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PERSPECTIVE

Psychiatric blood biomarkers: avoiding jumping to premature negative or positive conclusions

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Blood biomarkers may provide a scientifically useful and clinically usable peripheral signal in psychiatry, as they have been doing for other fields of medicine. Jumping to premature conclusions, negative or positive, can create confusion in this field. Reproducibility is a hallmark of good science. We discuss some recent examples from this dynamic field, and show some new data in support of previously published biomarkers for suicidality (SAT1, MARCKS and SKA2). Methodological clarity and rigor in terms of biomarker discovery, validation and testing is needed. We propose a set of principles for what constitutes a good biomarker, similar in spirit to the Koch postulates used at the birth of the field of infectious diseases.

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… 'With all thy getting, get understanding' …
—Proverbs 4:7 King James Version (KJV)

The identification of blood biomarkers for disease risk has emerged as an important area of translational research in medicine, particularly in cancer[1] and cardiovascular medicine,[2] in the quest for precision and individualization of preventive measures and of treatment. Although genetic tests may have a useful role as well[3] and can be done early on in life, biomarkers that look at gene expression, proteomic or metabolomic characteristics better reflect the gene–environment interactions that lead to disease manifestation. In psychiatry, in particular, it is impractical to directly access the target organ—the brain—in live individuals, and even its proxy fluid, the cerebrospinal fluid, is less accessible for routine use than the blood. Although it is clear that the blood is not the brain, there are common biological mechanisms, environmental and medication effects across tissues that can be identified with convergent approaches.[4,5] Upon demonstration of reproducibility and predictive ability in independent cohorts, the key litmus tests, such biomarkers should be rapidly moved into population testing and validation for clinical use. The unmet need in psychiatry is great, and the potential of biomarkers to revolutionize clinical management is commensurate with that. However, the burden of proof needs to be high as well.

We have recently published a study detailing the identification of blood gene expression biomarkers for suicidality.[6] The discovery was carried out using a powerful but hard to accomplish longitudinal within-subject design in male bipolar subjects, a high-risk group for suicide, identifying a small but very valuable subgroup of subjects who exhibited major switches in suicidal ideation at the time of different testing visits. Differential gene expression studies within each subject were carried out, factoring out any genetic and a majority of environmental effects. (Self-)report of phenotype is also more accurate in a within-subject design. In the effect, this design is arguably better than an identical twin study. What was changed in expression in common across the different subjects was carried forward in the analysis. A convergent functional genomics (CFG) approach was then used to prioritize the differentially expressed gene list, using independent lines of evidence implicating them in suicide (human genetic studies, human postmortem brain studies). Validation of the genes prioritized by CFG was carried out by testing for changes in expression in the blood of a demographically matched for gender and age cohort of male violent (non-overdose) suicide completers from the coroners' office. Six genes (SAT1, MARCKS, UBA6, PTEN, MAP3K and MT-ND6) showed a statistically significant, Bonferroni corrected, stepwise change in expression from live bipolar subjects in no suicidal states to high suicidal states to suicide completers. To further validate the markers and demonstrate their predictive ability, we examined in a larger male bipolar cohort whether biomarker levels can predict future psychiatric hospitalizations for suicidality, and demonstrated that they did. The panel of six markers had an AUC of 0.73, and a *P*-value of 0.04. This work has opened the field to active exploration, and raised hopes for progress. What was not known at the time, and is an area of active exploration by us and others, was: (1) whether these biomarkers work in diagnoses other than bipolar disorder or there are other biomarkers better suited for other diagnoses, (2) whether gender has a role, that is, would a different set of biomarkers work in females and (3) whether type of suicide (violent vs non-violent and impulsive vs planned) is underpinned by different sets of markers. The answer to these questions is likely to be at least partially yes. Gene expression is powerfully modulated by disease biology and medication effects in the first instance, by gender biology and sex hormones in the second instance, and by stress reactivity, drug abuse and toxicology in the third instance. Since the publication of our work in 2013, a study by Weinberger and colleagues[7] has replicated one of our six markers, MARCKS, in the brains of male violent suicide completers with schizophrenia, but not those with depression or with non-violent suicide. Another study by Kaminsky and colleagues[8] identified a new biomarker for suicide, SKA2, primarily in depression cohorts. Interestingly, SKA2 has effects upstream of our top biomarkers, SAT1, and may regulate its transcription.

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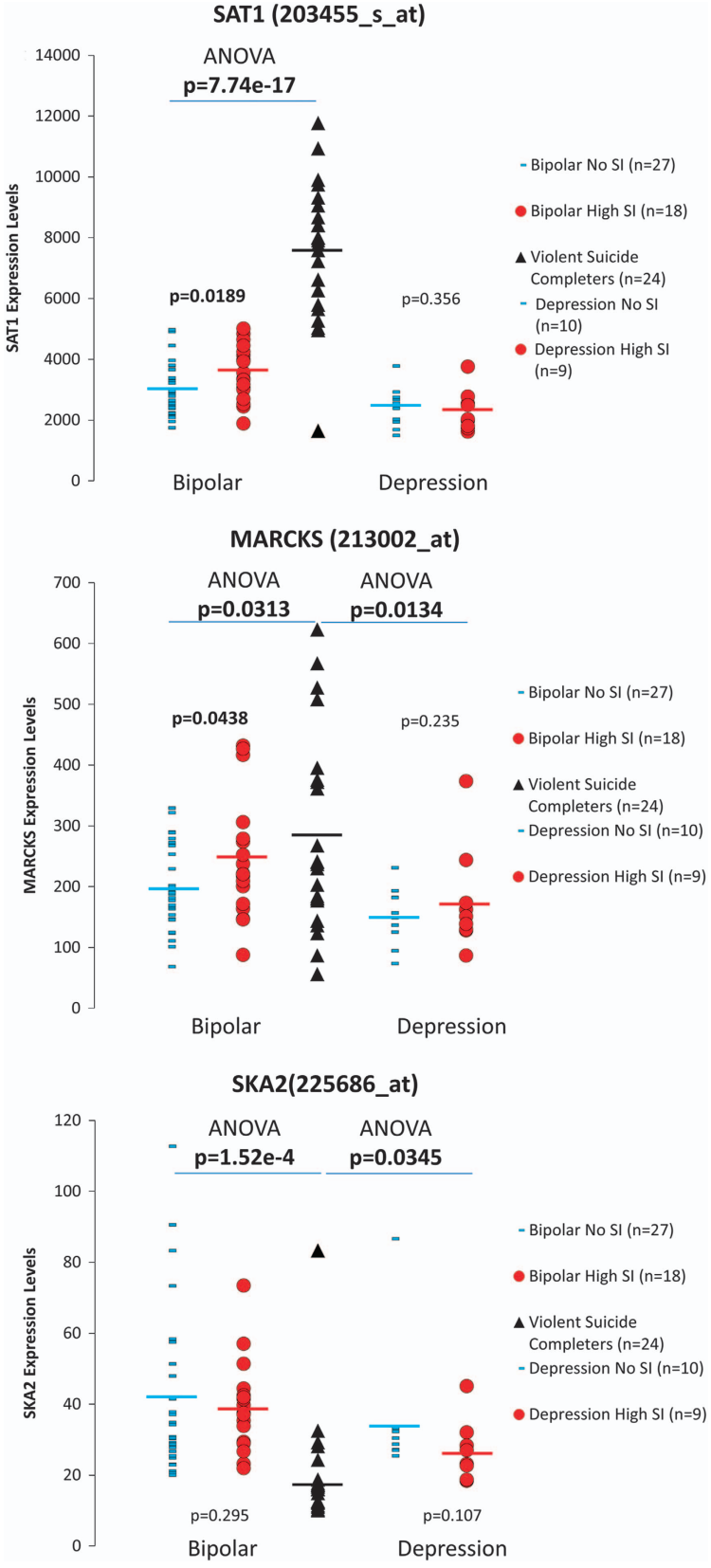

Figure 1. Reproducibility and diagnostic differences for top biomarkers for suicidality. Methods are as previously described.[6] Demographics of cohorts are presented in Supplementary Table S1. SAT1 and MARCKS, previously identified as top blood biomarkers increased in expression in suicidality by our group,[6] are significantly increased in violent suicide completers and in live subjects with high suicidal ideation (SI) in bipolar disorder, but not depression. SKA2, previously identified as a blood biomarker decreased in expression in suicidality by Kaminsky and colleagues,[8] is significantly decreased in violent suicide completers.

A study just reported by Mullins *et al*.[9] purports not to replicate our six top biomarkers. The authors jump to the conclusion of wanting to make a general statement in that regard. However, the study was carried out: (1) in depression as opposed to our bipolar work, (2) in a cohort that was 75% female as opposed to our exclusively male cohorts and (3) in a different phenotype/type of suicidality (treatment emergent suicidal ideation in response to antidepressant treatment). Moreover, the probands and controls were not matched for age (*P*-value for age difference was significant at $P = 0.02$). That may not matter much in the genetic (DNA) research arena in which the senior study authors have a good prior track record and expertise. For gene expression (RNA), however, age matters. Particularly unfortunate, given the fact that their sample was 75% females and thus female sex hormones come into play in terms of effects on gene expression, is the fact that their probands were on average menopausal, and their controls on average pre-menopausal. Thus, these data are hard to rely upon, and general conclusions based on it are unwarranted.

That being said, better designed biomarker discovery and validation studies in depression, in females, and in other types of suicidality are areas that should be encouraged and vigorously pursued. Our group and others are actively working on this. It seems clear that there may be differences between bipolar disorder and depression, as shown here in Figure 1, where we reproduce in larger cohorts our previous findings of increased SAT1 and MARCKS,[6] as well as independently reproduce the decreased SKA2 finding reported by Kaminsky and colleagues,[8] more so in bipolar disorder than in depression.

An example of possible premature jumping to positive conclusions was also published recently. Redei *et al*.[10] purport to have identified biomarkers for depression. These biomarkers were originally discovered in an animal model, of limited direct relevance or specificity to the human condition. There is a relative lack of transparency of why certain biomarkers were chosen and not others to carry forward in the current human study reported. Although there are some differences reported, there is no clear validation in independent human cohorts of a locked panel of markers, nor data on predictive ability for clinical course of the disorder in independent cohorts. In contrast, a panel of 10 biomarkers for mood state discovered by us in human studies of bipolar disorder subjects, locked and then validated by us in independent cohorts before publication,[4] was recently replicated completely independently by another group and shown to track the course of response to cognitive–behavioral therapy in depression.[11]

Moving forward, it may be useful for our field to have a set of rules or postulates for what constitute good biomarkers, similar to the Koch postulates used in infectious diseases. First, working on the right problem. One has to be clear about the population used for discovery: for which well-defined phenotype, in what clinical diagnostic group, of what gender, the biomarker(s) have been discovered. Second, using the right approach. The experimental design used to discover biomarkers is crucial for signal detection. A within-subject design is more powerful than a case–case design with extremes of distribution, which in turn is more powerful than a case–control design, that would require a much higher number of subjects. Metabolomics might be more direct[12] (albeit more limited) than proteomics, which in turn, if used in an unbiased discovery fashion,[13] may be more powerful than gene expression, which in turn is more powerful than genetics, as thousands of single-nucleotide polymorphisms can converge in the regulation of expression of a gene. A convergent functional genomics[3–6] or integrative approach to prioritize biomarkers based on multiple prior lines of evidence would ensure a fit to disease rather than a fit to cohort that may occur with machine learning approaches. Third, demonstrating reproducibility the right way. The validation of an *a priori* selected biomarker or locked panel of biomarkers in non-overlapping, completely independent cohorts with robust phenotypes is a must before making any believable claims. Fourth, the right use of the biomarkers. Showing prospectively that the biomarker(s) have predictive ability for future clinical course is necessary for the field to start adopting the biomarkers and to begin translating them into clinical practice, which should be a clear and present goal for all engaged in this type of research.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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

SUPPLEMENTARY INFORMATION:

Table S1. Demographic information

A. Individual

Cohort 1: Live bipolar subjects cohort (n=15) (45 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
phchp088v2	Bipolar I Disorder	45	M	Caucasian	0
phchp088v3	Bipolar I Disorder	45	M	Caucasian	0
phchp088v4	Bipolar I Disorder	49	M	Caucasian	3
phchp088v5	Bipolar I Disorder	50	M	Caucasian	4
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
phchp109v1	Bipolar Disorder	22	M	Caucasian	0
phchp109v2	Bipolar Disorder	25	M	Caucasian	3
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
phchp140v2	Bipolar II Disorder	38	M	Caucasian	3
phchp140v3	Bipolar II Disorder	38	M	Caucasian	2
phchp140v4	Bipolar II Disorder	40	M	Caucasian	0
phchp142v1	Bipolar I Disorder	55	M	Caucasian	0
phchp142v2	Bipolar I Disorder	55	M	Caucasian	0

phchp142v3	Bipolar I Disorder	55	M	Caucasian	0
phchp142v4	Bipolar I Disorder	57	M	Caucasian	2
phchp142v5	Bipolar I Disorder	57	M	Caucasian	0
phchp142v6	Bipolar I Disorder	58	M	Caucasian	0
phchp153v1	Bipolar II Disorder	55	M	Caucasian	0
phchp153v2	Bipolar II Disorder	55	M	Caucasian	2
phchp153v3	Bipolar II Disorder	56	M	Caucasian	0
phchp153v4	Bipolar II Disorder	57	M	Caucasian	0
phchp153v6	Bipolar II Disorder	58	M	Caucasian	0
phchp179v1	Bipolar Disorder NOS	36	M	Caucasian	0
phchp179v2	Bipolar Disorder NOS	37	M	Caucasian	0
phchp179v4	Bipolar Disorder NOS	37	M	Caucasian	3
phchp183v1	Bipolar I Disorder	48	M	Caucasian	3
phchp183v2	Bipolar I Disorder	48	M	Caucasian	0
phchp293v1	Bipolar NOS	43	M	Caucasian	0
phchp293v2	Bipolar NOS	44	M	Caucasian	2
phchp296v1	Bipolar II Disorder	48	M	Caucasian	0
phchp296v2	Bipolar II Disorder	49	M	Caucasian	2

Cohort 2: Live depression subjects cohort (n=7) (19 chips)

Subject ID visit	Diagnosis	Age	Gender	Ethnicity	SI
phchp155v1	Major Depressive Disorder	37	M	Caucasian	3
phchp155v2	Major Depressive Disorder	37	M	Caucasian	0
phchp161v1	Depressive Disorder NOS	54	M	African American	3
phchp161v2	Depressive Disorder NOS	54	M	African American	0
phchp161v3	Depressive Disorder NOS	54	M	African American	0
phchp182v1	Major Depressive Disorder	39	M	Caucasian	2
phchp182v2	Major Depressive Disorder	39	M	Caucasian	0
phchp182v3	Major Depressive Disorder	40	M	Caucasian	3
phchp194v1	Major Depressive Disorder	47	M	Caucasian	2

phchp194v2	Major Depressive Disorder	47	M	Caucasian	0
phchp194v3	Major Depressive Disorder	47	M	Caucasian	0
phchp198v1	Major Depressive Disorder	61	M	Caucasian	4
phchp198v2	Major Depressive Disorder	61	M	Caucasian	0
phchp198v4	Major Depressive Disorder	62	M	Caucasian	0
phchp236v1	Depressive Disorder NOS	51	M	Caucasian	0
phchp236v2	Depressive Disorder NOS	51	M	Caucasian	3
phchp304v1	Depressive Disorder NOS	52	M	Caucasian	2
phchp304v2	Depressive Disorder NOS	52	M	Caucasian	0
phchp304v3	Depressive Disorder NOS	52	M	Caucasian	2

Cohort 3: Coroner's office cohort-violent suicide completers (n=24) (24 chips)

Subject ID	Psychiatric Diagnosis	Age (years)	Gender	Ethnicity	Suicide by
INBR009	Bipolar/Schizophrenia	59	Male	Caucasian	Hanging
INBR011	Depression/ADHD	26	Male	Caucasian	GSW to chest
INBR012	Unknown	39	Male	Caucasian	GSW to head
INBR013	Depression	68	Male	African American	GSW to mouth
INBR014	None	27	Male	Caucasian	Hanging
INBR015	None	40	Male	Caucasian	Hanging
INBR016	Anxiety, TBI	68	Male	Caucasian	GSW to head
INBR017	Depression, alcohol abuse	56	Male	Caucasian	GSW to chest
INBR018	None	65	Male	Caucasian	Slit wrist
INBR019	Depression	55	Male	Caucasian	GSW to chest and head
INBR021	None	23	Male	African American	Hanging

INBR022	Depression	38	Male	Hispanic	GSW to head
INBR023	None	18	Male	Caucasian	Hanging
INBR024	None	23	Male	Caucasian	Hanging
INBR025	None	31	Male	African American	GSW to head
INBR028	Alcoholism	67	Male	Caucasian	GSW to chest
INBR030	None	22	Male	African American	GSW to head
INBR033	Depression	26	Male	Caucasian	GSW to chest
INBR035	Depression	58	Male	Caucasian	Electrocution
INBR036	None	59	Male	Caucasian	GSW to chest
INBR039	None	53	Male	Caucasian	Hanging
INBR040	None	36	Male	Caucasian	GSW to head
INBR044	None	23	Male	Caucasian	Hanging
INBR048	Psychosis	26	Male	Caucasian	GSW to head

B. Aggregate

SI Score	No SI (0)	High SI (2-4)	Overall
Cohort 1: Live bipolar subjects cohort (n=15)			
Number of subjects (number of chips)	15 (27)	15 (18)	15 (45)
Age			
Mean	47.5	43.8	46
s.d.	9.18	9.52	9.39
Range	22-58	25-57	22-58
Ethnicity (Caucasian/African American)	(15/0)	(15/0)	(15/0)
Cohort 2: Live depression subjects cohort (n=7)			
Number of subjects (number of chips)	7 (10)	7 (9)	7 (19)
Age			
Mean	50.4	48.1	49.3
s.d.	8.22	8.01	7.98
Range	37-62	37-61	37-62
Ethnicity (Caucasian/African American)	(6/1)	(6/1)	(6/1)
Cohort 3: Coroner's office cohort-violent suicide completers (n=24)			
Number of Subjects (number of chips)			24 (24)

Age	
Mean	42.08
s.d.	17.9
Range	18-68
Ethnicity (Caucasian/African American/Hispanic)	(19/4/1)

T-tests for age differences non-significant (BP vs. Depression p=0.161, BP vs. Suicide Completers p=0.294 , Depression vs. Suicide Completers p=0.074).
Abbreviations: M, male; NOS, not otherwise specified; ADHD, attention-deficit hyperactivity disorder; TBI, traumatic brain injury; GSW, gunshot wound; SI, suicidal ideation.
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.

For gene expression evaluation, methodology used was as previously described[1]. RMA normalization was done by groups (bipolar disorder, depression, and suicide completers), to avoid potential artifacts of normalization due to different ranges of expression.

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