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–acyltransferase 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 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...
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 diatomic 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) 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.

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 | 0  |
| 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 disorder NOS           | 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  | Silt 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   | 57  | M      | Caucasian | 2437.40                | 3.00                | 0                           | 0                                 | 0.00                                        | 0.00                                        |
| phchp053v1       | Bipolar I disorder   | 51  | M      | Caucasian | 2538.90                | 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                                        |
| phchp136v1       | 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 | 2730.90                | 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 I 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                           | 0                                 | 0.00                                        | 0.00                                        |
| phchp109v1       | Bipolar I disorder   | 22  | M      | Caucasian | 3200.80                | 3.00                | 1                           | 2                                 | 0.33                                        | 0.67                                        |
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.

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 |
| phchp066v1 | Bipolar II disorder | 39 | M | Caucasian | 4214.70 | 5.58 | 0 | 0 | 0.00 | 0.00 |
| phchp142v2 | 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
---|---|---|---
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 | 8.1 | 8.7
Range | 29–56 | 28–55 | 28–56
Ethnicity (Caucasian/African American) | (9/0) | (9/0) | (9/0)

Coroner’s office test cohort—suicide completers (n = 9)

Number of subjects (number of chips) | 9 (9)
Age (years)
Mean | 49.8
s.d. | 17
Range | 26–68

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

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 | 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–acyetyltransferase 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.
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 RNA 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 Suite 6.6 software package (Partek Incorporated, St Louis, MO, USA). Using only the perfect match values, we ran a robust multi-array analysis (RMA), background corrected with quantile normalization and a median polish of expression levels of all probesets for each chip. Then, to establish a list of differentially expressed probesets we ran two analyses.

1. 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 54675 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 54675 total probesets) had an absolute score of 11 and greater, and received an internal CFG score of 3 points.

2. 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 NetAffx (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 NetAffx, 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://compgen.rutgers.edu/mapinterpolator. For linkage convergence, the start of the gene had to map within 5 CM of the location of a marker linked to the disorder.

CFG scoring. For CFG analysis (Figure 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
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. 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 | Prior human genetic evidence | Prior human brain expression evidence | Total CFG score |
|----------------------------------------|-----------|--------|------------------------------|----------------------------------------|-----------------|
| SAT1, Spermidine/spermine N1–acetyltransferase 1 | 203455_s_at | I 2    | (Association) Suicide attempt<sup>45</sup> suicide<sup>46</sup> | Suicide in depression (D) PFC<sup>47</sup> | 8               |
| CD24                                   | 209772_s_at | D 4    | (Association) Suicide<sup>53</sup> | Suicide in mood disorders (D) PFC<sup>55</sup> | 8               |
| FOXN3                                  | 230790_x_at | I 2    | Suicide in mood disorders (D) PFC<sup>54</sup> | Suicide in mood disorders (D) PFC<sup>55</sup> | 8               |
| Forkhead box N3                         |           |        | Suicide (I) PFC<sup>56</sup> | Suicide in mood disorders (D) PFC<sup>57</sup> | 6               |
| GP1                                    | 231577_s_at | I 2    | Suicide (I) PFC<sup>57</sup> | Suicide in mood disorders (D) PFC<sup>57</sup> | 6               |
| Phosphoinositide-3-kinase, regulatory subunit 5 | 227553_s_at | I 4    | Suicide in mood disorders (D) PFC<sup>57</sup> | Suicide in mood disorders (D) PFC<sup>57</sup> | 8               |
| APOL2                                  | 221653_x_at | I 2    | Suicide PFC (I)<sup>54</sup> | Suicide in schizophrenia (D) PFC<sup>55</sup> | 6               |
| ATP13A2                                 | 218608_s_at | D 2    | Suicide in depression (D) PFC<sup>57</sup> | Suicide in depression (D) PFC<sup>57</sup> | 6               |
| ATPase type 13A2                        | 214149_s_at | I 2    | Suicide (I) PFC<sup>57</sup> | Suicide in schizophrenia (D) PFC<sup>57</sup> | 6               |
| ATPase, H+ transporting, lysosomal 9 kDa, V0 subunit e1 | 214244_s_at | D 2    | Suicide in schizophrenia (D) PFC<sup>57</sup> | Suicide in schizophrenia (D) PFC<sup>57</sup> | 6               |
| EPHX1, Epoxide hydrolase 1, microsomal (benzobin) | 202017_at | D 2    | Suicide in schizophrenia (D) PFC<sup>57</sup> | Suicide in schizophrenia (D) PFC<sup>57</sup> | 6               |
| GCOM1                                   | 239099_at | I 2    | Suicide in depression (D) PFC<sup>57</sup> | Suicide in schizophrenia (D) PFC<sup>57</sup> | 6               |
| GRINL1A complex locus 1                 | 201185_at | D 2    | Suicide (I)<sup>55</sup> | Suicide in mood disorders (I) PFC<sup>55</sup> | 6               |
| HTRA1                                   | 39402_at  | I 2    | Suicide (I)<sup>55</sup> | Suicide in mood disorders (I) PFC<sup>55</sup> | 6               |
| Interleukin 1, beta                     | 211354_s_at | D 2    | Suicide (I) PFC<sup>55</sup> | Suicide in mood disorders (I) PFC<sup>55</sup> | 6               |
| Leptin receptor                         |           |        | Suicide (I) PFC<sup>55</sup> | Suicide in mood disorders (I) PFC<sup>55</sup> | 6               |
| LHFP                                    | 218656_s_at | I 2    | Suicide PFC (I)<sup>54</sup> | Suicide in schizophrenia (I) PFC<sup>55</sup> | 6               |
| Lipoma HMGIC fusion partner            | 236156_at | I 2    | Suicide PFC (I)<sup>54</sup> | Suicide in schizophrenia (I) PFC<sup>55</sup> | 6               |
| Lipase A                                | 213002_at | I 2    | Suicide PFC (I)<sup>54</sup> | Suicide in schizophrenia (I) PFC<sup>55</sup> | 6               |
| Myristoylated alanine-rich protein kinase C substrate | 230699_at | I 2    | Suicide PFC (I)<sup>54</sup> | Suicide in schizophrenia (I) PFC<sup>55</sup> | 6               |
| PGLS                                   | 222176_at | I 2    | Suicide PFC (I)<sup>54</sup> | Suicide in schizophrenia (I) PFC<sup>55</sup> | 6               |
| Phosphatase and tensin homolog RECK    | 216153_s_at | I 2    | Suicide PFC (I)<sup>54</sup> | Suicide in schizophrenia (I) PFC<sup>55</sup> | 6               |
| 6-Phosphogluconolactonase               | 200671_s_at | D 2    | Suicide PFC (D)<sup>54</sup> | Suicide in mood disorders (I) PFC<sup>55</sup> | 4               |
| Phosphatase and tensin homolog          | 202688_s_at | I 2    | Suicide PFC (D)<sup>54</sup> | Suicide in mood disorders (I) PFC<sup>55</sup> | 4               |
| Reversion-inducing-cysteine-rich protein with kazal motifs | 202687_s_at | I 2    | Suicide PFC (D)<sup>54</sup> | Suicide in mood disorders (I) PFC<sup>55</sup> | 4               |
| SPTBN1                                  | 214329_s_at | I 2    | Suicide PFC (D)<sup>54</sup> | Suicide in mood disorders (I) PFC<sup>55</sup> | 4               |
| Spectrin, beta, non-erythrocytic 1      | 203504_s_at | I 4    | Suicide PFC (D)<sup>54</sup> | Suicide in mood disorders (I) PFC<sup>55</sup> | 4               |
| TNFSF10                                 | 241631_at | I 4    | Suicide PFC (D)<sup>54</sup> | Suicide in mood disorders (I) PFC<sup>55</sup> | 4               |
| Tumor necrosis factor (ligand) superfamily, member 10 | 220168_at | I 4    | Suicide PFC (D)<sup>54</sup> | Suicide in mood disorders (I) PFC<sup>55</sup> | 4               |
| ABCA1                                   | 219799_s_at | I 4    | Suicide PFC (D)<sup>54</sup> | Suicide in mood disorders (I) PFC<sup>55</sup> | 4               |
| ATP-binding cassette, subfamily A (ABC1), member 1 | 202687_s_at | I 2    | Suicide PFC (D)<sup>54</sup> | Suicide in mood disorders (I) PFC<sup>55</sup> | 4               |
| ARHGGEFH40 (FLJ10357)                  | 214329_s_at | I 2    | Suicide PFC (D)<sup>54</sup> | Suicide in mood disorders (I) PFC<sup>55</sup> | 4               |
| Rho guanine nucleotide exchange factor (GEF) 40 | 203504_s_at | I 4    | Suicide PFC (D)<sup>54</sup> | Suicide in mood disorders (I) PFC<sup>55</sup> | 4               |
| CASCA1                                  | 219799_s_at | I 4    | Suicide PFC (D)<sup>54</sup> | Suicide in mood disorders (I) PFC<sup>55</sup> | 4               |

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Table 2. (Continued)

| Gene symbol/gene name | Probesets | Change | Differential expression score | Prior human genetic evidence | Prior human brain expression evidence | Total CFG score |
|-----------------------|-----------|--------|-------------------------------|-----------------------------|--------------------------------------|----------------|
| DISC1                 | 244642_at | I      | 2                             | (Association) Suicide$^{53}$ |                                      | 4              |
| EIF2AK2               | 204211_x_at | I    | 4                             |                             |                                      | 4              |
| Eukaryotic translation initiation factor 2-alpha kinase 2 LOC277820 | | | | | | |
| LOC277820 Uncharacterized LOC277820 MAPK3K  | 231247_s_at | I | 4                             |                             |                                      | 4              |
| Mitogen-activated protein kinase kinase 3 MBNL2 | 242117_at | I | 4                             |                             |                                      | 4              |
| Musc-blind-like 2 (Drosophila) MT-ND6 (ND6) | | | | | | |
| MT-ND6 (ND6)     | 205017_s_at | D | 2                             | (Association) Suicide$^{53}$ |                                      | 4              |
| Mitochondrially encoded NADH dehydrogenase 6 OR2J3 | | | | | | |
| Olfactory receptor, family 2, subfamily J, member 3 RBM47 | | | | | | |
| RNA binding motif protein 47 RHEB | 227633_at | D | 2                             | (Association) Suicide$^{53}$ |                                      | 4              |
| Ras homolog enriched in brain RICTOR | 228248_at | I | 4                             |                             |                                      | 4              |
| RPTOR independent companion of MTOR, complex 2 SAMD9L | | | | | | |
| Sterile alpha motif domain containing 9-like SCARF1 | 206995_x_at | I | 4                             |                             |                                      | 4              |
| Scavenger receptor class F, member 1 SLC36A1 | | | | | | |
| Solute carrier family 36 (proton/amino acid symporter), member 1 STAT1 | | | | | | |
| Signal transducer and activator of transcription 1, 91kDa UBA6 | 232375_at | I | 4                             |                             |                                      | 4              |
| Ubiquitin-like modifier activating enzyme 6 ZC3HAV1 | 236879_at | I | 4                             |                             |                                      | 4              |
| Zinc finger CCCH-type, antiviral 1 COX5B | 1563075_s_at | I | 4                             |                             |                                      | 4              |
| Cytochrome c oxidase subunit Vb SMARCA1 | 213736_at | I | 2                             | (Linkage) 2q11.2$^{44}$     |                                      | 3              |
| SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 1 DHR | 203874_s_at | I | 2                             | (Linkage) Xq25$^{50}$        |                                      | 3              |
| D-box binding protein SAT1 | 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 ($P = 7.27e-07$) and no SI ($P = 1.51e-07$) 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 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

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| CFG Score | Gene   | Direction of Change | P-Value (One-Way ANOVA) |
|-----------|--------|---------------------|------------------------|
| 8         | SAT1   | I                   | 2.91E-13               |
| 4         | UBA6   | I                   | 8.94E-05               |
| 6         | MARCKS | I                   | 0.000187221            |
| 6         | PTEN   | I                   | 0.000298958            |
| 4         | MT-ND6 | I                   | 0.000391061            |
| 4         | MAP3K3 | I                   | 0.000777774            |
| 6         | LHPF   | I                   | 0.00153921             |
| 4         | LOC727820 | I                  | 0.003706529            |
| 8         | CD24   | D                   | 0.006082698            |
| 6         | RECK   | I                   | 0.009302353            |
| 8         | FOXN3  | I                   | 0.01040264             |
| 4         | SCARF1 | I                   | 0.014880001            |
| 4         | RICTOR | I                   | 0.040726456            |
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 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.

### Table 3. Underlying biology

#### A. Pathways

| No. | Top canonical pathways | P-value | Ratio | Pathway name | Enrichment score | Enrichment P-value |
|-----|-----------------------|---------|-------|--------------|------------------|-------------------|
| 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 | 2/64 (0.034) | Endometrial cancer | 4.96787 | 0.006958 |
| 4   | DHA signaling | 1.18E – 03 | 2/49 (0.041) | Influenza A | 4.90223 | 0.007743 |
| 5   | Endometrial cancer signaling | 1.69E – 03 | 2/57 (0.035) | Phosphatidylinositol signaling system | 4.835448 | 0.007793 |

#### B. Disease and disorders

| No. | Diseases and disorders | P-value | Number of molecules |
|-----|-----------------------|---------|-------------------|
| 1   | Cancer | 1.22E – 06 to 4.14E – 03 | 14 |
| 2   | Connective tissue disorders | 2.19E – 04 to 5.52E – 03 | 12 |
| 3   | Inflammatory disease | 2.19E – 04 to 4.54E – 03 | 8 |
| 4   | Skeletal and muscular disorders | 2.19E – 04 to 4.42E – 03 | 8 |
| 5   | Gastrointestinal disease | 2.22E – 04 to 4.54E – 03 | 9 |

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.
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. 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, psychosis, alcoholism and anxiety disorders. Mice engineered to lack DBP were stress-reactive and displayed a behavioral phenotype similar...
to bipolar disorder and comorbid alcoholism.\textsuperscript{24} In addition to bipolar disorder, alcoholism increases risk for suicide.\textsuperscript{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,\textsuperscript{26} was also increased in the amygdala and was decreased in the prefrontal cortex of DBP knock-out mice subjected to stress.\textsuperscript{25} S100A8, another biomarker increased in the blood of suicide completers,\textsuperscript{22,28,29} was decreased in the prefrontal cortex of DBP knock-out mice. Treatment with omega-3 fatty acids 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,\textsuperscript{11} while DISC1 and MBNL2 are also among the top candidate genes for bipolar disorder based on a large-scale CFG analysis of bipolar disorder genome-wide association study we previously conducted.\textsuperscript{7} In addition, DISC1 has clear animal model data for the role of its interaction with environmental stress in the pathophysiology of psychotic depression.\textsuperscript{24} 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 biomarker\textsuperscript{6} and psychosis biomarker\textsuperscript{4} 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.\textsuperscript{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, risk factor for suicide.\textsuperscript{30} IL1B is also an inflammatory marker, and has previously been implicated by us in anxiety disorders.\textsuperscript{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\textsuperscript{2,23} (Supplementary Table S6). Clozapine is the only FDA-approved treatment for suicidality.\textsuperscript{21}

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).

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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.
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. 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.

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.
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. They seem to reflect an endowment for cellular and organismal activity and growth, key characteristics of mood. SAT1, which is increased in live suicidal 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. Turecki and colleagues have provided compelling evidence for changes in the polyamine system in the brain of suicide completers. CD24, our top biomarker decreased in suicidal completers. CD24, our top biomarker decreased in suicidal

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

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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 GE 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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predicting 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.
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