IMMEDIATE COMMUNICATION

Discovery and validation of blood biomarkers for suicidality

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Suicides are a leading cause of death in psychiatric patients, and in society at large. Developing more quantitative and objective ways (biomarkers) for predicting and tracking suicidal states would have immediate practical applications and positive societal implications. We undertook such an endeavor. First, building on our previous blood biomarker work in mood disorders and psychosis, we decided to identify blood gene expression biomarkers for suicidality, looking at differential expression of genes in the blood of subjects with a major mood disorder (bipolar disorder), a high-risk population prone to suicidality. We compared no suicidal ideation (SI) states and high SI states using a powerful intrasubject design, as well as an intersubject case–case design, to generate a list of differentially expressed genes. Second, we used a comprehensive Convergent Functional Genomics (CFG) approach to identify and prioritize from the list of differentially expressed gene biomarkers of relevance to suicidality. CFG integrates multiple independent lines of evidence—genetic and functional genomic data—as a Bayesian strategy for identifying and prioritizing findings, reducing the false-positives and false-negatives inherent in each individual approach. Third, we examined whether expression levels of the blood biomarkers identified by us in the live bipolar subject cohort are actually altered in the blood in an age-matched cohort of suicide completers collected from the coroner's office, and report that 13 out of the 41 top CFG scoring biomarkers (32%) show step-wise significant change from no SI to high SI states, and then to the suicide completers group. Six out of them (15%) remained significant after strict Bonferroni correction for multiple comparisons. Fourth, we show that the blood levels of SAT1 (spermidine/spermine N1-acetyltransferase 1), the top biomarker identified by us, at the time of testing for this study, differentiated future as well as past hospitalizations with suicidality, in a live cohort of bipolar disorder subjects, and exhibited a similar but weaker pattern in a live cohort of psychosis (schizophrenia/schizoaffective disorder) subjects. Three other (phosphatase and tensin homolog (PTEN), myristoylated alanine-rich protein kinase C substrate (MARCKS), and mitogen-activated protein kinase kinase 3 (MAP3K3)) of the six biomarkers that survived Bonferroni correction showed similar but weaker effects. Taken together, the prospective and retrospective hospitalization data suggests SAT1, PTEN, MARCKS and MAP3K3 might be not only state biomarkers but trait biomarkers as well. Fifth, we show how a multi-dimensional approach using SAT1 blood expression levels and two simple visual-analog scales for anxiety and mood enhances predictions of future hospitalizations for suicidality in the bipolar cohort (receiver-operating characteristic curve with area under the curve of 0.813). Of note, this simple approach does not directly ask about SI, which some individuals may deny or choose not to share with clinicians. Lastly, we conducted bioinformatic analyses to identify biological pathways, mechanisms and medication targets. Overall, suicidality may be underlined, at least in part, by biological mechanisms related to stress, inflammation and apoptosis.

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INTRODUCTION

'To be, or not to be, that is the question'

W Shakespeare, *Hamlet*

Whatever its evolutionary, teleological and cultural reasons for existing, suicidal behavior is in most cases pathological and leads to irreversible tragedies.^{1,2} Paradoxically, given its importance, there are yet no reliable objective tools to assess and track changes in suicidal risk without asking the individuals directly. Such tools are desperately needed, as individuals at risk often choose not to share their ideation or intent with others, for

fear of stigma, hospitalization, or that in fact their plans may be thwarted.

A convergence of methods assessing the persons' internal subjective feelings and thoughts, along with external, more objective ratings of actions and behaviors, are used *de facto* in clinical psychiatry. Such an approach is insufficient and is lagging behind those used in other medical specialties. It lacks precision, objectivity and predictive ability.

Our group has previously provided the first proof-of-principle for the use of blood gene expression biomarkers to predict mood state³ and psychosis symptoms.⁴ As the target organ in psychiatry—the brain—cannot be biopsied in live patients, it is

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essential to be able to identify and validate peripheral biomarkers for subsequent practical implementation in clinical settings. We now present a comprehensive and highly reductionist approach for discovering and validating blood biomarkers for suicidality.

We used a Convergent Functional Genomics (CFG) approach to identify and prioritize biomarkers of relevance to suicidality. CFG is a powerful, combined approach for extracting signal from noise in genetic and gene expression studies. The CFG methodology has already been applied to help identify and prioritize candidate genes, pathways and mechanisms for neuropsychiatric disorders, such as bipolar disorder,^{5–8} alcoholism,⁹ anxiety¹⁰ and schizophrenia,¹¹ showing reproducibility and predictive ability in independent cohorts.

SUBJECTS AND METHODS

Human subjects

We present data from four cohorts: one live bipolar discovery cohort; one postmortem coroner's office test cohort; and two prospective follow-up live cohorts—one bipolar and one psychosis (schizophrenia/schizoaffective).

These live subjects are part of a larger longitudinal cohort being collected and studied by us. Subjects are recruited from the patient population at the Indianapolis VA Medical Center, the Indiana University School of Medicine, as well as various facilities that serve people with mental illnesses in Indiana. The subjects are recruited largely through referrals from care providers, the use of brochures left in plain sight in public places and mental health clinics, and through word of mouth. Subjects were excluded if they had significant medical or neurological illness or had evidence of active substance abuse or dependence. All subjects understood and signed informed consent forms detailing the research goals, procedure, caveats and safeguards. Subjects completed diagnostic assessments by an extensive structured clinical interview—Diagnostic Interview for Genetic Studies—at a baseline visit, followed by up to three testing visits, 3–6 months apart. At each testing visit, they received a series of psychiatric rating scales, including the Hamilton Rating Scale for Depression-17, which includes a suicidal ideation (SI) rating item (Figure 1), and the blood was drawn. Whole blood (10 ml) was collected in two RNA-stabilizing PAXgene tubes, labeled with an anonymized ID number, and stored at -80°C in a locked freezer until the time of future processing. Whole-blood (predominantly lymphocyte) RNA was extracted for microarray gene expression studies from the PAXgene tubes, as detailed below. We focused this initial study on a male population because of the demographics of our catchment area (primarily male in a VA Medical Center), and to minimize any potential gender-related effects on gene expression, which would have decreased the discriminative power of our analysis given our relatively small sample size.

Our intrasubject discovery cohort, from which the biomarker data were derived, consisted of nine male Caucasian subjects with bipolar disorder, with multiple visits, who each had a diametric change in SI scores from no SI to high SI from one testing visit to another testing visit. There were 6 subjects with 3 visits each, and 3 subjects with 2 visits each, resulting in a total of 24 blood samples for subsequent microarray studies (Table 1 and Figure 1).

Our postmortem cohort, in which the top biomarker findings were tested, consisted of an age-matched cohort of nine male suicide completers obtained through the Marion County coroner's office (eight Caucasians, one African American) (Table 1 and Supplementary Table S2). We required a last observed alive postmortem interval of 24 h or less, and the cases selected had completed suicide by means other than overdose, which could affect gene expression. Next of kin signed informed consent at the coroner's office for donation of tissues and fluids for research. The samples were collected as part of our INBRAIN initiative (Indiana Center for Biomarker Research in Neuropsychiatry).

The bipolar follow-up cohort ($n=42$) (Table 1) consisted of male Caucasian subjects in whom whole-genome blood gene expression data, including levels of SAT1 (spermidine/spermine N1-acetyltransferase 1), were obtained by us at testing visits over the years as part of our longitudinal study. If the subjects had multiple testing visits, the visit with the highest SAT1 level was selected for this analysis. The subjects' subsequent number of hospitalizations with or without suicidality was tabulated from electronic medical records. The psychosis (schizophrenia/schizoaffective) follow-up cohort ($n=46$) (Supplementary Table S9)

Figure 1. Discovery cohort: intrasubject and intersubject analyses. Phchp### is study ID for each subject. V# after it denotes visit number (1, 2 or 3). **(a)** Design and **(b)** suicidal ideation (SI) scoring. **(c)** Overlapping probesets and genes.

similarly consisted of Caucasian subjects in whom whole-genome blood gene expression data, including levels of SAT1, were obtained by us at testing visits over the years as part of our longitudinal study. If the subjects had multiple testing visits, the visit with the highest SAT1 level was selected for this analysis. The subjects' subsequent number of hospitalizations with or without suicidality was tabulated from electronic medical records. A hospitalization was deemed to be without suicidality if suicidality was not listed as a reason for admission, and no SI was described in the admission and discharge medical notes. Conversely, a hospitalization was deemed to be because of suicidality if suicidal acts or intent was listed as a reason for admission, and SI was described in the admission and discharge medical notes.

Medications

The subjects in the discovery cohort were all diagnosed with bipolar disorder (Table 1). Their psychiatric medications are listed in Supplementary Table S1. The subjects were on a variety of different psychiatric medications: mood stabilizer, antidepressants, antipsychotics, benzodiazepines and others. Medications can have a strong influence on gene expression. However, our discovery of differentially expressed genes was based on intrasubject analyses, which factor out not only genetic

Table 1. Demographics

A. Individual

Cohort 1: Live bipolar subjects discovery cohort (n = 9) (24 chips)

Subject ID visit	Diagnosis	Age	Gender	Ethnicity	SI
phchp023v1	Bipolar disorder NOS	52	M	Caucasian	0
phchp023v2	Bipolar disorder NOS	52	M	Caucasian	3
phchp023v3	Bipolar disorder NOS	52	M	Caucasian	0
phchp093v1	Bipolar I disorder	51	M	Caucasian	0
phchp093v2	Bipolar I disorder	51	M	Caucasian	0
phchp093v3	Bipolar I disorder	52	M	Caucasian	3
phchp095v1	Bipolar I disorder	28	M	Caucasian	3
phchp095v2	Bipolar I disorder	29	M	Caucasian	0
phchp095v3	Bipolar I disorder	29	M	Caucasian	2
phchp122v1	Bipolar disorder NOS	51	M	Caucasian	0
phchp122v2	Bipolar disorder NOS	51	M	Caucasian	2
phchp128v1	Bipolar I disorder	45	M	Caucasian	2
phchp128v2	Bipolar I disorder	45	M	Caucasian	0
phchp136v1	Bipolar I disorder	41	M	Caucasian	0
phchp136v2	Bipolar I disorder	41	M	Caucasian	0
phchp136v3	Bipolar I disorder	41	M	Caucasian	3
phchp153v1	Bipolar II disorder	55	M	Caucasian	0
phchp153v2	Bipolar II disorder	55	M	Caucasian	2
phchp153v3	Bipolar II disorder	56	M	Caucasian	0
phchp179v1	Bipolar disorder NOS	36	M	Caucasian	0
phchp179v2	Bipolar disorder NOS	37	M	Caucasian	0
phchp179v3	Bipolar disorder NOS	37	M	Caucasian	3
phchp183v1	Bipolar I disorder	48	M	Caucasian	3
phchp183v2	Bipolar I disorder	48	M	Caucasian	0

Cohort 2: Coroner's office test cohort-suicide completers (n = 9) (9 chips)

Subject ID	Psychiatric diagnosis	Age (years)	Gender	Ethnicity	Suicide by
INBR009	Bipolar/schizophrenia	59	M	Caucasian	Hanging
INBR011	Depression/ADHD	26	M	Caucasian	GSW to chest
INBR012	Unknown	39	M	Caucasian	GSW to head
INBR013	Depression	68	M	African American	GSW to mouth
INBR014	None	27	M	Caucasian	Hanging
INBR015	None	40	M	Caucasian	Hanging
INBR016	Anxiety/TBI	68	M	Caucasian	GSW to head
INBR017	Depression	56	M	Caucasian	GSW to chest
INBR018	None	65	M	Caucasian	Slit wrist

Cohort 3: Live bipolar subjects prospective follow-up cohort (n = 42)

Subject ID visit	Diagnosis	Age	Gender	Ethnicity	SAT1 levels at testing	Years since testing	Future hosp. w/o suicidality	Future hosp. due to suicidality	Frequency of future hosp. w/o suicidality	Frequency of future hosp. due to suicidality
phchp234v1	Bipolar II disorder	44	M	Caucasian	1955.20	0.83	0	0	0.00	0.00
phchp053v2	Bipolar I disorder	58	M	Caucasian	2178.30	5.67	4	0	0.71	0.00
phchp152v1	Bipolar I disorder	45	M	Caucasian	2178.80	2.33	0	0	0.00	0.00
phchp122v1	Bipolar disorder NOS	51	M	Caucasian	2245.60	0.58	0	0	0.00	0.00
phchp190v3	Bipolar disorder NOS	50	M	Caucasian	2300.60	1.25	0	0	0.00	0.00
phchp020v3	Bipolar disorder NOS	63	M	Caucasian	2342.60	4.08	0	0	0.00	0.00
phchp113v1	Bipolar I disorder	37	M	Caucasian	2437.40	3.00	0	0	0.00	0.00
phchp132v2	Bipolar I disorder	51	M	Caucasian	2558.90	2.33	0	0	0.00	0.00
phchp184v3	Bipolar disorder NOS	64	M	Caucasian	2575.40	1.33	0	0	0.00	0.00
phchp039v3	Bipolar I disorder	52	M	Caucasian	2580.10	5.75	0	0	0.00	0.00
phchp147v1	Bipolar II disorder	38	M	Caucasian	2582.80	2.25	0	0	0.00	0.00
phchp178v1	Bipolar I disorder	49	M	Caucasian	2616.80	1.00	0	0	0.00	0.00
phchp136v3	Bipolar I disorder	41	M	Caucasian	2635.90	2.00	0	0	0.00	0.00
phchp045v3	Bipolar I disorder	36	M	Caucasian	2721.00	5.42	0	0	0.00	0.00
phchp224v1	Bipolar I disorder	59	M	Caucasian	2748.10	1.08	1	1	0.92	0.92
phchp183v1	Bipolar I disorder	48	M	Caucasian	2750.90	0.42	2	1	4.80	2.40
phchp171v2	Bipolar disorder NOS	36	M	Caucasian	2795.70	1.50	0	0	0.00	0.00
phchp166v1	Bipolar disorder NOS	56	M	Caucasian	2829.60	1.92	0	0	0.00	0.00
phchp253v1	Bipolar disorder NOS	25	M	Caucasian	2888.50	1.00	0	0	0.00	0.00
phchp186v1	Bipolar II disorder	43	M	Caucasian	2901.50	1.67	0	0	0.00	0.00
phchp079v2	Bipolar disorder	44	M	Caucasian	3053.20	4.50	0	0	0.00	0.00
phchp128v1	Bipolar I Disorder	45	M	Caucasian	3118.60	2.67	0	0	0.00	0.00
phchp080v1	Bipolar I disorder	44	M	Caucasian	3153.60	5.00	0	0	0.00	0.00
phchp088v1	Bipolar I disorder	44	M	Caucasian	3194.10	4.58	0	10	0.00	2.18
phchp109v1	Bipolar I disorder	22	M	Caucasian	3200.80	3.00	1	2	0.33	0.67

Table 1. (Continued)*Cohort 3: Live bipolar subjects prospective follow-up cohort (n = 42)*

Subject ID visit	Diagnosis	Age	Gender	Ethnicity	SAT1 levels at testing	Years since testing	Future hosp. w/o suicidality	Future hosp. due to suicidality	Frequency of future hosp. w/o suicidality	Frequency of future hosp. due to suicidality
phchp134v3	Bipolar II disorder	59	M	Caucasian	3202.30	1.92	0	0	0.00	0.00
phchp153v1	Bipolar II disorder	55	M	Caucasian	3304.90	2.00	0	0	0.00	0.00
phchp274v2	Bipolar disorder NOS	48	M	Caucasian	3349.00	0.50	0	0	0.00	0.00
phchp140v3	Bipolar II disorder	38	M	Caucasian	3393.80	1.92	0	0	0.00	0.00
phchp030v3	Bipolar I disorder	49	M	Caucasian	3395.20	5.92	0	3	0.00	0.51
phchp124v1	Bipolar I disorder	53	M	Caucasian	3660.90	2.50	0	6	0.00	2.40
phchp095v3	Bipolar I disorder	29	M	Caucasian	3695.40	0.33	0	1	0.00	3.00
phchp100v1	Bipolar I Disorder	28	M	Caucasian	3767.80	1.58	0	0	0.00	0.00
phchp210v3	Bipolar I disorder	44	M	Caucasian	3844.60	0.50	0	0	0.00	0.00
phchp219v1	Bipolar disorder NOS	61	M	Caucasian	3845.10	1.17	0	0	0.00	0.00
phchp031v3	Bipolar I disorder	52	M	Caucasian	4080.70	4.08	1	0	0.24	0.00
phchp093v3	Bipolar I disorder	52	M	Caucasian	4137.40	2.67	0	1	0.00	0.38
phchp067v1	Bipolar II disorder	39	M	Caucasian	4214.70	5.58	0	0	0.00	0.00
phchp142v3	Bipolar I disorder	55	M	Caucasian	4310.70	1.92	0	0	0.00	0.00
phchp112v2	Bipolar I disorder	46	M	Caucasian	4410.40	1.33	0	0	0.00	0.00
phchp149v2	Bipolar disorder NOS	45	M	Caucasian	4586.90	2.00	1	0	0.50	0.00
phchp117v1	Bipolar I disorder	43	M	Caucasian	6531.10	3.00	0	0	0.00	0.00

B. Aggregate

SI score	No SI (0)	High SI (2–4)	Overall
<i>Live bipolar subjects discovery cohort (n = 9)</i>			
Number of subjects (number of chips)	9 (14)	9 (10)	9 (24)
Age (years)			
Mean	46.1	43.8	45.1
s.d.	8.1	9.7	8.7
Range	29–56	28–55	28–56
Ethnicity (Caucasian/African American)	(9/0)	(9/0)	(9/0)
<i>Coroner's office test cohort-suicide completers (n = 9)</i>			
Number of subjects (number of chips)	9 (9)		
Age (years)			
Mean	49.8		
s.d.	17		
Range	26–68		
<i>Live bipolar subjects prospective follow-up cohort (n = 42)</i>			
SAT1 Levels	Lower tertile	Upper tertile	Overall
Number of subjects	14	14	42
Age			
mean	48.5	45.3	46.2
(s.d.)	9	9.5	9.9
range	36–64	28–61	22–64
Ethnicity (Caucasian/African-American)	(14/0)	(14/0)	(42/0)

Abbreviations: M, male; NOS, not otherwise specified; ADHD, attention-deficit hyperactivity disorder; TBI, traumatic brain injury; hosp. hospitalization; GSW, gunshot wound.; SI, suicidal ideation; SAT1, spermidine/spermine N1-acetyltransferase 1.

Diagnosis established by comprehensive structured clinical interview. SI question is from the Hamilton Rating Scale for Depression obtained at the time of blood draw for each subject.

background effects but also medication effects, as the subjects had no major medication changes between visits. Moreover, there was no consistent pattern in any particular type of medication, or between any change in medications and SI, in the rare instances where there were changes in medications between visits.

Human blood gene expression experiments and analyses

RNA extraction. Whole blood (2.5–5 ml) was collected into each PaxGene tube by routine venipuncture. PaxGene tubes contain proprietary reagents

for the stabilization of RNA. The cells from whole blood were concentrated by centrifugation, the pellet washed, resuspended and incubated in buffers containing Proteinase K for protein digestion. A second centrifugation step was done to remove residual cell debris. After the addition of ethanol for an optimal binding condition, the lysate was applied to a silica-gel membrane/column. The RNA bound to the membrane as the column was centrifuged, and contaminants were removed in three wash steps. The RNA was then eluted using diethylpyrocarbonate-treated water. The protocol for RNA extraction is carried out on a QIAgen QIAcube.

Sample labeling. Sample labeling was performed using the Ambion MessageAmp II-BiotinEnhanced antisense RNA (aRNA) amplification kit. The procedure is briefly outlined below and involves the following steps:

1. Reverse transcription to synthesize first-strand cDNA was primed with the T7 oligo(dT) primer to synthesize cDNA containing a T7 promoter sequence.
2. Second-strand cDNA synthesis converted the single-stranded cDNA into a double-stranded DNA template for transcription. The reaction employed DNA polymerase and RNase H to simultaneously degrade the RNA and synthesize the second-strand cDNA.
3. cDNA purification removed RNA, primers, enzymes and salts that would have inhibited *in vitro* transcription.
4. *In vitro* transcription to synthesize aRNA with biotin-NTP Mix generated multiple copies of biotin-modified aRNA from the double-stranded cDNA templates; this is the amplification step.
5. aRNA purification removed unincorporated NTPs, salts, enzymes and inorganic phosphate to improve the stability of the biotin-modified aRNA.
6. aRNA fragmentation: the amplified RNA is fragmented in a reaction that employs a metal-induced hydrolysis to fragment the aRNA. The fragmented labeled aRNA is now ready for hybridization to the Affymetrix microarray chip (Affymetrix, Santa Clara, CA, USA).

Microarrays. Biotin-labeled aRNAs were hybridized to Affymetrix HG-U133 Plus 2.0 GeneChips (Affymetrix; with over 40 000 genes and expressed sequence tags), according to the manufacturer's protocols http://www.affymetrix.com/support/technical/manual/expression_manual.affx. Arrays were stained using standard Affymetrix protocols for antibody signal amplification and scanned on an Affymetrix GeneArray 2500 scanner with a target intensity set at 250. Quality-control measures, including 30/50 ratios for glyceraldehyde 3-phosphate dehydrogenase and β -actin, scale factors, background and Q-values, were within acceptable limits.

Analysis. We have used the subject's SI scores at the time of blood collection (0—no SI compared with 2 and above—high SI). We looked at gene expression differences between the no SI and the high SI visits, using both an intrasubject and an intersubject design (Figure 1).

Differential gene expression analyses in the discovery cohort

We imported all Affymetrix microarray data as cel files into Partek Genomic Suites 6.6 software package (Partek Incorporated, St Louis, MI, USA). Using only the perfect match values, we ran a robust multi-array analysis (RMA), background corrected with quantile normalization and a median polish probeset summarization of all 24 chips, to obtain the normalized expression levels of all probesets for each chip. Then, to establish a list of differentially expressed probesets we ran two analyses.

An intrasubject analysis using a fold change in expression of at least 1.2 between high- and no SI visits within each subject was performed. There were in total 15 comparisons. Probesets that had a 1.2-fold change were then assigned either a 1 (increased in high SI) or a -1 (decreased in high SI) in each comparison. These values were then summed for each probeset across the 15 comparisons, yielding a range of scores between -11 and 12. The probesets in the top 5% (1269 probesets, <5% of 54 675 total probesets) had an absolute (without sign) score value of 7 and greater, and received an internal CFG score of 1 point. The probesets in the top 0.1% (24 probesets, <0.1% of 54 675 total probesets) had an absolute score of 11 and greater, and received an internal CFG score of 3 points.

In addition, an intersubject analysis using *t*-test (two-tailed, unequal variance) was performed to find probesets differentially expressed between high SI and no SI chips (Figure 1), resulting in 648 probesets with $P < 0.05$. Probesets with a $P < 0.05$ received an internal CFG score of 1 point, whereas probesets with $P < 0.001$ received 3 points.

We further filtered results by only selecting probesets that overlapped between the intrasubject and the intersubject analyses, resulting in 279 probesets corresponding to 246 unique genes. Gene names for the probesets were identified using Partek and NetAffy (Affymetrix) for Affymetrix HG-U133 Plus 2.0 GeneChips, followed by GeneCards to confirm the primary gene symbol. In addition, for those probesets that were not assigned a gene name by Partek or NetAffy, we used the UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) to directly map them to

known genes. Genes were then scored using our manually curated CFG databases as described below (Figure 2).

Convergent Functional Genomics

Databases. We have established in our laboratory (Laboratory of Neurophenomics, Indiana University School of Medicine, www.neurophenomics.info) manually curated databases of all the human gene expression (postmortem brain, blood and cell cultures), human genetics (association, copy number variations and linkage), and animal model gene expression and genetic studies published to date on psychiatric disorders.¹² Only the findings deemed significant in the primary publication, by the study authors, using their particular experimental design and thresholds, are included in our databases. Our databases include only primary literature data and do not include review papers or other secondary data integration analyses to avoid redundancy and circularity. These large and constantly updated databases have been used in our CFG cross validation and prioritization (Figure 2).

Human postmortem brain gene expression evidence. Information about genes was obtained and imported in our databases by searching the primary literature with PubMed (<http://ncbi.nlm.nih.gov/PubMed>), using various combinations of keywords (gene name, suicide, suicide gene expression and human brain). Postmortem convergence was deemed to occur for a gene if there were published reports of human postmortem data showing changes in expression of that gene in brains from patients who died from suicide.

Human blood and other peripheral tissue gene expression data. For human blood gene expression, evidence was extracted from our database compiled by a similar method as above, performing a search of the primary literature by entering various combinations of keywords (gene name, suicide, suicide gene expression, lymphoblasts and blood). No matches were found for our final list of differentially expressed genes.

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

CFG scoring. For CFG analysis (Figure 2), two external cross-validating lines of evidence were weighted such that findings in human postmortem brain tissue, the target organ, were prioritized over genetic findings, by giving it twice as many points. Human brain expression evidence was given 4 points, whereas human genetic evidence was given a maximum of 2 points for association and 1 point for linkage. Each line of evidence was capped in such a way that any positive findings within that line of evidence result in maximum points, regardless of how many different studies support that single line of evidence, to avoid potential popularity biases.

In addition to our external score, we also prioritized genes based upon the initial differential expression analyses used to identify them. Probesets identified by differential expression analyses could receive a maximum of 6 points (1 or 3 points from intrasubject analyses, and 1 or 3 points from intersubject analyses).

Thus, the maximum possible total CFG score for each gene was 12 points (6 points for the internal score + 6 points for the external score), with the internal and external evidence weighted equally. The scoring system was decided upon before the analysis. It has not escaped our attention that other ways of scoring the lines of evidence may give slightly different results in terms of prioritization, if not in terms of the list of genes *per se*. Nevertheless, we feel this simple scoring system provides a good separation of genes based on differential expression and on independent cross-validating evidence in the field (Figure 2).

Pathway analyses

IPA 9.0 (Ingenuity Systems, www.ingenuity.com, Redwood City, CA, USA) was used to analyze the biological roles, including top canonical pathways and diseases, of the candidate genes resulting from our work (Table 3 and Supplementary Table S4), as well as to identify genes in our data sets that

Figure 2. Convergent Functional Genomics approach for identification and prioritization of genomic biomarkers for suicidality.

are the target of existing drugs (Supplementary Table S5). Pathways were identified from the IPA library of canonical pathways that were most significantly associated with genes in our data set. The significance of the association between the data set and the canonical pathway was measured in two ways: (1) A ratio of the number of molecules from the data set that map to the pathway divided by the total number of molecules that map to the canonical pathway is displayed. (2) Fisher's exact test was used to calculate a *P*-value determining the probability that the association between the genes in the data set and the canonical pathway is explained by chance alone. We also conducted a Kyoto Encyclopedia of Genes and Genomes pathway analysis through the Partek Genomic Suites 6.6 software package.

Validation analyses

We imported the nine Affymetrix microarray data files from the suicide completers cohort as cel files into the Partek Genomic Suites 6.6 software package (Partek Incorporated). We then ran a RMA, background corrected with quantile normalization, and a median polish probeset summarization of all the chips from the discovery and validation cohort ($24 + 9 = 33$ chips), to obtain the normalized expression levels of all probesets for each chip. Partek normalizes expression data into a log base of 2 for visualization purposes. We non-log-transformed expression data by taking 2 to the power of the transformed expression value. We then used the

non-log-transformed expression data to compare expression levels of biomarkers in the different groups (Figure 3). One-tail Student's *t*-tests with unequal variance, one-way ANOVA and Bonferroni corrections were used for statistical comparisons.

For live cohorts' future hospitalization analyses in bipolar disorder and schizophrenia/schizoaffective, we similarly RMA normalized each cohort, before looking at biomarker levels in individual subjects. One-tail Student's *t*-tests with equal variance were used for statistical comparisons. Receiver-operating characteristic curves were calculated using SPSS software for each of the four-dimensional analyses, predicting the state variable of hospitalizations due to suicidality.

RESULTS

Discovery

We conducted whole-genome gene expression profiling in the blood samples from a longitudinally followed homogeneous cohort of male subjects with a major mood disorder (bipolar disorder) that predisposes to suicidality. One in three individuals with bipolar disorder attempt suicide during their lifetime.¹³ The samples were collected at repeated visits, 3–6 months apart. State information about SI was collected from a questionnaire

Table 2. Top gene expression biomarkers for suicidality

Gene symbol/gene name	Probesets	Change	Differential expression score	Prior human genetic evidence	Prior human brain expression evidence	Total CFG score
<i>SAT1</i> Spermidine/spermine N1-acetyltransferase 1	203455_s_at	I	2	(Association) Suicide attempt; ⁴⁵ suicide ⁴⁶	Suicide in depression (D) PFC ⁴⁷ Suicide (D) AMY, PFC, HIP, THAL ³⁹ Suicide (D) PFC ⁴⁸ Suicide (D) PFC ⁴⁹ Suicide (D) PFC ⁵⁰ Suicide (D) PFC ⁵¹ Suicide (D) PFC ⁵² Suicide (D) PFC ⁴⁶	8
<i>CD24</i> CD24 molecule	209772_s_at	D	4		Suicide in mood disorders (D) NAC ¹⁵	8
<i>FOXN3</i> Forkhead box N3	230790_x_at	I	2	(Association) Suicide ⁵³	Suicide (I) PFC ⁵³	8
<i>GBP1</i> Guanylate binding protein 1, interferon-inducible, 67 kDa	231577_s_at 202269_x_at 202270_at	I	4 2 2		Suicide in mood disorders (D) NAC ¹⁵	8 6 6
<i>PIK3R5</i> Phosphoinositide-3-kinase, regulatory subunit 5	227553_at	I	4		Suicide in mood disorders (D) PFC ¹⁵	8
<i>APOL2</i> Apolipoprotein L2	221653_x_at	I	2		Suicide PFC (I) ⁵⁴	6
<i>ATP13A2</i> ATPase type 13A2	218608_at	D	2		Suicide (D) ¹⁵	6
<i>ATP6V0E1</i> ATPase, H ⁺ transporting, lysosomal 9 kDa, V0 subunit e1	214149_s_at 214244_s_at	I	2		Suicide (D) PFC ⁴⁶	6
<i>EPHX1</i> Epoxide hydrolase 1, microsomal (xenobiotic)	202017_at	D	2		Suicide in schizophrenia (D) PFC ⁵⁵	6
<i>GCOM1</i> GRINL1A complex locus 1	239099_at	I	2		Suicide in depression (D) PFC ⁵⁶	6
<i>HTRA1</i> HtrA serine peptidase 1	201185_at	D	2		Suicide (I) ¹⁵	6
<i>IL1B</i> Interleukin 1, beta	39402_at	I	2		Suicide (I) PFC ⁵⁷	6
<i>LEPR</i> Leptin receptor	211354_s_at	D	2		Suicide (D) PFC ⁵⁶ (D) PFC ⁵⁸ (D) HIP ⁵⁹ Suicide in depression (I) PFC ⁶⁰	6
<i>LHFP</i> Lipoma HMGIC fusion partner	218656_s_at	I	2		Suicide in mood disorders (I) NAC ¹⁵	6
<i>LIPA</i> Lipase A	236156_at	I	2		Violent suicide (I) PFC ⁶¹	6
<i>MARCKS</i> Myristoylated alanine-rich protein kinase C substrate	213002_at	I	2		Suicide in depression (I) ⁶²	6
<i>PGLS</i> 6-Phosphogluconolactonase	230699_at	I	2		Suicide PFC (D) ⁵⁴	6
<i>PTEN</i> Phosphatase and tensin homolog	222176_at	I	2		Suicide PFC, HIP (I) ²⁶	6
<i>RECK</i> Reversion-inducing-cysteine-rich protein with kazal motifs	216153_x_at	I	2		Suicide (I) PFC ¹⁵	6
<i>SPTBN1</i> Spectrin, beta, non-erythrocytic 1	200671_s_at	D	2		Suicide in mood disorders (I) NAC ¹⁵	6
<i>TNFSF10</i> Tumor necrosis factor (ligand) superfamily, member 10	202688_at 202687_s_at 214329_x_at	I	2		Suicide in schizophrenia (I) PFC ⁵⁵	6
<i>ABCA1</i> ATP-binding cassette, subfamily A (ABC1), member 1	203504_s_at	I	4		Suicide in depression (I) PFC ⁶⁰	4
<i>ARHGEF40</i> (FLJ10357) Rho guanine nucleotide exchange factor (GEF) 40	241631_at	I	4			4
<i>CASC1</i> Cancer susceptibility candidate 1	220168_at	I	4			4
<i>DHRS9</i> Dehydrogenase/reductase (SDR family) member 9	219799_s_at	I	4			4

Table 2. (Continued)

Gene symbol/gene name	Probesets	Change	Differential expression score	Prior human genetic evidence	Prior human brain expression evidence	Total CFG score
<u>DISC1</u> Disrupted in schizophrenia 1	244642_at	I	2	(Association) Suicide ⁵³		4
<u>EIF2AK2</u> Eukaryotic translation initiation factor 2-alpha kinase 2	204211_x_at	I	4			4
<u>LOC727820</u> Uncharacterized LOC727820	231247_s_at	I	4			4
<u>MAP3K3</u> Mitogen-activated protein kinase kinase 3	242117_at	I	4			4
<u>MBNL2</u> Muscleblind-like 2 (Drosophila)	205017_s_at	D	2	(Association) Suicide ⁵³		4
<u>MT-ND6 (ND6)</u> Mitochondrially encoded NADH dehydrogenase 6	1553575_at	I	4			4
<u>OR2J3</u> Olfactory receptor, family 2, subfamily J, member 3	217334_at	D	4			4
<u>RBM47</u> RNA binding motif protein 47	1565597_at	I	4			4
<u>RHEB</u> Ras homolog enriched in brain	227633_at	D	2	(Association) Suicide ⁶³		4
<u>RICTOR</u> RPTOR independent companion of MTOR, complex 2	228248_at	I	4			4
<u>SAMD9L</u> Sterile alpha motif domain containing 9-like	243271_at; 230036_at	I	4			4
<u>SCARF1</u> Scavenger receptor class F, member 1	206995_x_at	I	4			4
<u>SLC36A1</u> Solute carrier family 36 (proton/ amino acid symporter), member 1	213119_at	I	4			4
<u>STAT1</u> Signal transducer and activator of transcription 1, 91kDa	232375_at	I	4			4
<u>UBA6</u> Ubiquitin-like modifier activating enzyme 6	236879_at	I	4			4
<u>ZC3HAV1</u> Zinc finger CCCH-type, antiviral 1	1563075_s_at	I	4			4
<u>COX5B</u> Cytochrome c oxidase subunit Vb	213736_at	I	2	(Linkage) 2q11.2 ⁶⁴		3
<u>SMARCA1</u> SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 1	203874_s_at	I	2	(Linkage) Xq25 ⁵⁰		3
<u>DBP</u> D-box binding protein	209782_s_at	D	2			2

Abbreviations: I, increased in expression; D, decreased in expression; AMY, amygdala; PFC, prefrontal cortex; THAL, thalamus; HIP, hippocampus; NAC, nucleus accumbens.

The underlined gene names have human genetic association evidence.

Figure 3. Testing of biomarkers in suicide completers. **(a)** Upper: SAT1 (spermidine/spermine N1-acetyltransferase 1) expression is significantly increased ($P = 0.0057$) in our discovery work between subjects with high suicidal ideation (SI) (mean = 3413.37) and those reporting no SI (mean = 2642.97). Our test cohort of suicide completers (mean = 7171.51) showed significantly greater expression of SAT1 than both high SI ($P = 7.27 \times 10^{-7}$) and no SI ($P = 1.51 \times 10^{-7}$) groups from the discovery cohort. Lower: a suicide risk score was calculated by scoring the s.d. band a subject fell within as derived from the high SI discovery cohort, starting from the mean of the high-SI discovery cohort. A score of 0 indicates the subject falling between the means of the high SI and no SI subjects in the discovery cohort. A score of 1 means between the mean of the high SI and the first s.d. above it, score of 2 between the first and second s.d., score of 3 between the second and third s.d., and so on. Red line marks where the average SAT1 gene expression in high SI subjects would fall. **(b)** Upper: CD24 (CD24 molecule/small cell lung carcinoma cluster 4 antigen) expression was significantly decreased ($P = 0.0044$) within the discovery cohort between subjects reporting high SI (mean = 73.01) and no SI (mean = 108.634). The test cohort of suicide completers (mean = 71.61) was also significantly decreased ($P = 0.0031$) when compared with subjects reporting no SI. Lower: suicide risk score defined as the s.d. band in which the subject expression fell below the mean of the high-SI discovery cohort. Red line marks where the average CD24 gene expression in high SI subjects would fall. **(c)** Testing of top candidate biomarkers for suicidality. Thirteen out of the 41 CFG top-scoring biomarkers from Figure 2b (32%) showed step-wise significant change from no SI to high SI, to the validation suicide completers group. Six out of them (15%) remained significant after strict Bonferroni correction for multiple comparisons. The top CFG scoring biomarker SAT1 remained the top biomarker after validation.

administered at the time of each blood draw (Table 1). Out of 75 bipolar subjects (with a total of 174 visits) followed longitudinally in our study, there were 9 subjects that switched from a no SI (SI

score of 0) to a high SI state (SI score of 2 and above) at different visits, which was our intended study group. We used a powerful intrasubject design to analyze data from these 9 subjects and their

Table 3. Underlying biology

A. Pathways						
INGENUITY pathways				KEGG pathways		
No.	Top canonical pathways	P-value	Ratio	Pathway name	Enrichment score	Enrichment P-value
CFG score≥6.0; N = 21 genes						
1	Role of tissue factor in cancer	2.63E − 04	3/115 (0.026)	Apoptosis	6.69102	0.001242
2	Dendritic cell maturation	9.83E − 04	3/207 (0.014)	Measles	6.06369	0.002326
3	Melanoma signaling	1.13E − 03	2/46 (0.043)	Endometrial cancer	4.96787	0.006958
4	DHA signaling	1.18E − 03	2/49 (0.041)	Influenza A	4.90223	0.00743
5	Endometrial cancer signaling	1.69E − 03	2/57 (0.035)	Phosphatidylinositol signaling system	4.85448	0.007793
CFG score≥4.0; N = 41 genes						
1	NF-κB signaling	4.42E − 04	4/175 (0.023)	Measles	8.7667	0.000156
2	Dendritic cell maturation	5.38E − 04	4/207 (0.019)	Influenza A	6.87308	0.001035
3	PDGF signaling	7.5E − 04	3/85 (0.035)	mTOR signaling pathway	6.34986	0.001747
4	Role of pattern recognition receptors in recognition of bacteria and viruses	1.14E − 03	3/106 (0.028)	Apoptosis	4.75687	0.008592
5	Role of tissue factor in cancer	1.78E − 03	3/115 (0.026)	Toll-like receptor signaling pathway	4.37269	0.012617
B. Disease and disorders						
INGENUITY						
No.	Diseases and disorders	P-value		Number of molecules		
CFG score≥6.0; N = 21 genes						
1	Cancer	1.22E − 06 to 4.54E − 03		14		
2	Connective tissue disorders	2.19E − 04 to 3.41E − 03		8		
3	Inflammatory disease	2.19E − 04 to 4.54E − 03		8		
4	Skeletal and muscular disorders	2.19E − 04 to 4.42E − 03		9		
5	Gastrointestinal disease	2.22E − 04 to 4.54E − 03		12		
CFG score≥4.0; N = 41 genes						
1	Cancer	4.51E − 06 to 6.45E − 03		20		
2	Inflammatory response	2.70E − 05 to 6.45E − 03		12		
3	Antimicrobial response	9.95E − 05 to 6.45E − 03		4		
4	Infectious disease	1.25E − 04 to 5.52E − 03		6		
5	Connective tissue disorders	1.53E − 04 to 6.45E − 03		11		
Abbreviations: KEGG, Kyoto Encyclopedia of Genes and Genomes; CFG, Convergent Functional Genomics; DHA, docosahexaenoic acid; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor-κB.						

Abbreviations: KEGG, Kyoto Encyclopedia of Genes and Genomes; CFG, Convergent Functional Genomics; DHA, docosahexaenoic acid; mTOR, mammalian target of rapamycin; NF- κ B, nuclear factor- κ B.

24 visits. An intrasubject design factors out genetic variability, as well as some medications, lifestyle and demographic effects on gene expression, permitting identification of relevant signal with Ns as small as 1.¹⁴ An ancillary benefit of an intrasubject design may be accuracy/consistency of self-report of psychiatric symptoms ('phone expression'), similar in rationale to the signal-detection benefits it provides in gene expression. We also used an overall intersubject case–case analysis, to identify genes differentially expressed in the blood in no SI states versus high SI states (Figure 1). The number of subjects that met our criteria and were analyzed is small, but comparable to those in human postmortem brain gene expression studies of suicide.¹⁵ We are indeed treating the blood samples as surrogate tissue for brains, with the caveat that they are not the real target organ. However, with the blood samples from live human subjects we have the advantages of *in-vivo* accessibility, better knowledge of the mental state at the time of collection, less technical artifacts and especially of being able to do powerful intrasubject analyses from visit to visit. We considered and differentially scored only the very top 0.1 and 5% of the gene expression probesets distributions, and also required overlap

between the intrasubject and intersubject analyses of gene expression changes. Such a restrictive approach was used as a way of minimizing false positives, even at the risk of having false negatives (Figure 1c). For example, there were genes on each of the two lists, from intra- and intersubject analyses, that had clear prior evidence for involvement in suicidality, such as MT1E¹⁵ and GSK3B, respectively,¹⁶ but were not included in our subsequent analyses because they were not in the overlap.

We then used a CFG approach (Figure 2) to cross match the list of 246 overlapping top differentially expressed genes from the blood samples with other key lines of evidence (human postmortem brain data and human genetic data) implicating them in suicidality, as a way of identifying and prioritizing disease-relevant genomic biomarkers, extracting generalizable signal out of potential cohort-specific residual noise and genetic heterogeneity. We have built in our lab manually curated databases of the psychiatric genomic and proteomic literature to date, for use in CFG analyses.^{12,17–19} The CFG approach is thus a *de facto* field-wide collaboration. We use in essence, in a Bayesian fashion, the whole body of knowledge in the field to leverage findings from

Figure 4. SAT1 (spermidine/spermine N1-acetyltransferase 1) expression in the bipolar discovery cohort: relationship with suicidal ideation (SI), mood, psychosis, anxiety and stress. (a) SAT1 expression and SI item from Hamilton Rating Scale for Depression (HAMD) (scores of 0–4). (b) SAT1 expression and visual-analog scale for mood (0–100). High mood is to the left on the x-axis, low mood is to the right. (c) SAT1 expression and Hallucinations item from Positive and Negative Symptoms Scale (PANSS; scores of 1–7). Higher score indicates higher symptoms. (d) SAT1 expression and Delusions item from PANSS (scores of 1–7). Higher score indicates higher symptoms. (e) SAT1 expression and visual-analog scale for anxiety (0–100). Higher score indicates higher symptoms. (f) SAT1 expression and self-rating scale for stress (1–10). Higher score indicates higher symptoms. Only 20 out of 24 visits had stress data collected. * $P < 0.05$ between highest symptoms and lowest symptoms group.

our discovery data sets. Unlike our use of CFG in previous studies, for the current one we did not use any human peripheral tissue evidence from the literature, as there was none directly matching our genes, reflecting perhaps the dearth of peripheral gene expression work done so far on suicides, and the need for a study like ours. We also did not use animal model evidence, as there are to date no clear studies in animal models of self-harm or suicidality. SAT1 was the top-scoring blood biomarker, with the most extensive convergent evidence, increased in suicidal states identified by our work (that is, the top risk marker). CD24 (CD24 molecule/small cell lung carcinoma cluster 4 antigen) was the top blood biomarker decreased in suicidal states (that is, the top protective marker; Figure 2 and Table 2).

Testing in suicide completers

In order to know whether our findings relate to actual completed suicide, we then tested SAT1 levels in the blood samples from a heterogeneous cohort of nine consecutive male suicide completers obtained from the coroner's office, with the following characteristics: we required that the cases included in our analysis had a postmortem interval from last observed alive under 24 h, and that they had committed suicide by means other than overdoses, which could alter gene expression. Remarkably, we found SAT1 gene expression levels to be elevated in nine out of nine (100%) subjects who committed suicide, that we tested. In each of the suicide completers, the increase in SAT1 was at least

three s.d. above the average levels in high SI subjects, which constitutes a very stringent threshold for use as a predictive biomarker (Figure 3). We also examined other top candidate biomarkers for suicidality (Figure 3 and Supplementary Figure S3). Remarkably, 13 out of the 41 CFG top-scoring biomarkers from Figure 2b (32%) showed step-wise significant change from no SI to high SI, to the test suicide completers group. Six out of them (15%) remained significant after strict Bonferroni correction for multiple comparisons (Figure 3). The top CFG scoring biomarker SAT1 remained the top biomarker after validation.

Mechanistic understanding

Pathway analyses of our suicidality biomarker data identified among the top pathways the omega-3 docosahexaenoic acid signaling pathway. Low omega-3 levels have been correlated with increased suicidality in human epidemiological studies.^{20,21} Several of the biomarkers from our current study (SAT1, S100A8, IL1B and 16 others) were changed in expression by omega-3 treatment in the blood of the circadian clock gene DBP (D-box binding protein) knock-out mouse model in opposite direction to our human suicidality data (Supplementary Table S6). DBP is also one of the biomarkers identified to be decreased in high suicidal states in the current analysis. Serendipitously, previous work by our group has implicated DBP in mood disorders,²² psychosis,²³ alcoholism⁹ and anxiety disorders.¹⁰ Mice engineered to lack DBP were stress-reactive and displayed a behavioral phenotype similar

Figure 5. Prospective validation of SAT1 (spermidine/spermine N1-acetyltransferase 1): follow-up of future psychiatric hospitalizations due to suicidality. We analyzed in 42 bipolar subjects whether their SAT1 levels at the time of initial testing differentiated those who had subsequent hospitalizations due to suicidality in the years since the testing occurred. Range was 0.33–5.92 years of follow-up, average 2.48 years. **(a)** Upper half of SAT1 scores versus lower half of SAT1 scores. Twenty-one subjects in each group. There were six psychiatric hospitalizations not due to suicidality, and eight psychiatric hospitalizations due to suicidality. **(b)** Upper tertile of SAT1 scores versus lower tertile of SAT1 scores. Fourteen subjects in each group. There were three psychiatric hospitalizations not due to suicidality, and four psychiatric hospitalizations due to suicidality.

to bipolar disorder and comorbid alcoholism.²⁴ In addition to bipolar disorder, alcoholism increases risk for suicide.²⁵ Phosphatase and tensin homolog (PTEN), a biomarker increased in suicidality in the current study in the blood, as well as in the brain of suicide completers,²⁶ was also increased in the amygdala and was decreased in the prefrontal cortex of DBP knock-out mice subjected to stress.²⁵ S100A8, another biomarker increased in suicidality in the current study, was also increased in the blood of DBP stressed mice. Treatment with omega-3 fatty acids normalized the phenotype of those mice.²⁷

Other circadian clock-modulated genes identified by our analysis as biomarkers for suicidality were PIK3R5, MARCKS, IL1B, CASC1, CCRN4L, H3F3B, RBCK1, TNK2 and UBE2B. Circadian genes are involved in sleep–wake cycles, as well as mood regulation.^{6,7,22,28,29} Abnormal sleep (insomnia) has been identified as a

risk factor for suicide.³⁰ IL1B is also an inflammatory marker, and has previously been implicated by us in anxiety disorders.¹⁰

In addition, S100A8, MBNL2 and three other biomarkers had evidence for modulation by clozapine in the blood in opposite direction to our human suicidality data in previous independent animal model pharmacogenomics studies conducted by us^{4,23} (Supplementary Table S6). Clozapine is the only FDA-approved treatment for suicidality.³¹

Thus, the convergent evidence for our biomarkers is strong in translational ways beyond those used for their discovery and selection. S100A8 may be a key biomarker to monitor in terms of response to treatment with classic (clozapine) and complementary (omega-3) agents. Other potential drugs to be studied for modulating suicidality were revealed by our analyses (Supplementary Tables S5 and S6).

SAT1, FOXN3, DISC1, MBNL2 and RHEB had genetic association evidence for suicidality, suggesting that they are not only state biomarkers but also trait factors influencing suicidal risk. DISC1 is also one of the top candidate genes for schizophrenia based on a large-scale CFG analysis of schizophrenia genome-wide association study we recently conducted,¹¹ while DISC1 and MBNL2 are also among of the top candidate genes for bipolar disorder based on a large-scale CFG analysis of bipolar disorder genome-wide association study we previously conducted.⁷ In addition, DISC1 has clear animal model data for the role of its interaction with environmental stress in the pathophysiology of psychotic depression.³² DISC1 and MBNL2 may thus be key state and trait factors for suicidality risk in psychotic mood disorder subjects, and an indication for clozapine treatment in such subjects.

We also looked at the overlap of our suicide biomarkers with our previous mood biomarker³ and psychosis biomarker⁴ work (Supplementary Table S7), as well as with the human postmortem brain literature for other psychiatric disorders (Supplementary Table S8). DOCK5 and four other biomarkers were changed in high suicidal states in the opposite direction to their change in high mood states, and DOCK5 and six other biomarkers were changed in the same direction as their change in high psychosis states, suggesting that suicidality could be viewed as a psychotic dysphoric state, and that DOCK5 may be an additional key biomarker reflecting that state. This molecularly informed view is consistent with the emerging clinical evidence in the field.³³

The convergence of evidence then suggests that at least in the population we studied, suicidality may be associated with dysphoric mood, as well as increased psychosis, anxiety and stress. In our own data, SAT1 blood gene expression levels showed a trend towards increase in low mood, high psychosis, high anxiety and high stress in our bipolar subjects (Figure 4).

Prospective validation

To further validate SAT1, our top marker, we also looked at subsequent hospitalizations with and without suicidality (Table 1 and Supplementary Table S9), and previous hospitalizations with and without suicidality (Supplementary Table S10), in two live cohorts, one bipolar ($n=42$) and one psychosis (schizophrenia/schizoaffective; $n=46$). Higher SAT1 levels compared with lower SAT1 levels at the time of testing differentiated future and past hospitalizations owing to suicidality in the bipolar disorder subjects (Figure 5). A similar but weaker pattern was exhibited in the psychosis (schizophrenia/schizoaffective) subjects (Supplementary Figure S2). Remarkably, besides SAT1, three other (PTEN, MARCKS and MAP3K3) of the six biomarkers that survived Bonferroni correction in the suicide completers cohort validation step also showed similar but weaker results (Supplementary Table S11 and Supplementary Figure S3). Taken together, the prospective and retrospective hospitalization data suggests SAT1,

Figure 6. Multi-dimensional prediction of future psychiatric hospitalizations due to suicidality. We analyzed in 42 bipolar subjects whether their SAT1 (spermidine/spermine N1-acetyltransferase 1), anxiety, mood and psychosis levels at the time of initial testing differentiated from those who had subsequent hospitalizations due to suicidality in the years since the testing occurred. Data in each dimension was normalized to a 0–100 scale (with the mood visual-analog scale (VAS) inverted, as the assumption was made that depressed mood states would more closely correlate with suicidality). The angle between dimensions was assumed to be 90°, and a simple Pythagorean distance from origin score was calculated. The distribution of this score in the test cohort was used to generate a receiver-operating characteristic curve for hospitalizations due to suicidality. **(a)** ROC curve. **(b)** Detailed results. **(c)** Three-dimensional visualization.

PTEN, MARCKS and MAP3K3 might be not only state markers but perhaps trait markers as well.

We also examined whether using a multi-dimensional approach enhanced our ability to predict future hospitalizations, by adding data about mood, anxiety and psychosis to the data about the SAT1 expression levels (Figure 6). We found that the receiver-operating characteristic curve improved in a step-wise fashion, from an area under the curve of 0.640 with SAT1 alone, to an area under the curve of 0.798 with SAT1 and anxiety, area under the curve of 0.813 with SAT1, anxiety and mood, and area under the curve of 0.835 with SAT1, anxiety, mood and psychosis. From our

preliminary work, we identified levels of SAT1 that provide different levels of sensitivity and specificity (Supplementary Table S12). The anxiety and mood information was obtained from simple visual-analog scales, previously described by us.³⁴ The psychosis information is based on the combining of the scores on the hallucinations and delusions in the Positive and Negative Symptoms Scale (Supplementary Figure S5). Of note, this simple clinical-genomic approach does not directly ask about SI, which some individuals may deny or choose not to share with clinicians. Similar data were obtained for the panel of six top markers as shown in Supplementary Figure S6.

DISCUSSION

Using discovery in live subjects and validation in suicide completers, we found possible biomarkers for suicidality. Our top biomarker finding, SAT1, as well as PTEN, MARCKS and MAP3K3, were additionally validated by prospective and retrospective analyses in live subjects, looking at the ability to predict and differentiate future and past hospitalizations due to suicidality in bipolar disorder and psychosis (schizophrenia/schizoaffective; Supplementary Table S11).

Apoptosis

Beyond predictions, as a window into the biology of suicidality, the current work shows overlap at a gene and pathway level with apoptosis (Table 3, Supplementary Table S3 and S4). SAT1, for example, is a key catabolic enzyme for polyamines. Polyamine levels within cells control cell viability, and significant decreases in polyamine levels can result in apoptosis.³⁵ They seem to reflect an endowment for cellular and organismal activity and growth, key characteristics of mood.^{3,7,36} SAT1, which is increased in live SI subjects and in suicide completers in our studies, is highly inducible by a variety of stimuli, including toxins, cytokines, heat shock, ischemia and other stresses. SAT1-overexpressing mice had alterations in their polyamine pool, hair loss, infertility and weight loss.^{37,38} Turecki and colleagues³⁹ have provided compelling evidence for changes in the polyamine system in the brain of suicide completers. CD24, our top biomarker decreased in suicidal subjects, also has roles in apoptosis. Mice lacking CD24 show an increased rate of apoptosis.⁴⁰ It could be that simpler mechanisms related to cellular survival and programmed cell-death decision have been recruited by evolution for higher mental functions, such as feelings, thoughts, actions and behaviors, leading to suicidality. In that sense, suicidality could be viewed as whole-organism apoptosis ('self-apoptosis'). Apoptosis mechanisms have previously been implicated in mood disorders, and their inhibition in affective resilience.⁴¹ Interestingly, lithium, a medication with clinical evidence for preventing suicidality in bipolar disorder,⁴² has anti-apoptotic effects at a cellular level.⁴³ Imaging studies have shown reduced gray matter volume in the brain of individuals with bipolar disorder and history of suicide attempts. Long-term lithium treatment was associated with increased gray matter volumes in the same areas where suicide was associated with decreased gray matter.⁴⁴

Conclusions and future directions

Taken together, our results have implications for the understanding of suicide, as well as for the development of objective laboratory tests and tools to track suicidal risk and response to treatment. First, our results open empirical avenues for future field trials, clinical testing and validation in various at-risk populations, including studies in individuals with major depressive disorder. The current work was based on subjects with bipolar disorder, psychosis (schizophrenia/schizoaffective disorder) and coroner's office cases, which may represent a more externalizing or impulsive population and type of suicidality. Other types are likely to exist. Second, more work also needs to be done to examine potential gender and ethnicity differences. Our current work is based on male Caucasian subjects. Third, predicting suicidal feelings and thoughts (ideation) may be different than predicting suicidal actions and behaviors. Our current work has focused on suicide completers and hospitalizations, admittedly a more emergent concern. Fourth, state versus trait issues and sensitivity versus specificity for suicidality, for the individual markers identified by us, as well as for panels of markers and multi-modal approaches, need to be studied more extensively in different populations. Fifth, past individual and family history, as well as environmental context, may help improve predictive

approaches. Our approach was very focused and reductionist, albeit with good results.

Given the fact that approximately one million people die of suicide worldwide each year, and this is a potentially preventable cause of death, the need for, urgency and importance of efforts such as ours cannot be overstated.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

ABN designed the study and wrote the manuscript. HLN, DFL and MA analyzed the data. LP, LMG, NJ, EW, SB and GS performed database work. EB, KO, HD, JV, RS and MR organized and conducted testing in bipolar disorder subjects. MY, AB, AS and GES organized and carried out postmortem sample collections. NJS, SMK and DRS conducted microarray experiments and provided input on data analyses. All authors discussed the results and commented on the manuscript.

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