozen biomarkers from male bipolars

Gene symbol *Gene name*	*Probesets*	*Discovery (direction of change) method/score*	*Prior human genetic evidence for suicide*	*Prior human brain expression evidence for suicide*	*Prior human peripheral expression evidence for suicide*	*Prioritization Total CFG score for suicide*	*Validation ANOVA P-value*	*Descriptor*
CACNA1I Calcium channel, voltage-dependent, T type, alpha 1I subunit					Our previous work in males (I) (208299_at) Blood[4]			
ADAL Adenosine deaminase-like	239711_at	(D) AP/4			Our previous work in males (D) (239711_at) Blood[4] Our previous work in females (D) (239711_at) Blood[5]	4.00	4.53E – 08	Top discovery AP decreased gene
Top biomarkers from prioritization—step 2								
HTR2A 5-Hydroxytryptamine (serotonin) receptor 2A, G protein-coupled	244130_at	(I) DE/2	Association[29–33,46]	(I) Prefrontal cortex[34] Frontal cortex[35,36]		10.00	NS	Top prioritization DE Increased Gene
CRHR1 Corticotropin-releasing hormone receptor 1	214619_at	(D) DE/1	Association[47–49]	(I) Pituitary[50]		7.00	NS	Top prioritization DE decreased gene
KSR1 Kinase suppressor of ras 1	213769_at	(I) AP/4		(I) Nucleus accumbens[20]	Our previous work in males (I) (213769_at) Blood[4]	8.00	NS	Top Prioritization AP increased gene
CLYBL Citrate lyase beta like	239683_at	(D) AP/4		(I) Brain[51]	Our previous work in males (D) (239683_at) Blood[4]	8.00	*0.008799*	Top prioritization AP decreased gene
Top biomarkers from validation—step 3								
C20orf27 Chromosome 20 open reading frame 27	218081_at	(D) DE/2		(I) Anterior Cingulate Cortex[20]	Our previous work in males (D) (218081_a) Blood[4]	6.00	1.09E – 34	Top validation DE decreased gene
SAT1 Spermidine/spermine N1-acetyltransferase 1	213988_s_at	(I) DE/2	Association[52,53]	(I) Prefrontal cortex (BA46)[54] (D) Prefrontal cortex[55] Prefrontal cortex (BA4, BA8/9, and BA11)[56] (BA44)[54] (BA9)[57]	Our previous work in males (I) (213988_s_at)Blood[4]	8.00	4.06E – 34	Top validation DE increased gene
SPTBN1 Spectrin, beta, non-erythrocytic 1	215918_s_at	(I) AP/1		(I) Nucleus accumbens[20]	Our previous work in females (I)(214856_at)Blood[5]	5.00	6.7E – 32	Top validation AP increased gene
POLR2D Polymerase (RNA) II (DNA directed) polypeptide D	214144_at	(D) AP/1		(I) Anterior cingulate cortex[20]	Our previous work in males (D)(214144_a)Blood[4] Our previous work in females (I) (214144_at)Blood[5]	5.00	2.1E – 08	Top validation AP decreased gene

Abbreviations: ANOVA, analysis of variance; AP, absent/present; DE, differential expression; NS, non-stepwise in validation. Italic *P*-values are nominally but not Bonferroni significant.



AUCs	All		Subtypes						Personalized (Gender/Dx)											
≥ 0.7	0	0	3	5	7	3	0	0	2	4	15	1	6	0	6	7	7	19	7	0	17	0	15	9
≥ 0.6	16	0	3	5	31	3	38	12	2	8	47	1	6	0	6	7	10	19	38	4	17	2	25	17
≥ 0.5	52	6	3	5	31	3	39	12	2	8	47	1	6	0	6	7	10	19	38	4	17	2	25	17

Figure 2. Step 4: Testing for Diagnostics. Best single universal biomarkers predictors. From Top Dozen List (bold) and from Bonferroni List (combined 154 biomarkers). Bar graph shows best predictive biomarkers in each group. *Nominally significant $P < 0.05$. **Survives correction for multiple comparisons testing of 154 biomarkers, $P < 0.000325$. Table underneath the figures displays the actual number of biomarkers for each group whose region of interest (ROC) area under the curve (AUC) *P*-values are at least nominally significant. Some female diagnostic groups are missing from the graph, as they did not have any nominally significant biomarkers, due at least in part to the small number of cases.

92%, $P = 7.42E-06$) and, in male bipolars, the highest risk group (AUC 96%, $P = 8.03E-08$). For predicting future hospitalizations, the results were stronger in the high anxiety subtype (AUC 79%, $P = 7.52E-03$) and in male depression (AUC 95%, $P = 4.88E-04$).

Step 5: Biological vulnerability understanding

Evidence for involvement in other disorders. We conducted Convergent Functional Genomics analyses using the Top Dozen and Bonferroni biomarkers (Supplementary Table S5), which suggest that a majority (89%) of suicide biomarkers are involved in other psychiatric disorders, providing a basis for co-morbidity and increased vulnerability. A number of biomarkers (18%) were also involved in aging and longevity, possible substrates for our 'life switch' hypothesis.

Biological pathways. We conducted biological pathway analyses using the Bonferroni validated biomarkers, which suggest that neurotrophic factors, programmed cell death and insulin signaling are involved in the biology of suicide (Table 4).

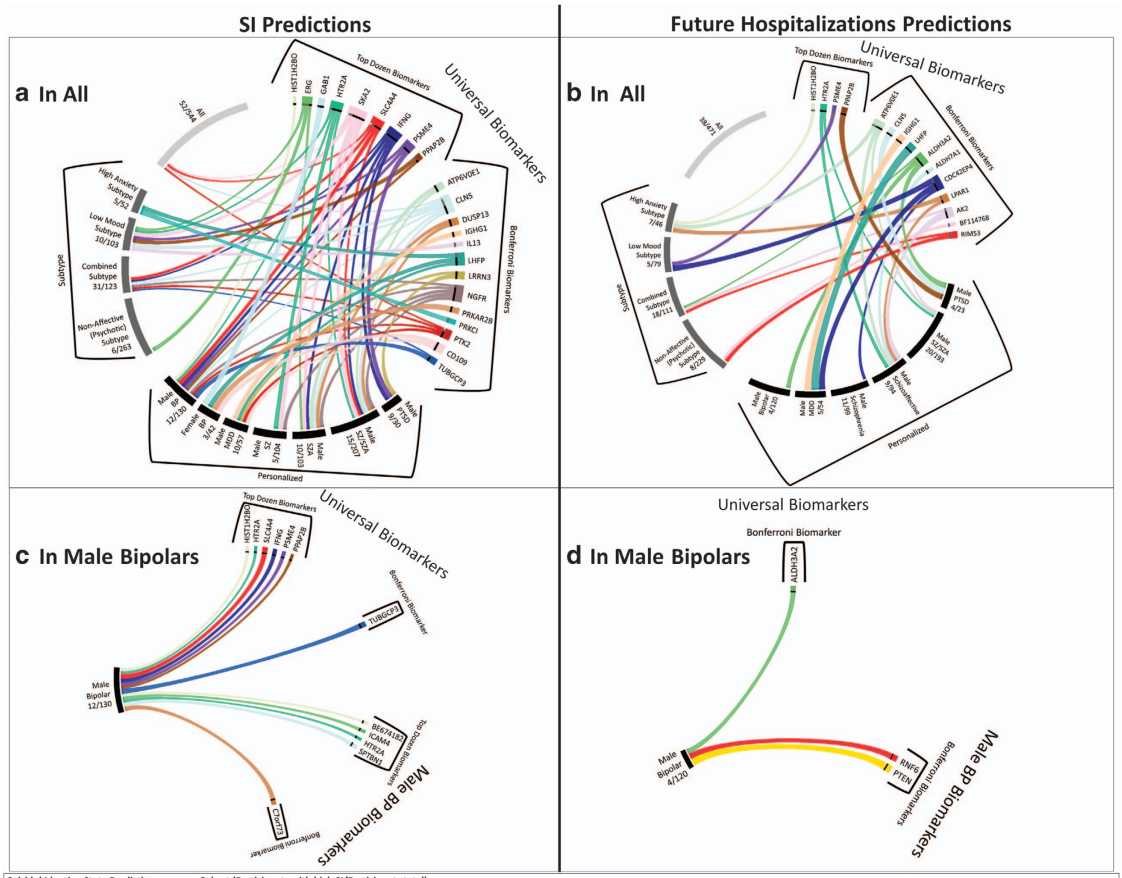
Networks and Interactions. We conducted STRING analyses that revealed groups of directly interacting genes, in particular HTR2 A/ARRB1/GSK3B, and SLC4A4/AHCYL1/AHCYL2 (Supplementary Figure S3). These networks may have biological significance and could be targeted therapeutically.

Circadian. A number of top biomarkers identified by us have biological roles that are related to the circadian clock (Supplementary Table S5). To be able to ascertain all the genes in our dataset 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 154 top biomarker genes, 18 had circadian evidence (11.7%) (Supplementary Table S5), suggesting a 1.7-fold enrichment for circadian genes. Circadian clock abnormalities are related to mood disorders[12,13] and sleep abnormalities have been implicated in suicide.[14]

Enrichment in suicide completers. Of the candidate biomarkers from the Prioritization step, 125/430 of the DE ones (29.1%) and 37/180 of the AP ones (20.6%) were Bonferroni validated in suicide completers. There is a 1.4-fold enrichment in DE vs AP, which suggests that completion of suicide may be due more to an incremental change in expression of genes rather than the complete turning on and off of genes.

Step 6: Therapeutics

Pharmacogenomics. A number of individual top biomarkers are known to be modulated by medications in current clinical use for treating suicidality, such as lithium (HTR2A, GSK3B, ITGB1BP1 and BCL2), clozapine (IL6, CD164, CD47, HTR2A, PGK1, DYRK2, IFNG and LPAR1) and omega-3 fatty acids (APOE, CD47, ACP1, GATM, LHFP and LPAR1) (Figure 7, Table 5 and Supplementary Table S5). In particular, HTR2A and CRYAB are at the overlap of lithium and clozapine, and MBP is at the overlap of all three treatments (Figure 7). Omega-3 fatty acids may be a widely deployable preventive treatment, with minimal side effects, including in women who are or may become pregnant. Of note, CD109, a Bonferroni list biomarker increased in expression in suicidality in our studies (Supplementary Table S5), has also reported to be increased in expression by treatment of lymphoblastoid cells with the SSRI paroxetine, and thus merits follow-up as a potentially useful biomarker for treatment-emergent SI.



Suicidal Ideation State Predictions		Cohort (Participants with high SI/Participants total)										
Universal Biomarkers	**All (52/544)**	**High Anxiety (5/52)**	**Low Mood (10/103)**	**Combined Subtype (31/123)**	**Non-Affective (6/263)**	**Male BP (12/130)**	**Female BP (3/42)**	**Male MDD (10/57)**	**Male SZ (5/104)**	**Male SZA (10/103)**	**Male SZSZA (15/207)**	**Male PTSD (9/30)**
Top Dozen List ERG			0.68/2.77E-02		0.75/1.93E-02					0.66/4.96E-02		
Top Dozen List GAB1							0.88/1.49E-02					
Top Dozen List HIST1H2BO						0.67/2.78E-02						
Top Dozen List HTR2A			0.66/4.74E-02			0.65/4.45E-02			0.79/1.58E-02		0.65/2.52E-02	
Top Dozen List IFNG				0.61/3.03E-02		0.71/9.22E-03				0.68/3.14E-02	0.66/1.75E-02	0.72/2.72E-02
Top Dozen List PPAP2B			0.75/4.15E-03			0.65/4.56E-02						
Top Dozen List PSME4			0.68/3.47E-02			0.69/1.41E-02						0.79/6.82E-03
Top Dozen List SKA2	0.61/3.35E-03		0.74/5.91E-03						0.79/1.35E-02	0.73/8.07E-03	0.75/5.97E-04	
Top Dozen List SLC4A4	0.64/3.83E-04			0.69/6.13E-04		0.77/9.27E-04					0.66/1.85E-02	
Bonferroni List ATP6V0E1										0.76/3.76E-03	0.68/9.28E-03	
Bonferroni List CD109							0.81/3.73E-02	0.73/1.11E-02				
Bonferroni List CLN5	0.65/1.86E-04		0.75/4.43E-03	0.68/1.27E-03		0.65/3.91E-02				0.68/3.47E-02	0.68/9.51E-03	
Bonferroni List DUSP13										0.72/9.96E-03	0.71/3.67E-03	
Bonferroni List IGHG1								0.79/2.47E-03				
Bonferroni List IL13			0.76/3.51E-03									
Bonferroni List LHFP		0.78/1.95E-02					0.79/4.60E-02	0.69/3.32E-02				
Bonferroni List LRRN3								0.68/3.56E-02				0.77/1.11E-02
Bonferroni List NGFR				0.66/4.27E-03		0.66/3.55E-02			0.72/4.96E-02	0.72/9.96E-03	0.72/1.92E-03	
Bonferroni List PRKAR2B							0.84/2.69E-02					
Bonferroni List PRKCI		0.8/1.55E-02										
Bonferroni List PTK2	0.61/4.53E-03			0.64/1.04E-02		0.66/3.64E-02		0.69/3.24E-02				
Bonferroni List TUBGCP3				0.61/3.28E-02		0.78/7.44E-04						
Male BP Biomarkers												
Bonferroni List C7orf73						0.75/2.38E-03						
Top Dozen List SPTBN1						0.72/6.62E-03						
Top Dozen List ICAM4						0.67/2.83E-02						
Top Dozen List BE674182						0.66/3.33E-02						
Top Dozen List HTR2A						0.65/4.45E-02						

Future Hospitalizations Predictions		Cohort (Participants with future hospitalizations for suicidality within the first year/Participants total)										
Universal Biomarkers	**All (38/471)**	**High Anxiety (7/46)**	**Low Mood (5/79)**	**Combined Subtype (18/111)**	**Non-Affective (8/229)**	**Male (BP 4/120)**	**Female BP (na)**	**Male MDD (5/54)**	**Male SZ (11/99)**	**Male SZA (9/94)**	**Male SZSZA (20/193)**	**Male PTSD (4/23)**
Top Dozen List HIST1H2BO		0.71/4.20E-02										
Top Dozen List HTR2A										0.72/1.47E-02	0.65/1.67E-02	
Top Dozen List PPAP2B												0.83/2.13E-02
Top Dozen List PSME4			0.72/4.73E-02									
Bonferroni List AK2	0.60/2.36E-02			0.68/6.72E-03						0.78/2.70E-03	0.64/2.34E-02	
Bonferroni List ALDH3A2				0.63/4.65E-02		0.77/3.38E-02						0.83/2.13E-02
Bonferroni List ALDH7A1										0.72/1.47E-02		
Bonferroni List ATP6V0E1		0.76/1.55E-02								0.73/1.21E-02	0.67/6.53E-03	
Bonferroni List BF114768					0.69/3.36E-02							
Bonferroni List CDC42EP4			0.84/5.28E-03					0.84/5.84E-03	0.67/3.15E-02			
Bonferroni List CLN5												0.87/1.16E-02
Bonferroni List IGHG1								0.9/1.64E-03				
Bonferroni List LHFP								0.98/2.54E-04				
Bonferroni List LPAR1		0.77/1.33E-02								0.7/2.22E-02		
Bonferroni List RIMS3				0.66/1.88E-02	0.73/1.37E-02							
Male BP Biomarkers												
Bonferroni List PTEN						0.90/3.27E-03						
Bonferroni List RNF6						0.81/1.58E-02						

Figure 3. Step 4: Testing for Diagnostics. Universal and male bipolar biomarkers. Best individual biomarkers from Top Dozen List and from Bonferroni List, for universal and for male bipolar. We only show biomarker results where region of interest (ROC) area under the curve (AUC) *P*-values are at least nominally significant and AUCs are at least 0.6 (60%). (**a**) Circos plot depicting the best individual biomarker predictions for suicidal ideation (SI) state in the independent cohort (across all participants, in subtypes and personalized by gender and diagnosis), using universal biomarkers. (**b**) Circos plot depicting the best individual biomarker predictions for future hospitalizations for suicidality in the first year following testing in the independent cohort (across all participants, in subtypes and personalized by gender and diagnosis), using universal biomarkers. (**c**) Circos plot depicting the best individual biomarker predictions for SI state in the independent male bipolar sub-cohort, using universal biomarkers and male bipolar biomarkers. (**d**) Circos plot depicting the best individual biomarker predictions for future hospitalizations for suicidality in the first year following testing in the independent male bipolar sub-cohort, using universal biomarkers and male bipolar biomarkers. The circumference bands represent and are proportional to the number of participants in each cohort. The ribbons represent and are proportional to the AUC of the predictions. Table underneath the figures displays the actual numerical results.

Table 3. Step 4: Diagnostics

A. SI state

Predictors	*Cohort*	*Participants visits with high SI/participants visits total*	*ROC AUC/P-value*	*Suicidality severity (HAMD SI score) correlation **R**/P-value*	*T-test P-value*
Universal					
Best Biomarkers					
SLC4A4	All	52/544	0.64/3.83E – 04	0.13/1.54E – 03	1.50E – 03
CLN5	All	52/544	**0.65/1.86E – 04**	– 0.11/6.13E – 03	3.90E – 04
BioM 148 Panel (Bonferroni List)	All	52/544	0.61/6.18E – 03	0.069/5.33E – 02	1.77E – 02
BioM 12 Panel (Top Dozen List)	All	52/544	0.61/3.66E – 03	0.12/3.02E – 03	3.08E – 03
BioM 2 Panel (SLC4A4 and CLN5)	All	52/544	**0.66/4.92E – 05**	0.14/7.82E – 04	1.90E – 04
Phenes					
Mood	All	52/544	**0.77/5.93E – 11**	– 0.38/3.17E – 20	1.95E – 10
Anxiety	All	52/544	**0.77/3.43E – 11**	0.31/8.60E – 14	2.03E–12
Mood and Anxiety (SASS)	All	52/544	**0.81/5.55E – 14**	0.40/3.66E – 22	3.57E – 14
CFI-S	All	52/523	**0.86/9.98E – 18**	0.43/1.03E – 24	5.46E – 16
Mood and Anxiety and CFI-S	All	52/523	**0.89/2.59E – 20**	0.49/1.60E – 33	1.08E – 18
Phenes and Biomarkers					
Mood and Anxiety and CFI-S and BioM 148	All	52/523	**0.89/1.36E – 20**	0.49/2.84E – 33	2.88E – 18
Mood and Anxiety, and CFI-S and BioM 12 (UP-Suicide)	*All*	*52/523*	***0.90/3.87E – 21***	*0.50/5.91-35*	*3.42E – 19*
Mood and Anxiety, and CFI-S and BioM2	All	52/523	**0.89/4.56E – 21**	0.50/4.07E – 34	2.83E – 18
Male Bipolar					
Best Biomarkers					
SPTBN1	M-BP	12/130	0.72/6.62E – 03	0.21/8.54E – 03	9.05E – 03
C7orf73	M-BP	12/130	0.75/2.38E – 03	– 0.17/2.76E – 02	1.08E – 04
BioM 54 Panel (Bonferroni List)	M-BP	12/130	0.49/5.29E – 01	0/4.90E – 01	7.12E – 01
BioM 12 Panel (Top Dozen List)	M-BP	12/130	0.57/2.08E – 01	0.08/1.78E – 01	8.79E – 02
BioM 2(SPTBN1 and C7orf73)	M-BP	12/130	0.80/3.54E – 04	0.23/4.77E – 03	6.62E – 05
Phenes					
Mood	M-BP	12/130	0.8/3.65E – 04	– 0.47/6.83E – 09	1.65E – 03
Anxiety	M-BP	12/130	0.86/2.19E – 05	0.41/7.09E – 07	1.91E – 05
Mood and Anxiety (SASS)	M-BP	12/130	0.86/1.66E – 05	0.5/7.15E – 10	5.66E – 05
CFI-S	M-BP	12/128	0.92/1.10E – 06	0.5/6.11E – 10	1.31E – 06
Mood and Anxiety, and CFI-S	M-BP	12/128	0.94/2.82E – 07	0.61/1.24E – 14	3.01E – 06
Phenes and Biomarkers					
Mood and Anxiety, and CFI-S and BioM 54	M-BP	12/128	0.93/5.30E – 07	0.61/1.78E – 14	5.54E – 06
Mood and Anxiety, and CFI-S and BioM 12	*M-BP*	*12/128*	*0.95/1.62E – 07*	*0.62/1.92E – 15*	*8.31E – 07*
Mood and Anxiety, and CFI-S and BioM 2	M-BP	12/128	0.97/5.14E – 08	0.64/2.29E – 16	2.59E – 07

B. Future hospitalizations for suicidality in the first year following assessment

Predictors	*Cohort*	*Participants visits with future hospitalizations for suicidality within the first year/particpants visits total*	*ROC AUC/P-value*	*Frequency of future hospitalizations for suicidality within the first year correlation R/P-value*	*T-test P-value*	*Cox regression hazard ratio /P-value*
Universal						
Best Biomarkers						
PSME4	All	38/471	0.59/2.62E – 02	0.08/4.12E – 02	6.20E – 02	1.23/ 1.56E – 01
AK2	All	38/471	0.60/2.31E – 02	– 0.06/9.70E – 02	9.39E – 03	1.35/ 7.22E – 02
BioM 148 Panel (Bonferroni List)	All	38/471	0.52/3.37E – 01	– 0.02/6.67E – 01	4.18E – 01	1.09/8.27E – 01
BioM 12 Panel (Top Dozen List)	All	38/471	0.58/4.20E – 02	0.05/1.47E – 01	5.02E – 02	1.88/1.41E – 01
BioM 2 Panel (PSME4 and AK2)	All	38/471	0.65/1.10E – 03	0.10/1.29E – 02	1.35E – 03	1.68/0.018

Table 3. (Continued)

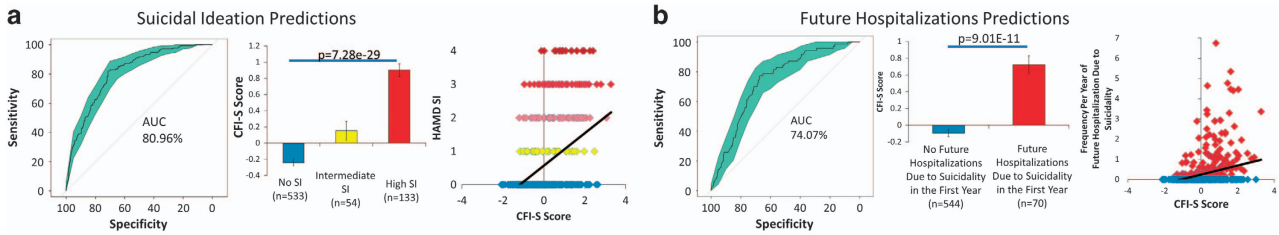
B. Future hospitalizations for suicidality in the first year following assessment

Predictors	*Cohort*	*Participants visits with future hospitalizations for suicidality within the first year/particpants visits total*	*ROC AUC/P-value*	*Frequency of future hospitalizations for suicidality within the first year correlation* R/P-*value*	T-*test* P-*value*	*Cox regression hazard ratio /*P-*value*
Phenes						
Mood	All	38/471	0.65/1.00E – 03	– 0.16/3.63E – 04	1.03E – 03	1.69/1.47E – 03
Anxiety	All	38/471	**0.69/3.70E – 05**	0.16/3.43E – 04	2.30E – 04	1.82/2.62E – 04
Mood and Anxiety (SASS)	All	38/471	**0.71/9.78E – 06**	0.18/4.89E – 05	7.73E – 05	1.45/ 8.11E – 05
CFI-S	All	38/470	**0.75/1.79E – 07**	0.2/5.11E – 06	1.40E – 06	2.02/7.11E – 07
Mood and Anxiety and CFI-S	All	38/470	**0.76/6.34E – 08**	0.22/4.18E – 07	2.22E – 06	1.40/1.13E – 07
HAMD SI	All	35/458*	**0.81/5.27E – 10**	0.40/1.57E – 19	2.64E – 06	2.10/1.11E – 15
Mood and Anxiety, and CFI-S and HAMD SI	All	35/458*	**0.82/9.96E – 11**	0.35/4.11E – 15	4.34E – 08	1.36/1.83E – 13
Phenes and Biomarkers						
Mood and, Anxiety and CFI-S and BioM 148	All	38/470	**0.76/6.65E – 08**	0.21/1.29E – 06	2.29E – 06	1.37/2.01E – 07
Mood and Anxiety, and CFI-S and BioM 12 (UP-Suicide)	*All*	*38/470*	***0.77/2.87E – 08***	*0.23/2.81E – 07*	*9.11E – 07*	*1.40/5.31E – 08*
Mood and Anxiety, and CFI-S and BioM 2	All	38/470	**0.76/3.87E – 08**	0.24/1.17E – 07	1.02E – 06	1.39/3.98E – 08
Mood and Anxiety, and CFI-S and HAMD SI, and BioM 2	All	35/458*	**0.82/9.38E – 11**	0.35/3.20E – 15	3.39E – 08	1.35/1.83E – 13
Male bipolars						
Best biomarkers						
PTEN	M-BP	4/120	0.9/3.27E – 03	0.22/6.76E – 03	3.12E – 02	1.73/2.73E – 02
RNF6	M-BP	4/120	0.82/1.58E – 02	– 0.14/5.89E – 02	9.14E – 03	6.24/7.19E – 02
BioM 54 Panel (Bonferroni List)	M-BP	4/120	0.75/4.23E – 02	0.11/1.23E – 01	4.71E – 02	4.58/2.52E – 01
BioM 12 Panel (Top Dozen List)	M-BP	4/120	0.56/3.41E – 01	0.05/2.85E – 01	3.08E – 01	2.57/5.73E – 01
BioM 2 (PTEN and RNF6)	M-BP	4/120	0.94/1.50E – 03	0.23/5.17E – 03	3.06E – 03	2.68/1.19E – 02
Phenes						
Mood	M-BP	4/120	0.69/1.04E – 01	– 0.14/6.08E – 02	1.75E – 01	2.10/1.32E – 01
Anxiety	M-BP	4/120	0.70/9.29E – 02	0.12/9.74E – 02	1.12E – 01	1.87/2.09E – 02
Mood and Anxiety (SASS)	M-BP	4/120	0.72/7.19E – 02	0.15/5.27E – 02	1.34E – 01	1.52/1.18E – 01
CFI-S	MBP	4/120	0.80/2.10E – 02	0.15/5.22E – 02	3.46E – 03	1.95/1.21E – 01
Mood and Anxiety, and CFI-S	M-BP	4/120	0.78/2.77E – 02	0.18/2.36E – 02	6.78E – 02	1.41/5.54E – 02
Phenes and biomarkers						
Mood and Anxiety, and CFI-S and BioM 54	M-BP	4/120	0.81/1.64E – 02	0.2/1.61E – 02	5.13E – 02	1.45/4.04E – 02
Mood and Anxiety, and CFI-S and BioM 12	*M-BP*	*4/120*	*0.79/2.59E – 02*	*0.19/1.88E – 02*	*7.92E – 02*	*1.44/4.72E – 02*
Mood and Anxiety, and CFI-S and BioM 2	M-BP	4/120	0.86/7.02E – 03	0.25/3.48E – 03	2.22E – 02	1.55/1.18E – 2

Biomarkers, phenes and combined predictions. Top increased and decreased individual predictive biomarkers. Underlined are individual biomarkers from the Top Dozen list, the others are from the Bonferroni list. For Universal, the panel of Top Dozen biomarkers is called BioM 12, and the panel of Bonferroni biomarkers is called BioM148, reflecting the number of markers in the panel. For Male Bipolar, the panel of Top Dozen biomarkers is called BioM 12, and the panel of Bonferroni biomarkers is called BioM54, reflecting the number of markers in the panel. Bold, Bonferroni significant in the diagnostic testing. Italic, *a priori* primary endpoint (UP-Suicide). Abbreviations: AUC, area under the curve; BP, bipolar; CFI-S, Convergent Functional Information for Suicidality; ROC, receiver operating characteristic; SASS, Simplified Affective State Scale; SI, suicidal ideation. Bold *P*-value of AUC survives correction for multiple testing for predictions. ROC AUC is our *a priori* primary predictive tool. HAMD SI is the suicide rating question from the Hamilton Rating Scale for Depression.*Smaller cohort, as not everybody had HAMD SI information.

New drug discovery/repurposing. Bioinformatic analyses using the gene expression signature of panels of top biomarkers identified new potential therapeutics for suicidality, such as ebselen (a lithium mimetic with anti-inflammatory and antioxidant properties),[15,16] piracetam (a nootropic cyclic derivative of GABA),[17] chlorogenic acid (an antioxidant polyphenol from coffee)[18] and metformin (an antidiabetic and possible longevity promoting drug)[19] (Table 6).

Phenomenology. SASS can be used to identify possible subtypes of suicidality (low mood, high anxiety, combined and non-affective) that may have practical utility, as we have shown in this



Predictor	ROC AUC	AUC p-value	t-test	Correlation R	Correlation p-value
CFI-S	0.810	3.20E-29	p=7.28e-29	0.443	2.47E-36

Item	Description	Stepwise	ANOVA No SI vs Intermediate SI vs High SI
10	**Dissatisfaction with present life**	**Y**	**5.58E-16**
16	**Chronic stress: lack of positive relationships, social isolation**	**Y**	**2.14E-15**
18	**Lack of coping skills (cracks under pressure)**	**Y**	**2.29E-14**
13	**Past history of suicidal acts/gestures**	**Y**	**1.96E-12**
12	**Current substance abuse**	**Y**	**2.88E-06**
11	**Lack of hope for the future**	**Y**	**6.93E-06**
9	**History of excessive introversion, conscientiousness**	**Y**	**7.41E-06**
7	**Acute stress: losses, grief**	**Y**	**1.33E-05**
15	**Acute stress: rejection**	**Y**	**1.86E-05**
8	**Chronic stress: perceived uselessness, not feeling needed, burden to extended kin**	**Y**	**7.15E-05**
17	**History of excessive extroversion and impulsive behaviors (including rage, anger, physical fights, seeking revenge)**	**Y**	**8.37E-04**
6	**Acute/severe medical illness, pain**	**Y**	**1.07E-03**
3	Family history of suicide in blood relatives	Y	3.19E-03
4	Personally knowing somebody who committed suicide	Y	2.56E-02
5	History of abuse: physical, sexual, emotional, neglect	Y	1.78E-01
19	Lack of children	Y	6.27E-01
20	History of command hallucinations of self-directed violence	N	3.18E-08
14	Lack of religious beliefs	N	6.15E-04
2	With poor treatment compliance	N	1.57E-03
22	Gender: Male	N	1.55E-01
21	Age: Older >60 or Younger <25	N	2.84E-01
1	Psychiatric illness diagnosed and treated	All have dx	All have dx

Predictor	ROC AUC	AUC p-value	t-test	Correlation R	Correlation p-value
CFI-S	0.741	2.69E-11	9.01E-11	0.305	6.66E-16

Item	Description	Stepwise	T-Test (one tailed) No Future Hospitalizations Due to Suicidality in the First Year vs Hospitalizations Due to Suicidality in the First Year
13	**Past history of suicidal acts/gestures**	**Y**	**5.00E-14**
20	**History of command hallucinations of self-directed violence**	**Y**	**5.91E-05**
16	**Chronic stress: lack of positive relationships, social isolation**	**Y**	**1.42E-04**
15	**Acute stress: rejection**	**Y**	**4.09E-04**
6	Acute/severe medical illness, pain	Y	3.78E-03
9	History of excessive introversion, conscientiousness	Y	6.47E-03
10	Dissatisfaction with present life	Y	8.63E-03
11	Lack of hope for the future	Y	0.010596577
7	Acute stress: losses, grief	Y	0.011549309
18	Lack of coping skills (cracks under pressure)	Y	0.014967478
2	With poor treatment compliance	Y	0.025120503
3	Family history of suicide in blood relatives	Y	0.042731464
22	Gender: Male	Y	0.047852304
12	Current substance abuse	Y	0.069034807
5	History of abuse: physical, sexual, emotional, neglect	Y	0.104299343
4	Personally knowing somebody who committed suicide	Y	0.153942173
8	Chronic stress: perceived uselessness, not feeling needed, burden to extended kin	Y	0.156594082
21	Age: Older >60 or Younger <25	Y	0.168685211
14	Lack of religious beliefs	Y	0.236253954
17	History of excessive extroversion and impulsive behaviors (including rage, anger, physical fights, seeking revenge)	Y	0.288722724
19	Lack of children	N	0.489134681
1	Psychiatric illness diagnosed and treated	All have dx	All have dx

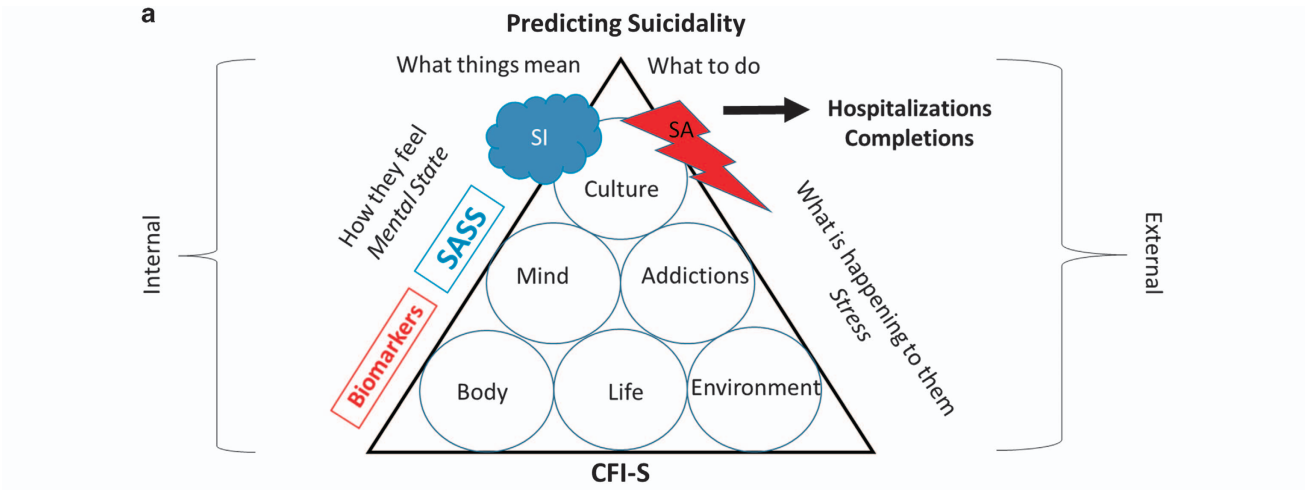
Figure 4. Convergent Functional Information for Suicide (CFI-S) testing. Testing in a large cohort that combines the discovery and test cohorts used for biomarker work. CFI-S was developed independently of any data from this study, by compiling known socio-demographic and clinical risk factors for suicide. 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 and cultural factors known to influence suicidal behavior, as well as two demographic factors, age and gender. (**a**) Prediction of high suicidal ideation (HAMD SI $>=2$). (**b**) Prediction of future hospitalizations due to suicidality within one year of follow-up. Table under **a** depicts individual items and their ability to differentiate between participants with No SI, Intermediate SI and High SI. Stepwise refers to gradual increase between the three groups (No SI, Intermediate SI, High SI). Table under **b** depicts individual items and their ability to differentiate between participants with and without future hospitalizations due to suicidality. Different items are positive in different individuals, providing leads for targeted (psycho)therapeutic interventions.

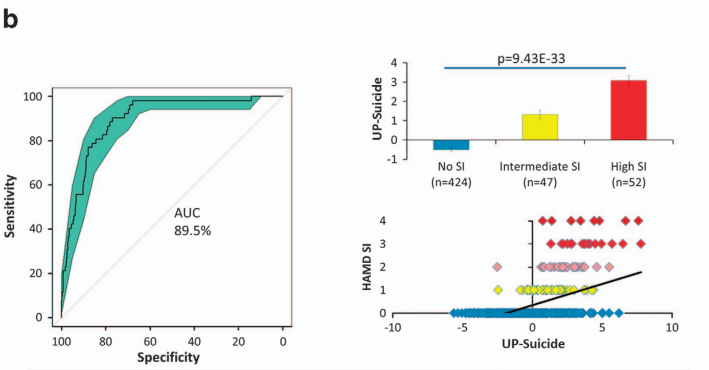
Figure 5. Predicting suicidality using a broad-spectrum predictor (UP-Suicide), which is an algorithm combining phenotypic information measures (Convergent Functional Information for Suicide (CFI-S) and Simplified Affective State Scale (SASS) (anxiety and mood)) and a panel of the Top Dozen universal biomarkers (BioM 12). (**a**) Model of various factors involved in suicidality (environmental stressors, life failures, body health issues, mind frailty, addiction problems and cultural examples). CFI-S, SASS and Biomarker panel are the components of our UP-Suicide. SA, suicide attempt; SI, suicidal ideation. (**b**) UP-Suicide predictions in the independent testing cohort, for High SI and for future hospitalizations for suicidality in the first year. (**c**) A dimensional view of risk stratification using phenotypic information measures and example of two high-risk participants. We calculated Euclidian distances from origin. Participant phchp158 is a divorced African American male in his late 20s with a long history of schizoaffective disorder, bipolar type and cannabis abuse. He was tested by us once (v1), while he was hospitalized for a suicide attempt by hanging. In the 5 years following testing, he has had two additional hospitalizations for suicidality: one for SI and one for attempt by overdose. He has had two hospitalizations for psychosis exacerbation without suicidality during this time span as well. Moved out of state, lost to follow-up since December 2015. Participant phchp328 was a divorced Caucasian female in her late 30s with a long history of depression, post-traumatic stress disorder (PTSD), borderline personality disorder and polysubstance abuse/dependence. She was first tested by us (v1) while inpatient for SI. Over the next year she subsequently had six psychiatric hospitalizations for suicidality: five due to SI and one due to a suicidal attempt by overdose. She also had one hospitalization for opioid withdrawal and depression during this time span as well. She committed suicide by overdose with pills, leaving behind a suicide note addressed to her mother. Her UP-Suicide score at Visit 1, composed of the panel of top dozen biomarkers (BioM12) scores and phenomic measures scores (CFI-S and SASS), was at the 100% of the scores of all the psychiatric participants visits in the current study. That testing was conducted during an inpatient hospitalization due to SI. Although 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 SI and one for opioid withdrawal/detox (the last one, which ended 2 weeks before the date of her committing suicide (T)).

work. The top CFI-S items distinguishing high SI from intermediate SI and no SI states were dissatisfaction with one's life, social isolation and lack of coping skills in the face of stress. The top CFI-S items distinguishing those that had future hospitalizations for suicidality versus those that did not were past history of suicidality, command auditory hallucinations and social isolation (Figure 4). This provides empirical evidence that, in general, reducing social isolation is a good behavioral therapeutic intervention for preventing suicidality. In different individuals different CFI-S items are positive, providing avenues for tailored and targeted (psycho)therapeutic interventions.

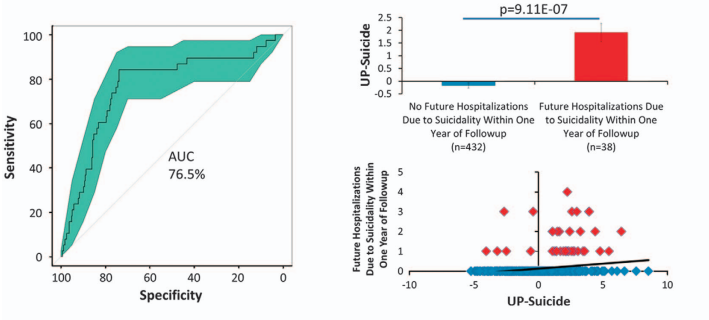
Final summation: overall evidence for establishment of clinical utility

For the universal biomarkers identified by us, combining all the available evidence from this current work and the published literature, into a convergent functional evidence score, brings to

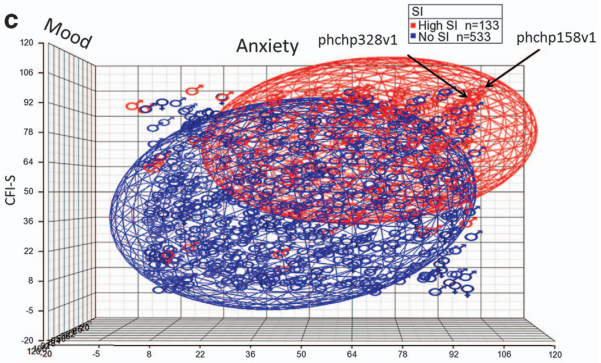




Predictor	Predicting	ROC AUC	AUC p-value	ANOVA	Correlation R	Correlation p-value
UP-Suicide	High SI	0.90	3.87E-21	9.43E-33	0.501463	5.91E-35

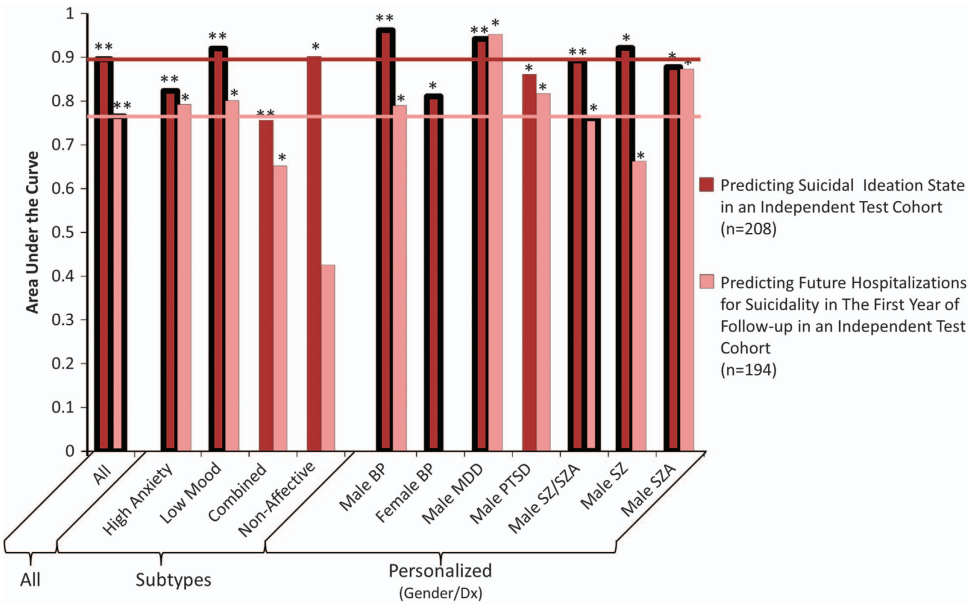


Predictor	Predicting	ROC AUC	AUC p-value	T-test	Correlation R	Correlation p-value
UP-Suicide	Future Hospitalizations for Suicidality	0.77	2.87E-08	9.11E-07	0.228367	2.81E-07



Dimensions	Test Result Variable(s)	ROC AUC	Std. Error	AUC p-value	Asymptotic 95% Confidence Interval	
					Lower Bound	Upper Bound
1D	CFIS	0.81	0.019	6.42E-29	0.772	0.847
2D	CFIS x Anxiety	0.833	0.019	4.16E-33	0.796	0.869
3D	CFIS x Anxiety x Mood	0.841	0.018	8.01E-35	0.807	0.876
4D	CFIS x Anxiety x Mood x BioM12	0.849	0.019	2.37E-36	0.812	0.886

	Participant 158			Participant 328				
Predictors	Visit 1 High SI HAMD-SI 4	+ 2 H		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
ARRB1	34.4			59.5		56.7		93.1		
LDLRAP1	21.4			99.8		59.3		29.8		
IFNG	91.4			88.5		76.6		18.2		
ERG	36.8			30.7		16.3		45.9		
CCL28	40.6			76.5		70.8		39.4		
SKA2	64.8			99.9		59.5		29.7		
SLC4A4	92.3			92.7		89.7		42.5		
PPAP2B	62.9			89.2		36.7		83.9		
HIST1H2BO	87.6			97.5		44.2		39		
PSME4	67.8			35.1		83.5		56.7		
GAB1	53.1			89.6		98.1		11.4		
HTR2A	8.6			62.7		43.6		57.4		
BioM12	70.3			99.4		90.3		46.1		
Mood	100			91.3		47.8		10.2		
CFIS	99			94.9		90.9		79.4		
Anxiety	99.1			99		94.1		73.1		
CFIS x Anxiety x Mood	100			99.4		87.7		56.6		
CFIS x Anxiety x Mood x BioM12	99.3			100		94.1		56.8		



	Participants Total visits/ High SI visits	High SI prediction ROC AUC ROC p-value	t-test (High SI vs. No SI)	Correlation R p-value	Participants Total visits/ First year hospitalized for suicidality/ All future hospitalized for suicidality	Predictions First year hospitalized for suicidality ROC AUC ROC p-value	T-test First year hospitalized for suicidality	Correlation R p-value First Year hospitalized for suicidality	Correlation R p-value All future hospitalized for suicidality
All	544/52	**0.8954 3.87E-21**	**3.42E-19**	**0.5015 5.91E-35**	470/38/98	**0.7654 2.87E-08**	**9.11E-07**	**0.2284 2.81E-07**	**0.2803 1.72E-10**
High Anxiety Subtype	50/5	0.8222 9.53E-03	1.22E-02	0.3457 6.97E-03	46/7/13	0.7912 7.52E-03	4.14E-03	0.2933 2.40E-02	0.1685 1.24E-01
Low Mood Subtype	99/10	**0.9191 7.42E-06**	**3.61E-06**	**0.4268 5.28E-06**	78/5/13	0.8 1.27E-02	1.15E-02	0.2756 7.29E-03	0.1808 4.99E-02
Combined Subtype	119/31	**0.7548 1.29E-05**	1.89E-05	**0.4672 4.25E-08**	111/18/38	0.6511 2.15E-02	1.87E-02	0.1712 3.62E-02	0.2077 1.33E-02
Non-Affective Subtype	252/6	0.9004 4.04E-04	3.94E-03	**0.3319 3.39E-08**	229/8/34	0.4242 7.67E-01	6.72E-01	-0.0139 5.83E-01	0.0322 3.12E-01
Male Bipolar	128/12	**0.9605 8.03E-08**	**4.79E-07**	**0.6322 6.05E-16**	120/4/9	0.7888 2.51E-02	6.30E-02	0.1927 1.75E-02	0.2765 1.07E-03
Female Bipolar	31/3	0.8095 4.12E-02	5.03E-02	0.4005 1.28E-02	4/0/0	NA NA	NA	NA NA	NA NA
Male Depression	57/10	**0.9404 7.02E-06**	4.35E-05	**0.6067 2.83E-07**	54/5/6	0.951 4.88E-04	**1.83E-07**	0.363 3.49E-03	0.3059 1.16E-02
Male PTSD	28/9	0.8596 1.24E-03	1.29E-03	0.6643 5.78E-05	23/4/14	0.8158 2.58E-02	2.72E-03	0.3493 5.12E-02	0.5951 5.30E-04
Male Schizophrenia/ Schizoaffective	206/15	**0.8918 2.22E-07**	**5.80E-08**	**0.4356 3.01E-11**	193/20/52	0.7598 7.20E-05	9.50E-04	**0.315 4.05E-06**	**0.3345 7.79E-07**
Male Schizophrenia	103/5	0.9204 7.86E-04	5.21E-04	0.389 2.44E-05	99/11/21	0.6612 4.12E-02	1.03E-01	0.2334 1.00E-02	0.3595 1.03E-04
Male Schizoaffective	103/10	0.8763 4.84E-05	3.01E-05	**0.4714 2.50E-07**	94/9/31	0.8719 1.28E-04	7.79E-05	0.3939 4.28E-05	0.3788 7.67E-05

Figure 6. UP-Suicide across all, by subtypes and personalized by gender/diagnosis. UP-Suicide composed of the panel of the Top Dozen universal biomarkers (BioM 12), Convergent Functional Information for Suicide (CFI-S) and Simplified Affective State Scale (SASS) (anxiety and mood). Plot depicts area under the curve (AUC) for the UP-Suicide predicting suicidal ideation (SI) and hospitalizations within the first year in all participants, as well as separately in subtypes, and by gender and diagnosis (Gender/Dx). Two asterisks indicate the comparison survived Bonferroni correction for all the multiple comparisons depicted. A single asterisk indicates nominal significance of $P < 0.05$. Bold outline indicates that the UP-Suicide was synergistic to its components, that is, performed better than the gene expression biomarkers or phenomic measures individually. Table contains descriptive statistics for all participants together, as well as separately by subtypes and by gender/dx. For female gender/dx groups, only the female bipolar subgroup had enough participants to yield at least a nominally significant AUC. Bold indicates the measure survived very stringent Bonferroni correction for all the multiple comparisons in our whole study (2737 biomarkers and phenes, resulting in a Bonferroni cutoff of 1.83E − 05). We also show Pearson's correlation data in the SI test cohort for HAMD-SI vs UP-Suicide, as well as Pearson's correlation data in the hospitalization test cohort for frequency of hospitalizations for suicidality in the first year and for frequency of hospitalizations for suicidality in all future available follow-up interval (which varies among participants, from 0.40 to 10.42 years).

the fore biomarkers that might have clinical utility, for future studies in the field (Figures 8, 9, and Supplementary Table S7).

Studies in male bipolars: personalization versus universal

As a comparator to the universal approach across gender and diagnoses, we also conducted within-participant longitudinal biomarker discovery analyses in male bipolars only, the largest subgroup ($n = 20$ participants, 65 testing visits) in our discovery cohort. Male bipolars are the highest risk group for suicide clinically and have been the focus of earlier suicide biomarker studies by us, with an N ($n = 9$) that was less than half of the current one. The discovery step was followed by prioritization and by validation in male suicide completers. We reproduced and

Table 4. Biological pathways

A. Universal biomarkers

Universal pathways	*DAVID GO functional annotation biological processes*					*KEGG pathways*				*Ingenuity pathways*		
	#	*Term*	*Count*	*%*	P-*value*	*Term*	*Count*	*%*	P-*value*	*Top Canonical Pathways*	P-*value*	*Overlap*
Validation Bonferroni significant ($n = 130$ genes, 148 probe sets)	1	Regulation of neurogenesis	8	6.6	2.10E – 04	Tryptophan metabolism	4	0.2	1.10E – 02	Protein kinase A signaling	4.36E – 06	0.031 12/386
	2	Negative regulation of apoptosis	11	9	2.60E – 04	Neurotrophin signaling pathway	6	0.3	1.40E – 02	IGF-1 signaling	2.86E – 05	0.062 35/582
	3	Negative regulation of programmed cell death	11	9	2.90E – 04	Insulin signaling pathway	6	0.3	1.90E – 02	Gap junction signaling	4.66E – 05	0.045 7/155
	4	Negative regulation of cell death	11	9	3.00E – 04	Butanoate metabolism	3	0.2	5.90E – 02	Renin-angiotensin signaling	5.52E – 05	0.055 6/109
	5	Regulation of cell morphogenesis	7	5.7	3.90E – 04	Endocytosis	6	0.3	6.10E – 02	Hepatic cholestasis	5.93E – 05	0.043 7/161

B. Male Bipolar biomarkers

Male bipolar pathways	*DAVID GO functional annotation biological processes*					*KEGG pathways*				*Ingenuity pathways*		
Validation Bonferroni significant (n = *50 genes, 54 probe sets)*	#	*Term*	*Count*	*%*	P-*value*	*Term*	*Count*	*%*	P-*value*	*Top Canonical Pathways*	P-*value*	*Overlap*
	1	Negative regulation of neuron differentiation	7	14.6	9.30E – 06	mTOR signaling pathway	3	6.2	1.60E – 02	G-protein coupled receptor signaling	1.14E – 14	0.113 29/256
	2	Negative regulation of neurogenesis	7	14.6	3.60E – 05	Small cell lung cancer	3	6.2	3.20E – 02	CREB signaling in neurons	1.98E – 14	0.14 24/171
	3	Negative regulation of nervous system development	7	14.6	5.50E – 05	Leukocyte transendothelial migration	3	6.2	5.80E – 02	Neuropathic pain signaling in dorsal horn neurons	4.82E – 13	0.18 18/100
	4	Positive regulation of protein localization to plasma membrane	4	8.3	1.10E – 04	Sphingolipid signaling pathway	3	6.2	6.00E – 02	14-3-3-mediated signaling	7.79E – 12	0.154 18/117
	5	Positive regulation of protein localization to cell periphery	4	8.3	1.10E – 04	NA	NA	NA	NA	Gap junction signaling	1.50E – 11	0.129 20/155

Abbreviations: GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; NA, not applicable.

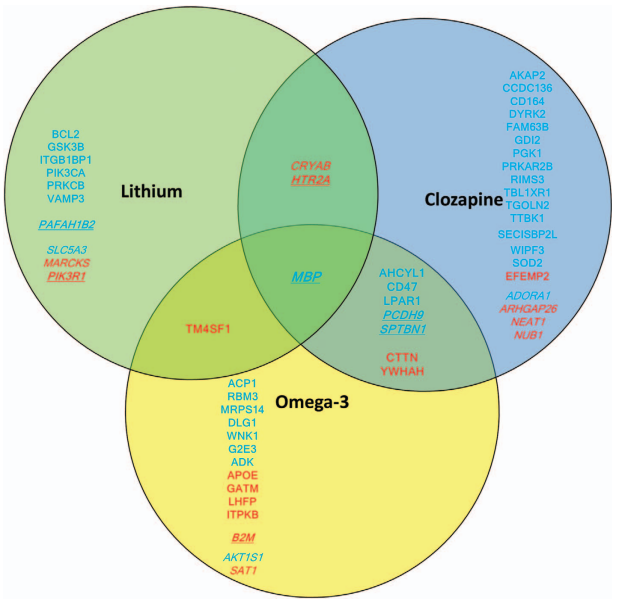


Figure 7. Step 6: Therapeutics. Pharmacogenomics. Individual biomarkers modulated by medications used in the treatment of suicidality. Universal biomarkers, bold; male bipolar, italic; both, bold and italic and underlined. Red, increased in expression in suicidality. Blue, decreased in expression in suicidality.

expanded some of our previous biomarker findings in bipolar disorder (Table 2B, Figure 3 and Supplementary Figure S4). We tested the male bipolar derived top dozen biomarkers (Table 2B) and all the biomarkers that survived Bonferroni correction after the validation step (Supplementary Table S8), for prediction of SI and for prediction of future psychiatric hospitalizations due to suicidality in the male bipolar subgroup ($n = 49$) in the independent test cohort (Figure 3 and Supplementary Table S10). We were successful in the identification of predictive biomarkers that might be more specific for suicidality in male bipolars. We also examined whether biomarkers discovered using just male bipolar subjects yields even better predictors for male bipolar subjects than using the universal biomarkers and found that to be the case for trait (hospitalizations) predictions (Figure 3). A number of top male bipolar biomarkers identified by us are targets of medications in current clinical use for treating suicidality (Supplementary Table S12). Bioinformatic drug repurposing analyses using the gene expression biosignature of panels of top biomarkers identified new potential therapeutics for suicidality in male bipolars (Table 6C and D). The top compounds identified include betulin (a natural plant compound with longevity and anticancer properties), naproxen (a non-steroidal anti-inflammatory), as well as chlorphenesin and baclofen (central nervous system acting muscle relaxants used to treat pain and spasms). Combining all the available evidence from this current work and the published literature, into a convergent functional evidence score (Figure 10 and Supplementary Table S13), leads to a prioritization of biomarkers for future studies in the field.

DISCUSSION

Was our quest for more universal predictors or our quest for more personalized predictors more informative? The answer seems to be both, for different and complementary reasons. The universal approach may illuminate a more specific core biology for suicide, the personalized approach by gender and diagnosis may provide a more sensitive and context dependent applicability. The new subtypes we identified seem to have some utility (Figure 2 and Supplementary Figure S2). They merit future exploration and are easy to assess in the general population using our SASS questionnaire/app.

The current work is more comprehensive in design and larger in size than our previous studies.[4,5,7] We used a systematic discovery, prioritization, validation, and testing approach.[2] For discovery, we used a hard to accomplish but powerful within-participant design, with an *N* of 66 participants with 193 visits. A within-participant design factors out genetic variability, as well as some medications, lifestyle and demographic effects on gene expression, permitting identification of relevant signal with *N* as small as 1.[6] 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. Just the male bipolar sub-component alone had twice as many participants in discovery (20 vs 9) and four times as many participants in validation (38 vs 9) than our original breakthrough study on suicide biomarkers in male bipolars published in 2013.[7] Out of the six Bonferroni-validated biomarkers in that study,[7] four were also Bonferroni validated in the current study (SAT1, BF114768/UBA6, MARCKS and PTEN), at the exact same probe sets. Moreover, LHFP, a biomarker that just missed the Bonferroni cutoff in the original male bipolar work,[7] is a universal Bonferroni validated biomarker in the current work, with predictive ability for SI state in the high anxiety subtype (AUC of 78%, *P*-value 1.95E − 02), female bipolars (AUC of 79%, *P*-value 4.6E − 02) and male depression (AUC 69%, *P*-value 3.32E − 02), as well as very strong predictive ability for future hospitalizations for suicidality in male depression (AUC 98%, *P*-value 2.54E − 04) (Figures 2 and 3). LHFP (lipoma HMGIC fusion partner), increased in expression in blood in our studies, is also previously reported to be increased in expression in brains of suicide completers.[20] Of note, SAT1, which is increased in expression in suicide in our studies, degrades spermidine, a compound recently implicated in longevity.[21] This is consistent with our overall 'life switch'[22] hypothesis.

Our Bayesian-like Convergent Functional Genomics platform used for prioritizing findings following the discovery step ensures robust built in reproducibility, as it is based on corroborating evidence by other groups, using different methodologies and cohorts. No single approach or study is perfect and datasets are especially powerful in combination.

The phenotypic measures apps scores by themselves ('digital biomarkers') were more precise predictors than the blood biomarkers by themselves, although their combination did show some synergy (Table 3). However, blood biomarkers may have usefulness for objective diagnostic testing and patient stratification, over and above clinical classifications. This may be particularly important when individuals choose not to share how they feel and do not seek help. We observed such possible discrepancies for different subtypes and gender/diagnostic groups (Supplementary Figure S2).

In terms of how our biomarker discoveries might be applied in clinical laboratory settings, we suggest that one might combine the best universal biomarkers with the best personalized biomarkers, to have the best of both worlds. In practice, every new patient tested would be normalized against the database of similar patients already tested and compared with them for ranking and risk prediction purposes, regardless of whether a platform such as Affymetrix or a more targeted one is used in the end clinically. As databases get larger, normative population levels can and should be established, similar to any other laboratory measures. Moreover, longitudinal monitoring of changes in biomarkers within an individual, measuring most recent slope of change, maximum levels attained and maximum slope of change attained, may be even more informative than simple cross-sectional comparisons of levels within an individual with normative populational levels and is the focus of future studies by our group.

Biomarkers may also be useful for patient stratification and measuring response to treatment (pharmacogenomics) (Figure 7,

Table 5. Step 6: Therapeutics

	Discovery (change) method/score	*Prioritization total CFG score for suicide*	*Validation ANOVA P-value*	*Omega-3*	*Lithium*	*Clozapine*	*Antidepressants*	*Other mood stabilizers*	*Other other antipsychotics*
Universal Top Dozen biomarkers									
HTR2A 5-Hydroxytryptamine (serotonin) receptor 2A, G protein-coupled	(I) DE/2	10	NS		(D) NT2.D1 cells Lithium[58]	(D) PFC Clozapine[59,60]	Blocked by: Mirtazapine, Amitriptyline	(D) NT2.D1 cells Valproate[58]	(D) PFC Haloperidol[59] Blocked by: Paliperidone, Risperidone, Iloperidone, Asenapine, Cariprazine, Lurasidone, Quetiapine, Olanzapine
IFNG Interferon, gamma	(D) AP/1	9	NS						(I) Saliva, Blood Olanzapine, Risperidone, Quetiapine, Aripiprazole[61]
SLC4A4 Solute carrier family 4 (sodium bicarbonate cotransporter), member 4	(I) AP/1	7	7.74E – 05					(D) AMY, CP Valproate[62]	
CCL28 Chemokine (C–C motif) ligand 28	(D) AP/4	4	NS				(I) Lymphoblastoid cell line Paroxetine[63]		
Male bipolar top dozen biomarkers									
HTR2A 5-Hydroxytryptamine (serotonin) receptor 2A, G protein-coupled	(I) DE/2	10	NS		(D) NT2.D1 cells Lithium[58]	(D) PFC Clozapine[59,60]	Blocked by: Mirtazapine, Amitriptyline	(D) NT2.D1 cells Valproate[58]	(D) PFC Haloperidol[59] Blocked by: Paliperidone, Risperidone, Iloperidone, Asenapine, Cariprazine, Lurasidone, Quetiapine, Olanzapine
SAT1 Spermidine/spermine N1-acetyltransferase 1	(I) DE/2	8	4.06E – 34	(D) blood Omega 3[64]					
SPTBN1 Spectrin, beta, non-erythrocytic 1	(I) AP/1	5	6.7E – 32	(D) blood Omega 3[64]					

Pharmacogenomics. Top Dozen lists biomarkers in our datasets that are targets of existing drugs and are modulated by them in opposite direction. Abbreviations: ANOVA, analysis of variance; CFG, Convergent Functional Genomics; NS, non-stepwise in validation; PFC, prefrontal cortex.

Table 6. Step 6: Therapeutics. New drug discovery/repurposing

A. CMAP analysis with the universal Top Dozen biomarkers signature

Rank	*Cmap name*	*Dose*	*Cell*	*Score*
6086	Torasemide	11 μM	MCF7	−0.9
6087	Tenoxicam	12 μM	MCF7	−0.9
6088	Halcinonide	9 μM	PC3	−0.902
6089	Tretinoin	1 μM	MCF7	−0.913
6090	NU-1025	100 μM	MCF7	−0.915
6091	Abamectin	5 μM	MCF7	−0.919
6092	Prednicarbate	8 μM	MCF7	−0.921
6093	Tolmetin	13 μM	MCF7	−0.924
6094	Alprostadil	10 μM	PC3	−0.925
6095	Amoxapine	13 μM	MCF7	−0.927
6096	Fenbufen	16 μM	MCF7	−0.929
6097	Sertaconazole	8 μM	PC3	−0.93
6098	Oxybuprocaine	12 μM	MCF7	−0.961
6099	**Piracetam**	28 μM	MCF7	−0.973
6100	**Ebselen**	15 μM	PC3	−1

B. CMAP analysis with the universal Bonferroni biomarkers signature

Rank	*Cmap name*	*Dose*	*Cell*	*Score*
6068	Fluoxetine	12 μM	HL60	−0.812
6069	Betulin	9 μM	HL60	−0.812
6070	Netilmicin	3 μM	HL60	−0.82
6071	Dl-alpha tocopherol	9 μM	HL60	−0.821
6072	Haloperidol	10 μM	HL60	−0.823
6073	Streptozocin	15 μM	HL60	−0.824
6074	Hesperidin	7 μM	HL60	−0.824
6075	Calcium folinate	8 μM	MCF7	−0.825
6076	Harpagoside	8 μM	MCF7	−0.826
6077	Trimipramine	10 μM	HL60	−0.836
6078	Dapsone	16 μM	HL60	−0.844
6079	Fulvestrant	10 nM	HL60	−0.845
6080	Rilmenidine	8 μM	HL60	−0.845
6081	Atractyloside	5 μM	HL60	−0.849
6082	Tenoxicam	12 μM	HL60	−0.851
6083	Chlorpromazine	11 μM	HL60	−0.852
6084	Harman	18 μM	HL60	−0.858
6085	Homatropine	11 μM	HL60	−0.863
6086	Ramifenazone	14 μM	HL60	−0.864
6087	Clozapine	10 μM	HL60	−0.866
6088	Diphenhydramine	14 μM	HL60	−0.873
6089	Metrizamide	5 μM	HL60	−0.874
6090	Prochlorperazine	7 μM	HL60	−0.874
6091	Pirenperone	10 μM	HL60	−0.876
6092	Hydrastinine	16 μM	HL60	−0.88
6093	Carbimazole	21 μM	HL60	−0.884
6094	Asiaticoside	4 μM	HL60	−0.886
6095	Procainamide	15 μM	HL60	−0.894
096	Ozagrel	15 μM	HL60	−0.903
6097	Adiphenine	11 μM	HL60	−0.923
6098	Merbromin	5 μM	HL60	−0.924
6099	**Metformin**	24 μM	HL60	−0.983
6100	**Chlorogenic acid**	11 μM	HL60	−1

C. CMAP analysis with the male bipolar Top Dozen biomarkers signature

Rank	*Cmap name*	*Dose*	*Cell*	*Score*
6086	Harmaline	14 μM	MCF7	−0.822
6087	Foliosidine	13 μM	MCF7	−0.834
6088	3-Nitropropionic acid	10 μM	MCF7	−0.844
6089	Biperiden	11 μM	PC3	−0.844
6090	Carisoprodol	15 μM	HL60	−0.848
6091	Nalidixic acid	15 μM	MCF7	−0.861

Table 6. (Continued)

C. CMAP analysis with the male bipolar Top Dozen biomarkers signature

Rank	*Cmap name*	*Dose*	*Cell*	*Score*
6092	Eucatropine	12 μM	PC3	−0.869
6093	Solasodine	10 μM	PC3	−0.873
6094	Dicoumarol	12 μM	PC3	−0.875
6095	Pivampicillin	9 μM	MCF7	−0.879
6096	Gabexate	10 μM	PC3	−0.885
6097	Dacarbazine	22 μM	PC3	−0.892
6098	Prestwick-692	7 μM	MCF7	−0.927
6099	**Carteolol**	12 μM	HL60	−0.946
6100	**Betulin**	9 μM	HL60	−1

D. CMAP analysis with the male bipolar Bonferroni biomarkers signature

Rank	*Cmap name*	*Dose*	*Cell*	*Score*
6068	Valproic acid	50 μM	MCF7	−0.799
6069	Ondansetron	12 μM	PC3	−0.802
6071	NU-1025	100 μM	MCF7	−0.807
6073	Glycopyrronium bromide	10 μM	PC3	−0.814
6074	Triflupromazine	10 μM	HL60	−0.817
6075	Suxibuzone	9 μM	MCF7	−0.819
6076	Mepyramine	10 μM	MCF7	−0.822
6079	Hydrocortisone	11 μM	MCF7	−0.837
6080	Benfotiamine	9 μM	PC3	−0.839
6082	Deferoxamine	6 μM	PC3	−0.841
6084	Homatropine	11 μM	MCF7	−0.845
6085	Nifedipine	12 μM	PC3	−0.849
6086	Alpha-ergocryptine	7 μM	MCF7	−0.862
6088	Meclozine	9 μM	MCF7	−0.875
6089	Acacetin	14 μM	PC3	−0.882
6095	Fenoprofen	7 μM	PC3	−0.933
6096	CP-690334-01	1 μM	MCF7	−0.936
6097	Baclofen	19 μM	PC3	−0.94
6098	Chlorambucil	13 μM	MCF7	−0.945
6099	**Naproxen**	16 μM	PC3	−0.96
6100	**Chlorphenesin**	16 μM	HL60	−1

Connectivity Map[65,66] (CMAP) analysis, drugs that have opposite gene expression profile effects to our suicide biomarkers signatures. A score of −1 indicates the perfect opposite match, that is, the best potential therapeutic for suicidality. Underlined, drugs known to treat mood disorders and suicidality, which thus serve as a *de facto* positive control for our approach. Bold means top compounds of interest, pharmaceutical and natural.

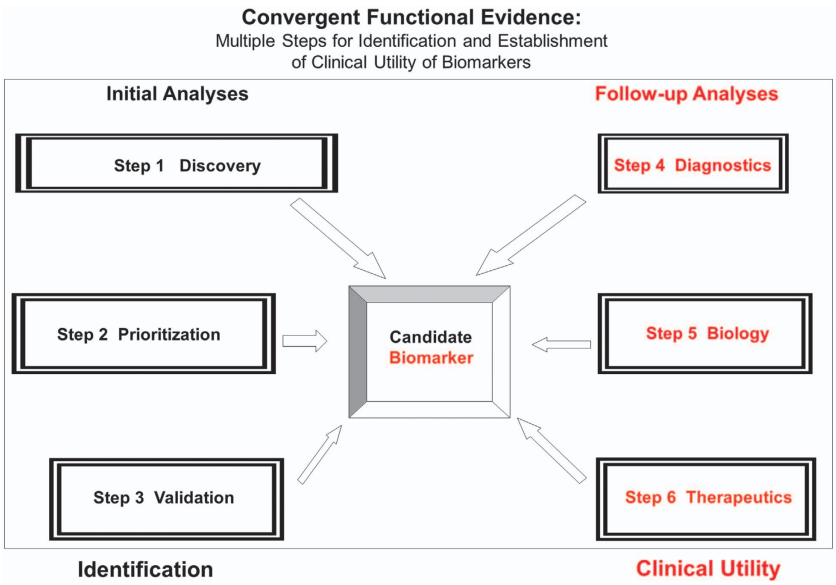
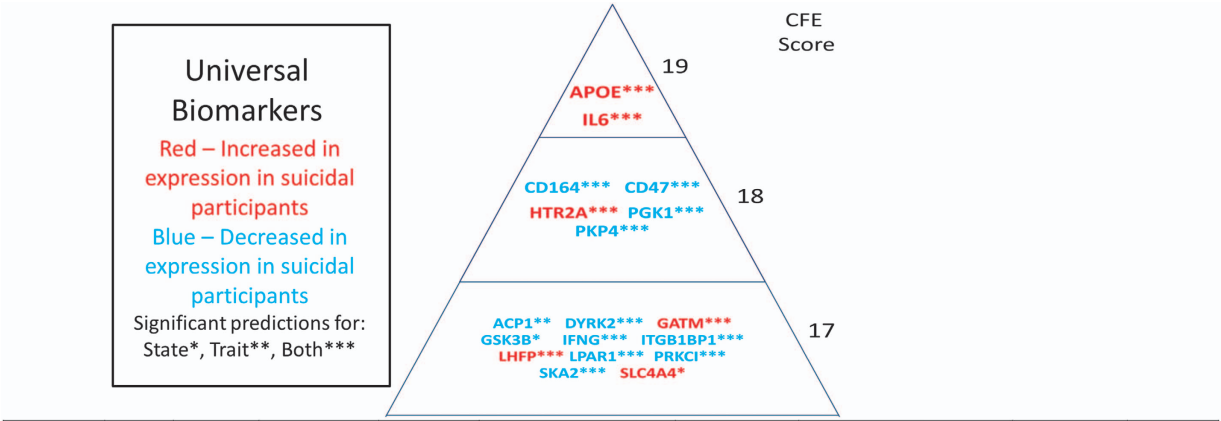
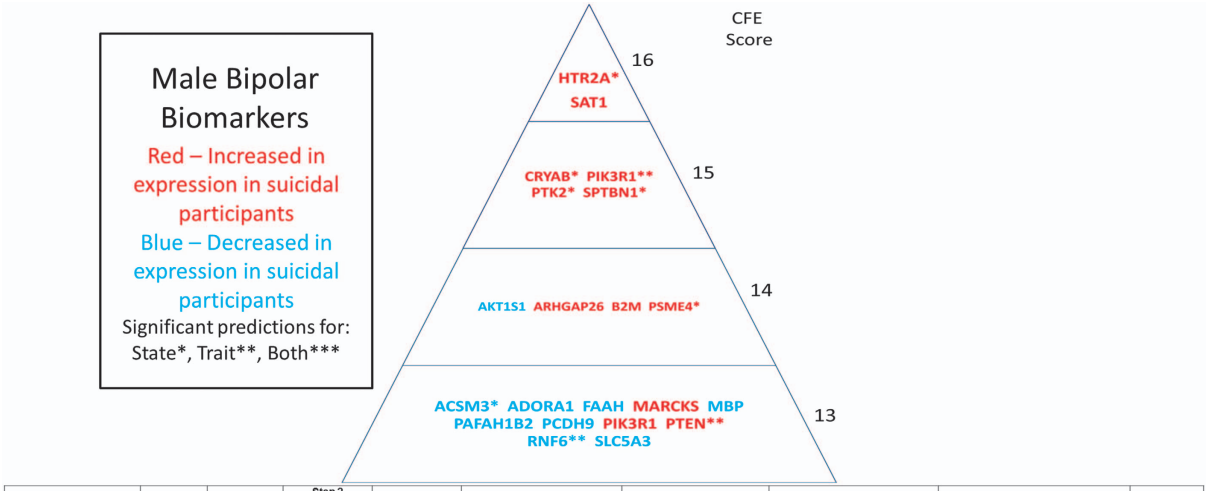


Figure 8. Convergent functional evidence—multiple steps for identification and establishment of clinically utility of biomarkers.



Gene Symbol/Gene Name	Probesets	Step 1 Discovery in Blood (Direction of Change) Method/ Score	Step 2 Convergent Functional Genomics (CFG) Evidence For Involvement in Suicide Score	Step 3 Validation in Blood ANOVA p-value/ Score	Step 4 Significant Prediction of Suicidal Ideation All Best in Subtypes Best in Individualized Gender/Dx ROC AUC/ p-value	Step 4 Significant Prediction of First Year Hospitalizations for Suicidality All Best in Subtypes Best in Individualized Gender/Dx ROC AUC/ p-value	Step 5 Other Psychiatric and Related Disorders Evidence	Step 6 Drugs that Modulate the Biomarker in Opposite Direction to Suicide	CFE Polyevidence Score
APOE apolipoprotein E	203382_s_at	(I) DE/1	6	3.44E-09/4	All: 0.58/2.26E-02 Combined Subtype: 0.62/1.99E-02 M-BP: 0.71/9.02E-03	M-PTSD 0.78/4.43E-02	Aggression,Aging, Alcohol Alzheimer's Disease, ASD, Dementia, Depression-related, Longevity, MDD, Psychosis ,PTSD, SZ	Omega-3	19
IL6 interleukin 6	205207_at	(I) AP/1	6	1.82E-15/4	All: 0.58/3.74E-02 Combined Subtype: 0.61/3.98E-02	M-PTSD 0.82/2.58E-02	Aggression, Antipsychotics Anxiety, BP, Cognition, Dementia Depression, Longevity, MDD, Mood Neurological, Panic, Personality, SZ/SZA, PTSD , Sleep, Stress, SZ	Antipsychotics, Antidepressants, Tocilizumab, Siltuximab	19
CD164 CD164 molecule, sialomucin	208654_s_at	(D) DE/2	4	3.01E-08/4	All: 0.59/1.80E-02 M-BP: 0.68/1.94E-02	M-PTSD 0.86/1.43E-02	BP, Cocaine Dependence, Stress	Clozapine	18
CD47 CD47 molecule	211075_s_at	(D) DE/2	4	1.62E-17/4	All: 0.6/9.71E-03 Depressed Subtype: 0.68/2.99E-02 M-SZA: 0.69/2.19E-02	M-PTSD 0.79/3.72E-02	MDD, Stress, SZ	Clozapine Omega-3	18
HTR2A 5-hydroxytryptamine (serotonin) receptor 2A, G protein-coupled	244130_at	(I) DE/2	8	NS	Depressed Subtype: 0.66/4.74E-02 M-SZ: 0.79/1.58E-02	M-SZA 0.72/1.47E-02	Alcohol, Anxiety, BP, MDD, SZ, OCD Response to Antidepressants	Clozapine, Lithium, Valproate, Antipsychotics, Antidepressants	18
PGK1 phosphoglycerate kinase 1	217383_at	(D) DE/2	4	4.07E-07/4	M-SZA: 0.73/8.31E-03	M-SZA 0.71/1.84E-02	Alcohol, BP, MDD, SZ, SZA	Clozapine, Diazepam	18
PKP4 plakophilin 4	201929_s_at	(D) DE/1	5	3.82E-08/4	Combined Subtype: 0.62/2.59E-02 M-SZ: 0.75/2.93E-02	Combined Subtype 0.68/8.75E-03	Alcohol, BP, MDD, SZ/SZA, SZ	Valproate	18
ACP1 acid phosphatase 1, soluble	1554808_at	(D) DE/1	6	3.82E-11/4		M-MDD 0.74/3.79E-02	BP, SZ	Omega-3, SSRIs, Olanzapine	17
DYRK2 dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2	202969_at	(D) DE/1	4	1.67E-13/4	All: 0.58/3.37E-02 Combined Subtype: 0.61/3.00E-02 M-SZ/SZA: 0.68/9.85E-03	M-PTSD 0.82/2.58E-02	Aging, BP, MDD, Sleep	Clozapine	17
GATM L-arginine:glycine amidinotransferase	1566861_at	(I) DE/1	4	1.80E-12/4	Combined Subtype: 0.6/4.84E-02 M-BP: 0.68/1.94E-02	M-PTSD 0.78/4.43E-02	Alzheimer's Disease, BP, MDD, PTSD	Omega-3	17
GSK3B glycogen synthase kinase 3 beta	226183_at	(D) DE/1	6	2.19E-36/4	M-SZA: 0.68/3.47E-02		Aging , Alcohol, BP, Dementia, Depression, Mood Stabilizers, Lithium response , MDD, SZ	Lithium, SSRI, Antipsychotics	17
IFNG interferon, gamma	210354_at	(D) AP/1	8	NS	All: 0.6/1.01E-02 Combined Subtype: 0.61/3.03E-02 M-PTSD: 0.73/2.72E-02	M-PTSD 0.82/2.58E-02	SZ, MDD, PTSD, Anxiety, SZ/SZA	Antipsychotics	17
ITGB1BP1 integrin beta 1 binding protein 1	203337_x_at	(D) DE/1	4	1.11E-08/4	Depressed Subtype: 0.67/4.21E-02 M-SZ: 0.78/1.64E-02	Non-Affective Subtype 0.7/2.59E-02	Alzheimer's Disease, BP, Mood, SZ	Lithium	17
LHFP lipoma HMGIC fusion partner	218656_s_at	(I) DE/1	4	3.97E-10/4	All: 0.57/5.00E-02 Anxious Subtype: 0.78/1.95E-02 F-BP: 0.79/4.60E-02	M-MDD 0.98/2.54E-04	SZ	Omega-3	17
LPAR1 lysophosphatidic acid receptor 1	204036_at	(D) AP and DE/1	4	1.35E-23/4	M-BP: 0.68/2.13E-02	Anxious Subtype 0.77/1.33E-02	Aging, BP, Longevity, MDD, Mood, PTSD, SZ	Clozapine, Omega-3, Antidepressants	17
PRKCI protein kinase C, iota	209677_at	(D) DE/1	4	2.71E-05/4	Anxious Subtype: 0.8/1.55E-02	Combined Subtype 0.64/2.64E-02	BP, Circadian abnormalities Cocaine Dependence, MDD, SZ	Ingenol mebutate	17
SKA2 spindle and kinetochore associated complex subunit 2	225686_at	(D) DE/1	8	4.55E-03/2	All: 0.61/3.35E-03 Depressed Subtype: 0.74/5.91E-03 M-SZ: 0.79/1.35E-02	M-PTSD 0.84/1.75E-02	PTSD, Stress		17
SLC4A4 sodium bicarbonate cotransporter	210739_x_at	(I) AP/1	6	7.74E-05/4	All: 0.64/3.83E-04 Combined Subtype: 0.69/6.13E-04 M-BP: 0.77/9.27E-04		Circadian abnormalities, Longevity, MDD, SZ	Valproate	17

Figure 9. Universal biomarkers—convergent functional evidence (CFE) for involvement in suicidality. Top Dozen and Bonferroni biomarkers. *Post-hoc* summation of all the evidence form discovery, validation, prioritization and testing (for state—SI and for trait-future hospitalizations), along with evidence for involvement in other psychiatric disorders and for being a target of drugs. This prioritization highlights for future studies biomarkers that may have broad applicability in the field, for diagnostics and therapeutics. ASD, autism spectrum disorder; BP, bipolar; MDD, major depressive disorder; PTSD, post-traumatic stress disorder; SZ, schizophrenia.



Gene Symbol/Gene Name	Probesets	Step 1 Discovery in Blood (Direction of Change) Method/Score	Step 2 Convergent Functional Genomics (CFG) Evidence For Involvement in Suicide Score	Step 3 Validation in Blood ANOVA p-value/ Score	Step 4 Significant Prediction of Suicidal Ideation in Male Bipolars ROC AUC/p-value	Step 4 Significant Prediction of First Year Hospitalizations for Suicidality in Male Bipolars ROC AUC/p-value	Step 5 Other Psychiatric and Related Disorders Evidence	Step 6 Drugs that Modulate the Biomarker in Opposite Direction to Suicide	CFE Polyevidence Score
HTR2A 5-Hydroxytryptamine Receptor 2A	244130_at	(I) DE/2	8.00	NS	0.65/4.45E-02	NA	Alcohol Anxiety BP MDD SZ OCD	Clozapine, Lithium, Valproate, Antipsychotics, Antidepressants	16
SAT1 spermidine/spermine N1-acetyltransferase 1	210592_s_at	(I) DE/2	6.00	4.00E-33/4	NA	NA	MDD Anxiety Mood Disorders NOS	Omega 3	16
CRYAB crystallin, alpha B	209283_at	(I) DE/1	4.00	3.49E-05	0.65/4.41E-02	NA	Autism Alcohol PTSD SZA BP SZ Insomnia Social Isolation Stress MDD	Lithium Clozapine Methamphetamine	15
PIK3R1 Phosphoinositide-3-Kinase Regulatory Subunit 1	239476_at	(I) DE/1	4.00	2.97E-12	NA	0.81/1.64E-02	Schizophrenia MDD Relaxation Response PTSD BP Longevity Stress Insomnia Anxiety	Mood Stabilizers	15
PTK2 Protein Tyrosine Kinase 2	241453_at	(I) DE/2	4.00	4.29E-16/4	0.66/3.64E-02	NA	Alcohol ASD BP Circadian abnormalities MDD Neurological SZ/SZA Stress SZ	CT-707	15
SAT1 spermidine/spermine N1-acetyltransferase 1	203455_s_at	(I) DE/1	6.00	9.99E-29/4	NA	NA	MDD Anxiety Mood Disorders NOS	Omega 3	15
SAT1 spermidine/spermine N1-acetyltransferase 1	213988_s_at	(I) DE/2	6.00	4.08E-34	NA	NA	MDD Anxiety Mood Disorders NOS	Omega 3	15
SPTBN1 spectrin, beta, non-erythrocytic 1	215918_s_at	(I) AP/1	4.00	6.7E-32	0.72/6.62E-03	NA	Aging BP Longevity MDD SZ	Clozapine Omega-3 **Diazepam**	15
AKT1S1 AKT1 substrate 1 (proline-rich)	1555821_a_at	(D) DE/2	4.00	8.69E-09/4	NA	NA	Circadian abnormalities Aging	Omega-3 fatty acids	14
AKT1S1 AKT1 substrate 1 (proline-rich)	224982_at	(D) AP/1 and DE/2	4.00	8.04E-11/4	NA	NA	Circadian abnormalities Longevity	Omega-3 fatty acids	14
ARHGAP26 Rho GTPase activating protein 26	205068_s_at	(I) DE/1	5.00	7.99E-08/4	NA	NA	BP MDD Panic Disorder SZ	Clozapine	14
B2M beta-2-microglobulin	232311_at	(I) DE/2	4.00	5.43E-06/4	NA	NA	Alcohol Effect of valproate MDD SZ	Omega-3	14
PSME4 Proteasome Activator Subunit 4	237180_at	(I) DE/2	4.00	2.02E-16/4	0.69/1.41E-02	NA	ASD MDD		14
ACSM3 acyl-CoA synthetase medium-chain family member 3	210377_at	(D) DE/1	4.00	2.31E-10/4	0.69/1.35E-02	NA	MDD Mood		13
ADORA1 adenosine A1 receptor	205481_at	(D) DE/1	4.00	1.19E-07/4	NA	NA	Alcohol SZ BP Mood, Stimulants Depression	Clozapine	13
FAAH fatty acid amide hydrolase	204231_s_at	(D) DE/1	4.00	7.47E-12/4	NA	NA	Alcohol SZ BP MDD Pain Placebo PTSD Stress Hallucinogens Social Isolation		13
MARCKS Myristoylated alanine-rich protein kinase C substrate	213002_at	(I) DE/1	4.00	7.35E-08/4	NA	NA	BP SZ MDD Yohimbine Alcohol Panic Disorder	Lithium	13
MBP myelin basic protein	225408_at	(D) AP/1	4.00	3.26E-06/4	NA	NA	Alcohol Alzheimer's Disease BP MDD Mood Neurological SZ	Clozapine Omega-3 Lithium	13
PAFAH1B2 platelet-activating factor acetylhydrolase 1b, catalytic subunit 2 (30kDa)	210160_at	(D) DE/1	4.00	4.85E-09/4	NA	NA	MDD	Lithium PCP Clozapine	13
PCDH9 Protocadherin 9	238919_at	(D) AP/1	4.00	4.52E-05/4	NA	NA	Aging MDD SZ/SZA SZ	Clozapine Omega-3	13
PIK3R1 phosphoinositide-3-kinase, regulatory subunit 1 (alpha)	212240_s_at	(I) DE/1	4.00	7.11E-14/4	NA	NA	SZ MDD Relaxation Response PTSD BP Longevity Stress Alcohol Insomnia Anxiety	Amygdala mood stabilizers	13
PTEN phosphatase and tensin homolog	222176_at	(I) DE/1	4.00	4.88E-05/4	NA	0.9/3.27E-03	SZ MDD BP PTSD Longevity Stress		13
RNF6 ring finger protein (C3H2C3 type) 6	210932_s_at	(D) DE/1	4.00	1.25E-05/4	NA	0.82/1.58E-02	BP Social Isolation		13
SLC5A3 sodium/myo-inositol cotransporter	213167_s_at	(D) DE/1	4.00	4.89E-14/4	NA	NA	Chronic Stress MDD Alcohol	Lithium	13

Figure 10. Male bipolar biomarkers—convergent functional evidence for involvement in suicidality. Top Dozen and Bonferroni biomarkers. *Post-hoc* summation of all the evidence form discovery, validation, prioritization and testing, along with evidence for involvement in other psychiatric disorders and for being a target of drugs. This prioritization highlights for future studies biomarkers that may have broad applicability in the field, for diagnostics and therapeutics. ASD, autism spectrum disorder; BP, bipolar; MDD, major depressive disorder; PTSD, post-traumatic stress disorder; SZ, schizophrenia.

Table 5 and Supplementary Table S5), and drug discovery/repurposing (Table 6). In terms of therapeutics, ebselen, although discovered decades ago for other indications and shown to be safe and well tolerated in human studies, has been recently proposed as a treatment for bipolar disorder.[15] The fact that it was the compound that had the strongest/perfect score on the Connectivity Map analyses as having the opposite gene expression effects to the biosignature of the Top Dozen universal biomarkers for suicidality is a tantalizing result. Piracetam and chlorogenic acid are compounds that can be classified as nutraceuticals, already in use and relatively safe and innocuous, which may facilitate adoption, along with omega-3 fatty acids, for pre-emptive population-level approaches. Metformin, besides being originally an antidiabetic compound, may act on promoting longevity by mimicking the effects of calorie restriction. The fact that it may have anti-suicidal properties is consistent with a possible suicide/longevity 'life switch' machinery, as previously proposed by us.[22] Interestingly, clozapine and fluoxetine, two known medications used to treat suicidality, were identified by our bioinformatics approach, but with lower priority than metformin (Table 6B). They serve as reassuring positive controls.

The biomarkers also provide a window toward a more nuanced understanding of the biology of suicide. The universal biomarkers may be pointing to a core common biology, such as inflammation and stress response, and the balance between neuronal cell survival and apoptosis, that is, a cellular level 'life switch'. The personalized biomarkers (such as male bipolar biomarkers), may be more reflective of the psychiatric co-morbidity driving suicidality and medication effects in different gender/diagnostic groups, such as mTOR signaling in male bipolars, which is modulated by mood regulating drugs, including a new one (ketamine),[23] which also shows promise for treating suicidality.[24]

Among the Top Dozen universal biomarkers (Table 2A) was HTR2A, which was increased in expression and is targeted by most modern antipsychotics, and ARRB1, which was decreased in expression and is downstream of HTR2A in the signaling cascade, and also interacts with GSK3B,[25] which was decreased in expression as well (Supplementary Figure S3). The combination of these expression effects may provide for excessive/unbridled signaling[26] as a key pathological step in suicidality. For male bipolars, similar STRING analysis revealed a network centered on PTK2 (Supplementary Figure S4).

Finally, a *post-hoc* convergent functional evidence score of the totality of evidence in these series of studies, including testing for predictive ability, evidence for biological predisposition/involvement in psychiatric disorders, and for being potential drug targets, identifies APOE and IL6 as the top overall biomarkers of interest, pointing to stress, inflammation and accelerated aging (Figure 9 and Supplementary Table S7). It is unclear at this point whether they are just markers of that co-occurring pathology or are direct drivers of suicide as well.

Overall, we believe this work is a major step forward towards understanding, diagnosing and treating suicidality. Suicide occurs maladaptively in the face of negative life events, with the individual who commits suicide being vulnerable due to a psychiatric illness and addictions, misperceiving circumstances and/or overreacting in an impulsive manner. Related to that, suicide can be an attempt to assuage perceived guilt or an attempt to harm (through social opprobrium or guilt) the individual(s) perceived to be the source of the suffering and lack of success of the suicidal person. Conversely, a well-balanced and functioning mind is protective. To predict behaviors, it may be important to provide context to the biomarkers, as to how the person feels (SASS) and who the person is (CFI-S) (Figure 5a). That is why the combined UP-Suicide predictor is robust across all subjects, in subtypes and personalized by gender and diagnosis, with AUCs over 90% (Figure 6). We hope that our risk assessment tools may become self-cancelling predictions once tested and deployed in the general population, as they can lead to precision prevention with early targeted interventions: biological, psychological and social. Indeed, beyond biological markers and drug treatments, SASS can help identify different subtypes and in different individuals different CFI-S items are positive, providing avenues for tailored and targeted (psycho)therapeutic interventions. Culture may have a particularly important role (Figure 5a). Negative behaviors in the past, negative examples from the environment and negative cultural values can lead to a downward spiral of failure. Reversing negative cultural influences on behaviors involves coming to terms with or forgetting the past, starting to accumulate positive precedents, changing the environment (or moving to a different environment) and gravitating to/adopting a different set of cultural values. Given that one person dies from suicide every 40 seconds worldwide, the importance and urgency of efforts such as ours cannot be overstated.[27] We demonstrate how this field can develop precision, move towards personalization, and have tools to pre-empt suicide acutely, as well as prevent suicide in the long term. We envision the participation of individuals at risk in such efforts initially, followed by the pervasive deployment of the tools in society at large in the long term. It has not escaped our attention that, if our 'life switch' hypothesis between suicide and longevity is correct,[22] the possibility exists to transform a negative into a positive.

CONFLICT OF INTEREST

The authors declare no conflict of interest. ABN is listed as inventor on a patent application filed by Indiana University.

NOTES

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

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AUTHOR CONTRIBUTIONS

ABN designed the study and wrote the manuscript. DFL, HLN, PLP, EMN, HLD and KR analyzed the data. VV performed database work. JN, AW, DLG and TJ organized, conducted and scored testing in psychiatric participants. AB, MY, AS, GES and ABN organized and carried out postmortem samples collection. TG, SMK, NS 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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