RESEARCH ARTICLE

REVISED Predicting lithium treatment response in bipolar patients using gender-specific gene expression biomarkers and machine learning [version 3; referees: 1 approved, 2 approved with reservations]

Andy R. Eugene [1,2], Jolanta Masiak[3], Beata Eugene[4]

[1]Independent Researcher, Kansas, USA
[2]Department of Pharmacogenomics, Bernard J. Dunn School of Pharmacy, Inova Center for Personalized Health, Shenandoah University, Fairfax, VA, 22031, USA
[3]Independent Neurophysiology Laboratory, Department of Psychiatry, Medical University of Lublin, Lublin, 20-439, Poland
[4]Marie-Curie Sklodowska University, Lublin, 20-400, Poland

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Abstract

Background: We sought to test the hypothesis that transcriptome-level gene signatures are differentially expressed between male and female bipolar patients, prior to lithium treatment, in a patient cohort who later were clinically classified as lithium treatment responders.
Methods: Gene expression study data was obtained from the Lithium Treatment-Moderate dose Use Study data accessed from the National Center for Biotechnology Information's Gene Expression Omnibus via accession number GSE4548. Differential gene expression analysis was conducted using the Linear Models for Microarray and RNA-Seq (limma) package and the Decision Tree and Random Forest machine learning algorithms in R.
Results: Using quantitative gene expression values reported from patient blood samples, the RBPMS2 and LILRA5 genes classify male lithium responders with an area under the receiver operator characteristic curve (AUROC) of 0.92 and the ABRACL, FHL3, and NBPF14 genes classify female lithium responders AUROC of 1. A Decision Tree rule for establishing male versus female samples, using gene expression values were found to be: if RPS4Y1 ≥ 9.643, patient is a male and if RPS4Y1 < 9.643, patient is female with a probability=100%.
Conclusions: We developed a pre-treatment gender- and gene-expression-based predictive model selective for classifying male lithium responders with a sensitivity of 96% using 2-genes and female lithium responders with sensitivity=92% using 3-genes.

Keywords

lithium, treatment response, gene expression, machine learning, microarray, transcriptome, precision medicine, pharmacogenomics, psychiatry, genomic medicine

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1 **Ming-Fen Ho**, Mayo Clinic, USA

2 **Sunil V. Kalmady**, University of Alberta, Canada
Alberta Machine Intelligence Institute, Canada

3 **Duan Liu**, Mayo Clinic, USA

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Andy R. Eugene (andyeugene.md@gmail.com)

Author roles: Eugene AR: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Masiak J**: Project Administration, Writing – Review & Editing; **Eugene B**: Formal Analysis, Investigation, Methodology, Project Administration, Writing – Original Draft Preparation, Writing – Review & Editing

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REVISED **Amendments from Version 2**

The major differences between this new version and the previously published version of the article are:

1. We added a graphic illustration of the data analysis workflow to support text in the methods section of the manuscript (new Figure 1; old figures have been re-numbered)
2. We added a three decision-trees detailing the machine learning classification steps using the gene expression data that classifies sample gender, male lithium responders, and female lithium responders (new Figure 5).
3. We expanded the introduction to detail information on pharmacogenomics and therapeutic drug monitoring as well as, added text in various sections in the manuscript to better explain our findings and put them in context of advancing genomic medicine with increasing clinical pharmacology trained physicians in healthcare systems.
4. We provide an updated Supplementary File 1.

Also, author Andy R. Eugene is no longer at Shenandoah University, and is now listed as an Independent Researcher.

See referee reports

Introduction

Lithium is the most well-established mood-stabilizer in the practice of psychiatry (Jermain *et al.*, 1991; Landersdorfer *et al.*, 2017). A recent propensity-score adjusted and matched longitudinal cohort-study evaluating the effectiveness of the newer mood stabilizers: olanzapine (n=1477), quetiapine (n=1376), and valproate (n=1670), in comparison to lithium (n=2148), found that patients treated with lithium experienced reduced rates of both unintentional injury and self-harm (Hayes *et al.*, 2016). However, due to lithium's narrow index of 0.5–1.2 mEq/mL, Therapeutic Drug Monitoring (TDM) is the standard-of-care to ensure patient safety using pharmacokinetic principles in medical practice (Hiemke *et al.*, 2011). Actually, if TDM is applied broadly among medical specialties, pharmacogenomic reports that focus on pharmacokinetic-based gene-drug interactions (e.g. CYP2D6-Paroxetine or CYP2C19-Clopidogrel) may not be necessary in all cases and insurance reimbursement would not be a rate-limiting step in advancing genomic medicine. Although, this approach alone would not account for the hypersensitivity-type pharmacogenomic reactions; however, a TDM pharmacogenomic-hypersensitivity reaction hybrid approach may be an option when concerns about the electronic medical record costs, genotyping and/or sequencing machine costs, and data server infrastructure costs are prohibitive factors causing hospital systems and primary care clinics not to implement pharmacogenomic testing.

A limitation of TDM-only approach, rather than a gene-drug testing, is that one would need to administer the drug and measure a blood concentration after the drug is administered, which may not be an option in life-threatening cases (e.g. stent thrombosis and Clopidogrel). Contrastingly, a profound area of concern for pharmacogenomic testing reports are that hospitals are not implementing actionable pharmacogenomic alerts in the patient medical records if the patient did not have the pharmacogenomic testing at their hospital laboratory due concerns of being a certified genomics laboratory and concerns of litigation when knowingly prescribing a drug that the patient cannot metabolize and scanned into the medical record.

It is important to note that pharmacogenomic reports do not necessarily account for drug-drug-gene interactions – which are often the case – when patients are prescribed three or more medications. In such cases, hospital systems should embed clinical pharmacologist physicians, as is done by leading hospitals globally (e.g. Karolinska Institute in Stockholm Sweden awarding the Nobel Prize, the Mayo Clinic, and more) that aim to maintain high rates of patient drug safety and hospital quality outcome measures (Eichelbaum *et al.*, 2018; Eugene & Eugene, 2018). However, even after accounting for drug doses and drug selection to avoid adverse drug reactions, divergent clinical response rates, among genders, are well-known and reported in psychiatric patients treated with lithium (Viguera *et al.*, 2000).

In a 1986, Zetin and colleagues published the results of a study that evaluated four methods for predicting lithium daily dosages, and the final equation resulted in a 147.8mg/day increased dosage-adjustment for male patients (Zetin *et al.*, 1986). Similarly, a later study by Lobeck and colleagues corroborated the 147.8 mg/day male increase dose requirement for the lithium maintenance dose in bipolar patients (Lobeck *et al.*, 1987). However, neither do the current dosing guidelines recommend a gender-based dose adjustment using pharmacometrics methods, to avoid toxicity, nor are gender-specific gene expression screening panels available to predict lithium efficacy currently available and implemented.

A recent large-scale meta-analysis of human body-tissue gene expression reported that the body organ with the most abundant gender-biased gene expression is the anterior cingulate cortex within the frontal cortex of the brain (Mayne *et al.*, 2016). Thus, these findings suggest that therapeutic drug response may be influenced not only via drug absorption, distribution, metabolism, and elimination, but also within the underlying gene signatures across the human transcriptome and mechanisms of gene-gene interactions that regulate physiology. Beech and colleagues conducted a study to identify gene expression differences from the peripheral blood in patients classified as lithium responders and non-responders (Beech *et al.*, 2014). However, the study reported that no significant gender-biased gene expression differences were found (p-value=0.941) in patients who were randomized to optimal therapy (control), defined as one FDA-approved mood stabilizer, versus patients treated with lithium plus optimal therapy (Beech *et al.*, 2014). Despite these initially reported findings, a recent study by Labonté and colleagues, which used RNA-Seq to evaluate the transcriptome in patients diagnosed with major depressive disorder (MDD), concluded that gender dimorphism exists at the transcriptome-level in MDD patients and that gender-specific treatments should be investigated (Labonté *et al.*, 2017).

Therefore, there is a clinical need to investigate if indeed a gender dimorphism exists in lithium treatment by applying a combination of statistics and data science/engineering methods

to advance precision and genomic medicine in psychiatry. These findings may improve prediction of clinical drug response of lithium prior to initiating drug therapy in patients with bipolar or schizoaffective disorders, who often cannot risk drug inefficacy for obvious safety reasons. Therefore, the overall aim for our study is to define gender-specific transcriptional-level regulators of lithium treatment response that may influence treatment of bipolar or schizoaffective disorders. We will test the hypothesis that biologically plausible gene expression differences exist, prior to lithium treatment, in patients diagnosed with bipolar disorder in the following three patient subgroups: (1) male and female patients who were later clinically classified as lithium treatment responders; (2) male-responders versus male-non-responders; (3) female-responders versus female-non-responders.

Methods

Data

DNA microarray data analyzed in this study are originally referenced from the Lithium Treatment-Moderate dose Use Study placed in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) via accession number GSE45484 with the Illumina HumanHT12 V4.0 expression Beadchip GPL10558 platform file to associate gene names and descriptions. The original multisite clinical study recruited patients from Case Western Reserve University, Massachusetts General Hospital, Stanford University, Yale University, and the Universities of: Pittsburgh, Texas Health Science Center at San Antonio, and Pennsylvania (Beech *et al.*, 2014). From the original 120 peripheral blood samples used to generate probe and gene expression profiles, from patients diagnosed with bipolar disorder, the clinical phenotype of being either a treatment- responder or non-responder was assessed using the Clinical Global Impression Scale for Bipolar Disorder-Severity (CGI-BP-S) (Spearing *et al.*, 1997).

Study design

To assess for gender-specific differential gene signatures, in our first analysis we grouped patients based on gender alone and not on any other variables (i.e. optimal treatment versus lithium, or responder versus non-responder status). Then, we rationalized that from the results of the gender-specific transcriptome signatures from our first analysis, we will set the top two-hundred and fifty genes as controls in an effort to identify pharmacologic treatment-response transcriptome biomarkers that are not directly linked to the X or Y chromosome. Therefore, we *overlaid* the top two-hundred and fifty genes from all results that were reported in subsequent analyses to identify genes with lithium-specific transcriptional differences between genders associated with response to Lithium treatment. In our second analysis, we only selected patients who were classified as lithium treatment-responders, at baseline, and the results from the gene expression differences are reported excluding the sex-specific control genes identified in the first experiment. In our third and fourth analyses, we compared: male-responders vs. male non-responders, and female-responders vs. female non-responders, respectively.

Machine learning

A graphical depiction of the data analysis methods are shown in Figure 1. The *Decision Tree* and *Random Forest* machine

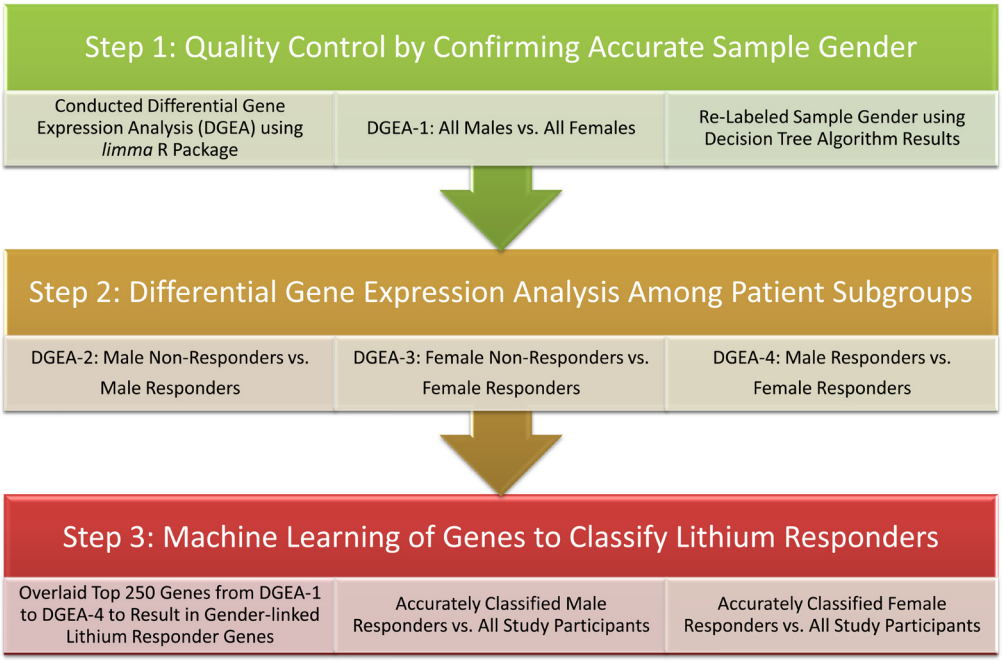

Figure 1. Data analysis workflow used to accurately classify and label sample-gender and gender-specific lithium treatment responders. Heatmaps were created following identification of the top differentially expressed genes and Variable Importance plots were produced following identification of gender-specific lithium treatment responders.

learning algorithms were used for classification following identification of statistically significant DNA microarray genes. This method sets the stage for subsequent analyses aiming to identify gender-specific responder genes with small sample size of three male-responders and six-female responders from the total of sixty patients. Thus, to reiterate, we first utilized the significant results obtained from the gene expression package implemented in the *limma* package in R and then applied the *Decision Tree* and *Random Forest* algorithms for classification and determined this to be novel.

To identify if patients were either male or female, we divided the dataset of 120 samples, pre-treatment and post-treatment, from sixty patients into three sub-datasets: (1) training dataset (60% of total sample), (2) validation dataset (20% of total sample), and the (3) test dataset (20% of total sample). However, due to having small lithium treatment responder sample-sizes, when identifying gender-specific responders versus 'All Other Patients', we simply used a training dataset (70% of total) and a test dataset (30% of total sample). We then reported the classification performance of the models using the following diagnostic parameters: sensitivity, specificity (not calculated for gender-specific lithium responders due sample size), and an area under the receiver operator characteristic curve (AUROC). We selected the traditional Decision Tree algorithm to classify male versus female samples using the following parameters: complexity of 0.01, a max depth of 3, minimum bucket of 7, and a minimum split of 20 observations. Further, for classifying male-responders and female-responders, we selected the *Random Forest* algorithm and set the number of Trees to build at 500 with 7 variables at any time for dataset partitioning. Finally, we reported variable importance plots of genes throughout the paper that was used to explain which genes were most important for classifying patients into different reportable subgroups. Final results of the *Random Forest* processes for male- and female-responders are located in Supplementary File 1.

Gene expression analysis

Differential gene expression analysis of the DNA microarray data was conducted using the Empirical Bayes method implemented within the *limma* package (version 3.34.5) and utilizes the Biobase package (version 2.38.0) which both run within the R for Statistical Programming environment (version 3.4.3; R Foundation for Statistical Computing, Vienna, Austria) (Ritchie *et al.*, 2015; Team, 2013). Due to multiple testing of the peripheral blood transcriptome, the False-Discovery Rate was adjusted using the Benjamini-Hochberg method. A p-value of less 0.05 was considered to be statistically significant and a differential gene expression threshold of 0.5 was used and reported during the machine learning process.

Results

Table 1 provides the patient age and sample sizes used during subgroup analyses. In our first analysis, which aimed to group patients based on gender alone and not based on clinical variables detailed in the original study, data-driven gene analytics identified four female-labeled patient samples with gene expression levels similar to that found in male patients for the following

Table 1. Patient age and sample sizes used during subgroup analyses.

Lithium treated patient population			
Baseline	**Mean age**	**S.D.**	**Sample size (n)**
Male-responder	36	8.1	3
Female-responder	31	11.8	6
Male-non-responder	40	10	7
Female-non-responder	44	9.2	12
***General mood stabilizers patient population**			
Baseline	**Mean age**	**S.D.**	**Sample size (n)**
Male-responder	51	--	1
Female-responder	49	10.5	3
Male-non-responder	43	12.5	9
Female-non-responder	37	14.5	19
Total patient population			
Gender	**Mean age**	**S.D.**	**Sample size (n)**
Male	41	10.8	20
Female	39	13.1	40
Study population	40	12.3	60

***Note:** United States Food and Drug Administration approved Mood Stabilizers.

Y-chromosome genes: *RPS4Y1, EIF1AY, KDM5D, RPS4Y2*; and the *XIST* gene located on the X-chromosome. Therefore, all subsequent hypothesis-testing were analyzed with the updated male-gender classification for the following NCBI GEO patient samples: GSM1105526 (baseline lithium-non-responder), GSM1105528 (1-month lithium-non-responder), GSM1105546 (baseline lithium-non-responder), and GSM1105548 (1-month lithium-non-responder). Figure 2 illustrates the gene expression findings resulting in re-classification for the aforementioned patient samples. The Decision Tree rule states: if *RPS4Y1* < 9.643 then the patient is a female with a probability of 100%. Whereas, if the *RPS4Y1* ≥ 9.643 then the patient is a male with a probability of 9%. After proceeding with the machine learning analysis of both the 'training' and 'validation' datasets, the final 'test' dataset resulted in the following diagnostic test evaluation parameters: Sensitivity=100% (95% C.I. 66.37%-100.00%), Specificity=100% (95% C.I. 78.20%-100.00%), and an AUROC of 1. Figure 3 illustrates the variable importance plots used in the machine learning process for classifying patients as being a male-lithium-responder or female-lithium-responder relative to the full patient population. The results show, in descending order of predictive power, the genes selective for male lithium-responders versus the full patient population being RBPMS2, CDH23, and SIDT2. Similarly, in descending order of predictive power, for female lithium-responders versus the entire patient population, the FHL3, ABRACL, RPL10A, and RPS23 genes are most selective.

Table 2 provides the results for the gender-specific differentially expressed genes from the entire study population using

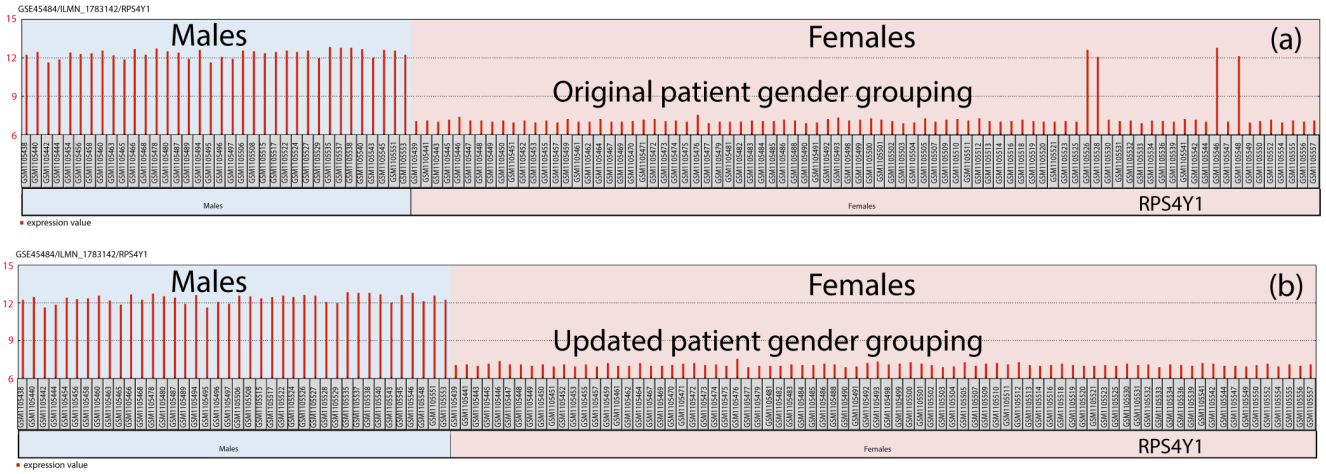

Figure 2. Gene expression levels for the Ribosomal protein S4, Y-linked 1 (RPS4Y1) gene illustrating 4 patient samples as labeled as female and were re-assigned to the male patient gender group.

a fold-change (FC) threshold of 0.5. A total of five genes met the *a priori* FC requirements and were found to be *RPS4Y1, EIF1AY, KDM5D, RPS4Y2*, and *EIF1AY*. These five down-regulated male-biased genes were all found on the Y-chromosome. Contrastingly, a total of 10 upregulated female-biased genes were found to be: *XIST, S100P, IFIT3, TNFAIP6, IFITM3, IFIT2, CHURC1, ANXA3, ADM*, and *PROK2*. The *RPS4Y1* gene in males (FC= -4.9807, p=7.36E-47) and the *XIST* gene (FC=1.7615, p=2.98E-36), found on the X-chromosome, in females resulted in the greatest expression changes between genders. The male-favored genes resulted in a larger expression change than compared to the females.

Table 3 provides the results for the differentially expressed genes that were found between male and female responders prior to initiation of lithium and optimal therapy, meeting the FC criteria of at least 0.5. In male lithium responders, we found 5 differentially expressed while the RNA binding protein with multiple splicing 2 (*RBPMS2*) gene ranked with the greatest FC of -1.351 (unadjusted p=0.00111). Whereas, 9 genes were associated with female lithium responders, with greatest expression change being the major histocompatibility complex class-1-H (HLA-H) at 1.602 (unadjusted p-value=0.00099). The neuroblastoma breakpoint family member-14 (*NBPF14*) gene met the Benjamani-Hochberg adjusted p-value criteria and resulted with an expression change of 0.586 (adjusted p=0.0462). Figure 4 illustrates the heat-map and dendrogram overview of the two-way unsupervised hierarchical cluster analysis of the reported differentially expressed genes among male and female responders to lithium therapy at baseline that correspond to values reported in Table 3.

Using the baseline blood sample microarray data, the predictive modeling results for identifying lithium-responders from the complete study population of male and female controls and treatment samples, resulted in a validation/test sample cohort for males of: Sensitivity=95.83% (95% C.I. 78.88%-99.89%), Specificity=not calculated due sample size of test dataset, and an AUROC = 0.92 using the *RBPMS2* and *LILRA5* genes. Likewise, in the test dataset for females: Sensitivity=91.67% (95% C.I. 61.52%-99.79%), Specificity= not calculated due sample size of test dataset, and an AUROC = 1 with the *ABRACL*, FHL3, and the *NBPF14* genes. Therefore, we developed a 2-gene predictive model for men and a 3-gene predictive model for women classifying lithium response in bipolar patients from a general population of bipolar patients using transcriptional signatures at baseline, prior to prescribing and treating a patient with lithium.

Table 4 provides the list of 10 differentially expressed genes found in male lithium responders (5-genes) and male lithium-non-responders (5-genes). The RNA binding protein with multiple splicing 2 (*RBPMS2*) gene (FC= -1.326, unadjusted p=0.001358) in male lithium responders and the Ribosomal protein S23 (*RPS23*) gene (FC=1.521, unadjusted p=0.013306) were found to result in the largest expression change differences between subgroups. However, in female responders and female non-responders, the Family with Sequence Similarity 117 Member B (*FAM117B*) gene (FC=0.5257, unadjusted p=0.0048554) and the Golgin B1 (*GOLGB1*) gene (FC= -0.6536, unadjusted p=0.0003716) were differentially expressed, respectively and shown in Table 5.

Discussion

The purpose of this investigation was to define gender-specific transcriptome-level regulators of lithium treatment response prior to the initiation of lithium treatment. We first established the gender-relevant transcriptional control genes across all study-participant blood samples and specifically to male- and female-responders using a differential gene expression threshold of 0.5. We found that in the downloaded data from the Gene Expression Omnibus, some patients were mislabeled as males and females. Therefore in our first quality control analysis that established the methodology for subsequent gender-specific lithium responders, the following Decision Tree rule for accurate classifying of gender: if RPS4Y1 < 9.643, then patient is female with a probability of 100% and if RPS4Y1 ≥ 9.643,

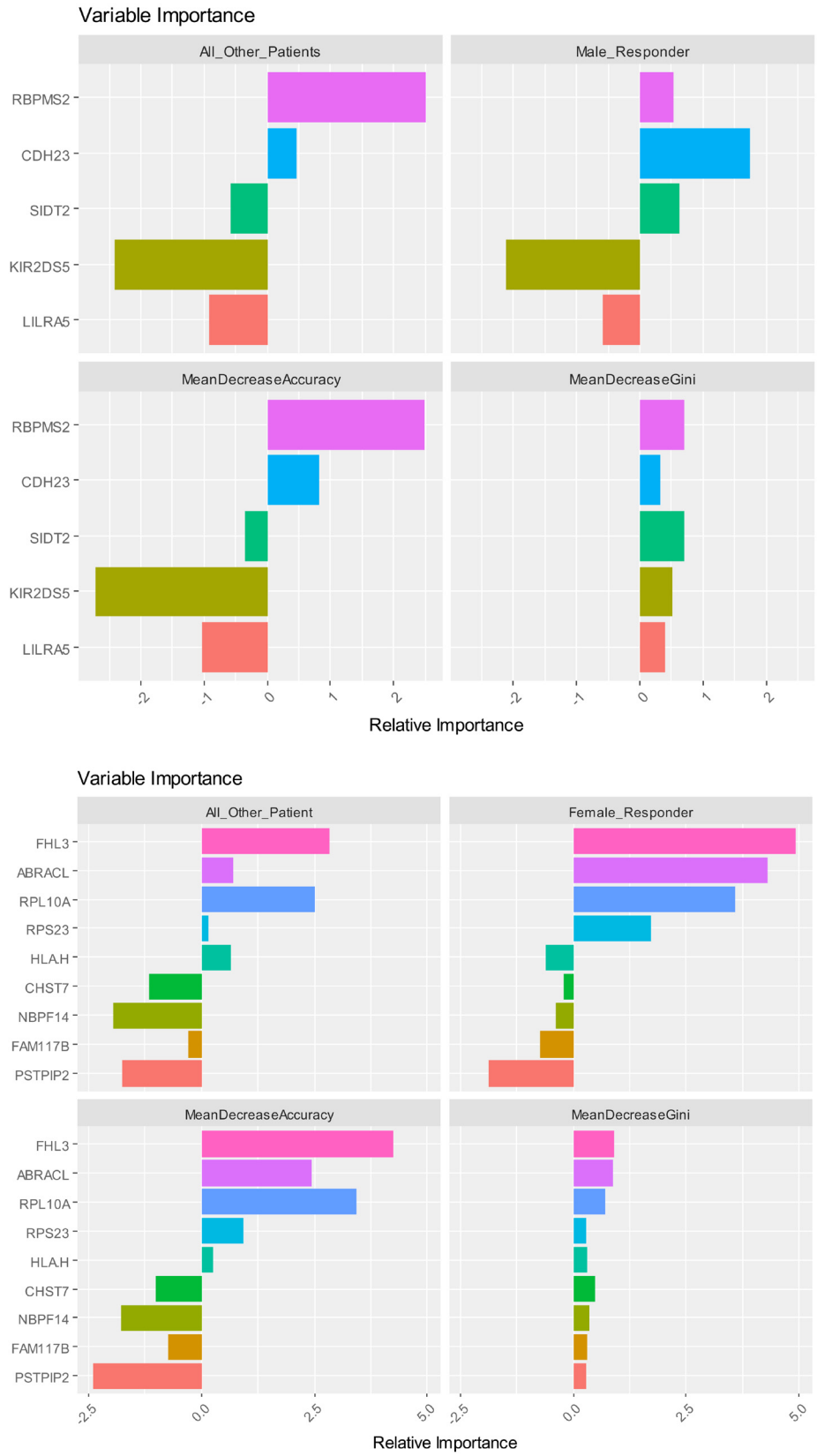

Figure 3. Variable importance ratings of genes selective (above) of male lithium responders versus the entire population of treated and untreated patient men and women; and (below) female lithium responders versus the entire population of treated and untreated men and women.

Table 2. Differentially expressed genes between genders across all study participants with a log fold-change threshold of 0.5.

Male-associated genes					
Gene	**Adjusted P-value**	**P-value**	**Log fold change**	**Gene description**	**Location**
RPS4Y1	7.36E-47	2.81E-51	-4.9807	Ribosomal Protein S4, Y-linked 1	Yp11.3
EIF1AY	1.02E-41	8.61E-46	-2.5861	Eukaryotic Translation Initiation Factor 1A, Y-linked	Yq11.223
KDM5D	7.36E-47	4.67E-51	-1.6658	Lysine Demethylase 5D	Yq11
HLA-DRB1	0.016	0.0000362	-1.7072	Major Histocompatibility Complex, Class II, DR Beta 1	
RPS4Y2	1.35E-40	1.43E-44	-1.5014	Ribosomal Protein S4, Y-linked 2	Yq11.223
EIF1AY	9.38E-31	1.98E-34	-0.9443	Eukaryotic Translation Initiation Factor 1A, Y-linked	Yq11.223
Female-associated genes					
Gene	**Adjusted P-value**	**P-value**	**Log fold change**	**Gene description**	**Location**
XIST	2.98E-36	5.03E-40	1.7615	X Inactive Specific Transcript (non-protein coding)	Xq13.2
S100P	1.70E-02	3.31E-05	1.028	S100 Calcium Binding Protein P	4p16
IFIT3	5.00E-03	5.82E-06	0.8765	Interferon Induced Protein with Tetratricopeptide Repeats 3	10q24
TNFAIP6	3.73E-04	2.52E-07	0.7304	TNF Alpha Induced Protein 6	2q23.3
IFITM3	4.51E-02	1.69E-04	0.7284	Interferon Induced Transmembrane Protein 3	11p15.5
IFIT2	4.91E-02	1.95E-04	0.6739	Interferon Induced Protein with Tetratricopeptide Repeats 2	10q23.31
CHURC1	6.30E-02	3.18E-04	0.6678	Churchill Domain Containing 1	14q23.3
ANXA3	2.33E-03	2.26E-06	0.6218	Annexin A3	4q21.21
ADM	8.69E-04	6.80E-07	0.5986	Adrenomedullin	11p15.4
PROK2	2.16E-02	4.79E-05	0.5189	Prokineticin 2	3p13

then the patient is a male with a lower probability. The differential gene expression threshold of 0.5 was found to be adequate and corroborated with similar studies that used a similar threshold for establishing gene transcription signatures (Jansen *et al.*, 2014; Mayne *et al.*, 2016). However, when comparing the male-responders to male non-responders, as well as, the female responders to female non-responders, we set an inclusion fold-change threshold to 0.3. This approach is not unusual, since it is already established that both large and subtle expression changes produce to significant biological and physiological processes (Wurmbach *et al.*, 2002). Our results are hypothesis-generating and establish a computational methodology that provides insight to the importance of subgroup analysis in genomic medicine, irrespective of patient small sample-sizes. The end-goal of such analyses serves as a testing methodology for establishing gene screening panels to improve precision medicine in vulnerable and high-risk patient populations. In these patient populations, it is often not feasible to wait for weeks to determine whether a prescribed medication will work and in some cases manic patients are neither able to fully comprehend and be objectively assessed using the CGI-BP-S (Spearing *et al.*, 1997).

When reviewing the heat-map and dendrogram hierarchical cluster analysis patterns, specifically the numerous non-responders clinically-labeled and illustrated in Figure 6, they suggest that the underlying etiology resulting in clinical symptoms (e.g. mania) that led to the diagnosis of bipolar disorder may need re-classification. Further, the subsequent treatments may need to be tailored in data-driven computational psychiatry approaches. In Figure 6, for the females, the samples in the center cluster illustrates that a group of patients are clear non-responders while the patients clustered in the far-right are partial-responders, from a molecular perspective. The natural questions that arise are: (1) How to best convert the non- and partial-responders to treatment-responders? (2) Is a behavioral intervention, in this

Table 3. Differentially expressed genes between male and female responders prior to Lithium pharmacotherapy with a log fold-change threshold of 0.5.

Genes associated with male lithium responders					
Gene	**Adjusted P-value**	**P-value**	**Log fold change**	**Gene description**	**Highest gene tissue expression**
RBPMS2	1	0.00111	-1.351	RNA Binding Protein with Multiple Splicing 2	Heart, Urinary Bladder
SIDT2	1	0.00932	-0.82	SID1 Transmembrane Family Member 2	Stomach, Prostate
CDH23	1	0.00388	-0.674	Cadherin-Related 23	Ovary, Fat
LILRA5	1	0.00359	-0.592	Leukocyte Immunoglobulin Like Receptor A5	Appendix, Bone Marrow
KIR2DS5	1	0.00431	-0.506	Killer Cell Immunoglobulin Like Receptor, Two Ig Domains and Short Cytoplasmic Tail 5	--
Genes associated with female lithium responders					
Gene	**Adjusted P-value**	**P-value**	**Log fold change**	**Gene description**	**Highest gene tissue expression**
HLA-H	1	0.000996	1.602	Major Histocompatibility Complex, Class I, H (pseudogene)	Lymph Node, Bone Marrow
RPS23	1	0.00308	1.471	Ribosomal Protein S23	Ovary, Bone Marrow
FHL3	1	0.000751	0.893	Four and a Half LIM Domains 3	Esophagus, Endometrium
RPL10A	1	0.00299	0.628	Ribosomal Protein L10a	Ovary, Bone Marrow
****NBPF14**	**0.0462	0.00000782	0.586	Neuroblastoma Breakpoint Family Member 14	Skin, Ovary
PSTPIP2	1	0.000473	0.569	Proline-Serine-Threonine Phosphatase Interacting Protein 2	Bone Marrow, Spleen
FAM117B	1	0.00949	0.556	Family with Sequence Similarity 117 Member B	Testis, Adrenal
CHST7	1	0.00812	0.529	Carbohydrate Sulfotransferase 7	Spleen, Fat
ABRACL	1	0.00396	0.505	ABRA C-Terminal Like	Colon, Lymph Node

Notes: **The NBPF14 gene reached the Benjamani-Hochberg adjusted p-value.

select group of patients, for whom lithium is not effective, the best answer because the symptoms maybe of a different etiology? If indeed the symptoms are of a different etiology (e.g. inflammatory), from the lithium treatment-responders, then other diagnostic (e.g. electrophysiological neuroimaging) tools may be warranted and corresponding most efficacious treatments sought.

When differentiating between male and female patients, we found that the Ribosomal Protein S4, Y-linked 1 (*RPS4Y1*, adjusted p-value=7.36E-47) male-linked gene and the X Inactive Specific Transcript (*XIST*, adjusted p-value=2.98E-36) female-linked gene were the most differentially expressed among genders, which is consistent with previously published studies (Guillén *et al.*, 2014; Jansen *et al.*, 2014; Mayne *et al.*, 2016). The genes that are specific to male lithium responders, relative to female lithium responders, are *RBPMS2, SIDT2, CDH23, LILRA5*, and *KIR2DS5*. Using the same methodology, genes identifying female lithium responders, relative to male lithium responders, are *HLA-H, RPS23, FHL3, RPL10A, NBPF14, PSTPIP2, FAM117B, CHST7*, and *ABRACL*. The Neuroblastoma Breakpoint Family Member 14 (*NBPF14*, adjusted p-value=0.0462, Fold-change=0.586) achieved the Benjamani-Hochberg adjusted p-value of 0.0462, and has been reported to be associated with cortical neurogenesis (Suzuki *et al.*, 2017).

Computational psychiatry methods that analyze objective clinical signals (e.g. electroencephalography) and various data-types (e.g. gene expression [RNA], single-nucleotide polymorphisms [DNA], plasma drug concentrations) to classify patients in psychiatry, as advocated by the National Institute of Mental Health's Research Domain Criteria (RDoC), are essential in psychiatry, especially in patients with developmental delay, language difficulty, and conditions of potentially different etiologies than traditionally taught (Clark *et al.*, 2017; Eugene & Masiak, 2016). Ideally, in such cases, alternative FDA-approved

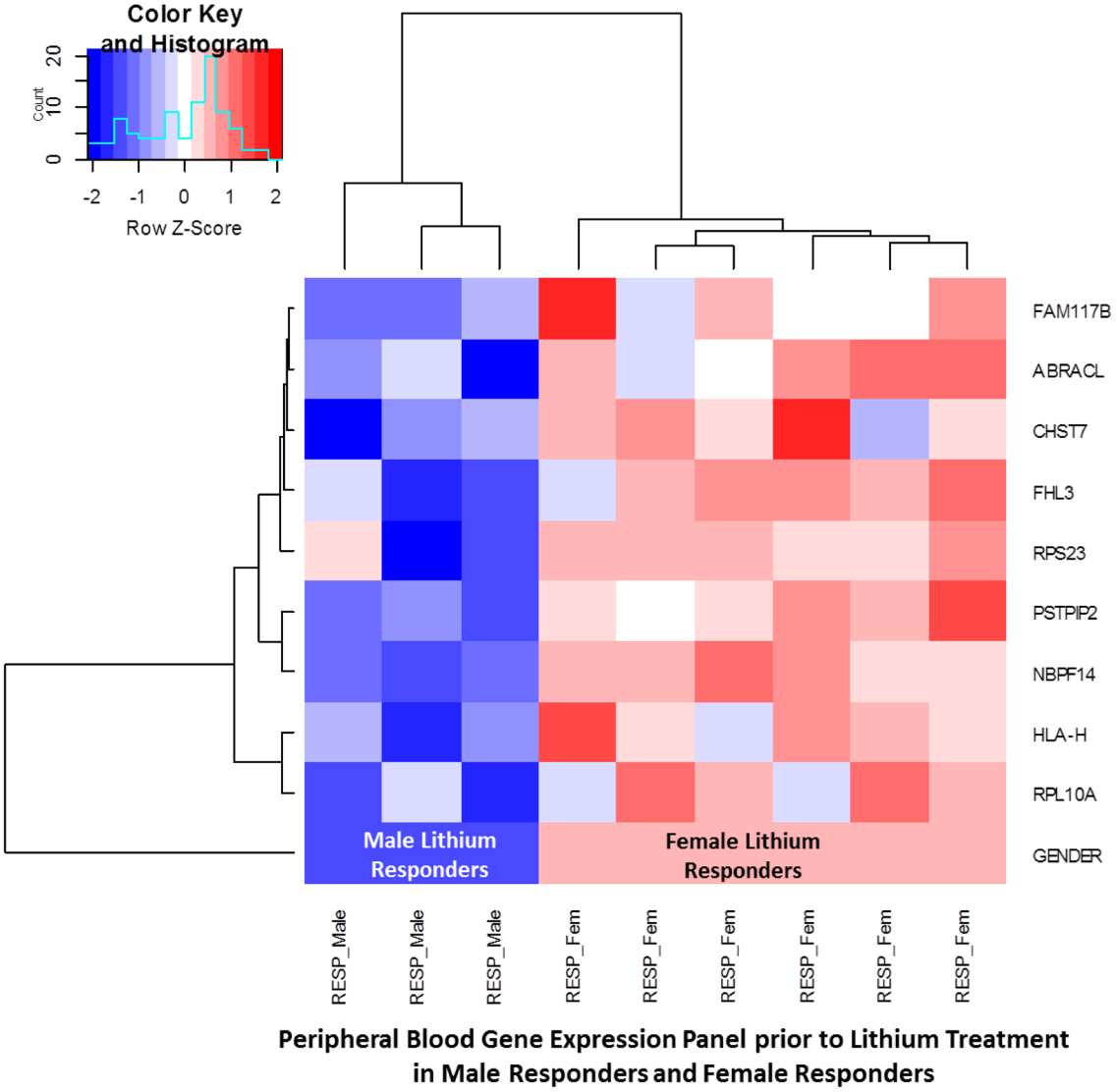

Figure 4. Heat-map and dendrogram overview of the two-way unsupervised hierarchical cluster analysis of differentially expressed genes in male (n=3) and female (n=6) lithium responders after overlaying the top 250 differentially expressed genes found gender biased genes.

mood stabilizers may be initially selected prior to any pharmacological intervention by simply using a blood test. Perhaps, a gene expression screening panel at baseline, prior to the initiation of lithium and/or other FDA-approved mood stabilizer, may be better in high-risk patient populations.

These findings suggest that when implementing genomic medicine, clinical research teams should move beyond the single-gene approach when screening for treatment response biomarkers. This approach is currently the standard when screening for patient toxicity at standard doses in poor or ultra-rapid metabolizers using drug pharmacokinetics; however, as more transcription factors are discovered that regulate the cytochrome (CYP) P-450 system of genes, multi-gene pharmacokinetic panels are inevitable and may be included in future Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines. Next, medical management of patients with mania and psychosis either with pharmacotherapy and/or behavioral intervention should be tailored to biological gender due to known neuronal circuitry differences in age-matched patients with psychosis (Eugene *et al.*, 2015). Further, as a result of lithium not being hepatically metabolized, but rather transported and renally excreted as well as, the known myriad drug-drug interactions, patient dose selection may benefit from pharmacometrics modeling by American Board of Clinical Pharmacology certified physicians in applied clinical pharmacology/clinical pharmacology (Perera *et al.*, 2014; Zetin *et al.*, 1986).

Further, clinical pharmacologist physicians are essential for advancing genomic medicine and providing consults in

Table 4. Differentially expressed genes between Male Responders and Male Non-Responders at baseline with a log fold-change threshold of 0.3.

Genes associated with male lithium responders					
Gene	**Adjusted P-value**	**P-value**	**Log fold change**	**Gene description**	**Highest gene tissue expression**
RBPMS2	1	0.001358	-1.326	RNA Binding Protein with Multiple Splicing 2	Heart, Urinary Bladder
SVBP	1	0.01366	-0.76	Small Vasohibin Binding Protein	Testis, Fat
LILRA5	1	0.011739	-0.714	Leukocyte Immunoglobulin Like Receptor A5	Appendix, Bone Marrow
CPA3	1	0.008048	-0.592	Carboxypeptidase A3	Gall Bladder, Lung
SLC45A3	1	0.016508	-0.455	Solute Carrier Family 45 member 3	Prostate, Stomach
ZNF234	1	0.003254	-0.41	Zinc Finger Protein 234	Spleen, Thyroid
DIDO1	1	0.008232	-0.385	Death Inducer-Obliterator 1	Ovary, Spleen
TPP2	1	0.013053	-0.385	Tripeptidyl Peptidase 2	Testis, Thyroid
KRT73	1	0.007333	-0.373	Keratin 73	Skin, Lymph Nodes
ZMYM3	1	0.00363	-0.372	Zinc Finger MYM-type Containing 3	Ovary, Testis
NOTCH2 NL	1	0.009657	-0.348	Notch 2 N-terminal Like	Testis, Skin
TIPRL	1	0.007794	-0.34	TOR Signaling Pathway Regulator	Endometrium, Brain
CAMK1D	1	0.005376	-0.333	Calcium/Calmodulin dependent Protein Kinase ID	Brain, Skin
EFNA1	1	0.00632	-0.324	Ephrin A1	Placenta, Lung
Genes associated with male lithium non-responders					
Gene	**Adjusted P-value**	**P-value**	**Log fold change**	**Gene description**	**Highest gene tissue expression**
RPS23	1	0.013306	1.521	Ribosomal Protein S23	Ovary, Bone Marrow
IRF2BPL	1	0.010952	1.005	Interferon Regulatory Factor 2 Binding Protein Like	--
HLA-C	1	0.003461	0.997	Major Histocompatibility Complex, Class I, C	Lung, Bone Marrow
RGPD1	1	0.001745	0.76	RANBP2-like and GRIP Domain Containing 1	Testis, Liver
ASGR2	1	0.019947	0.598	Asialoglycoprotein Receptor 2	Liver, Gall Bladder
LPAR1	1	0.01374	0.453	Lysophosphatidic Acid Receptor 1	Brain, Placenta
RRN3P1	1	0.017025	0.42	RRN3 homolog, RNA Polymerase I Transcription Factor Pseudogene 1	Thyroid, Lymph Node
TOMM34	1	0.016655	0.416	Translocase of Outer Mitochondrial Membrane 34	Testis, Adrenal
ACAD11	1	0.015882	0.405	Acyl-CoA Dehydrogenase Family Member 11	Kidney, Liver
CEBPE	1	0.00269	0.404	CCAAT/enhancer Binding Protein Epsilon	Bone Marrow, Small Intestine
CMIP	1	0.017203	0.394	C-Maf Inducing Protein	Brain, Small Intestine
IGSF6	1	0.011786	0.38	Immunoglobulin Superfamily Member 6	Spleen, Appendix
HDHD2	1	0.01764	0.361	Haloacid Dehalogenase Like Hydrolase Domain Containing 2	Brain, Thyroid
LMO4	1	0.012872	0.359	LIM Domain Only 4	Brain, Stomach
BACE2	1	0.000711	0.353	Beta-site APP-Cleaving Enzyme 2	Stomach, Gall Bladder
TPP1	1	0.00061	0.341	Tripeptidyl Peptidase 1	Spleen, Appendix
GALNS	1	0.007613	0.341	Galactosamine (N-acetyl)-6-Sulfatase	Bone Marrow, Testis
SYNM	1	0.019042	0.322	Synemin	Esophagus, Prostate

Table 5. Differentially expressed genes between Female Responders and Female Non-Responders at baseline with a log fold-change threshold of 0.3.

Genes associated with female lithium responders					
Gene	**Adjusted P-value**	**P-value**	**Log fold change**	**Gene description**	**Highest gene tissue expression**
FAM117B	0.998	0.0048554	0.5257	Family with Sequence Similarity 117 Member B	Testis, Adrenal
STAMBPL1	0.998	0.0074433	0.39	STAM Binding Protein Like 1	Adrenal, Testis
CD248	0.998	0.0038199	0.3626	CD248 Molecule	--
IFIH1	0.998	0.0075822	0.3453	Interferon Induced with Helicase C domain 1	Spleen, Appendix
GPR160	0.998	0.0071723	0.3394	G Protein-coupled Receptor 160	Small Intestine, Duodenum
STAP1	0.998	0.0053096	0.3222	Signal Transducing Adaptor Family Member 1	Lymph Node, Appendix
YEATS4	0.998	0.0089003	0.3103	YEATS Domain Containing 4	Testis, Bone Marrow
CD83	0.998	0.0004367	0.3014	CD83 Molecule	Bone Marrow, Lymph Node
TMOD2	0.998	0.0081514	0.3012	Tropomodulin 2	Brain, Appendix
Genes associated with female lithium non-responders					
Gene	**Adjusted P-value**	**P-value**	**Log fold change**	**Gene description**	**Highest gene tissue expression**
GOLGB1	0.998	0.0003716	-0.6536	Golgin B1	
RASA4CP	0.998	0.0030349	-0.4554	RAS p21 Protein Activator 4C, Pseudogene	Spleen, Endometrium
NACC2	0.998	0.0061286	-0.3803	NACC Family Member 2	Brain, Fat
EDARADD	0.998	0.0021425	-0.3553	EDAR Associated Death Domain	Urinary Bladder, Kidney
ZNF573	0.998	0.0058465	-0.3463	Zinc Finger Protein 573	Thyroid, Spleen
ALDH2	0.998	0.0031872	-0.335	Aldehyde Dehydrogenase 2 Family (mitochondrial)	Fat, Liver
TAPBPL	0.998	0.0032596	-0.3206	TAP Binding Protein Like	Duodenum, Small Intestine

pharmacogenomics. These physicians would confirm the applicability of embedding machine learning results integrated within artificial intelligence applications in the electronic medical record. Figure 5 shows the machine learning classification results of gene expression levels that determine (a) sample gender, (b) male lithium treatment responders, and (c) female lithium treatment responders. These very study results – though with a small treatment responder population – presents an approach for data science and engineering methods for use in genomics and medicine.

The limitations of our analysis – as in most pharmacogenomic clinical studies – are understandably due to a small patient sample size and multiple-comparison p-value adjustments (Dudoit *et al.*, 2003). The fundamental aims of our research questions were designed to answer biological questions of gender and clinical response to lithium and not meant to be driven exclusively by multiple comparisons adjusted p-values or limited by not having enough patients. This approach has led to various successes in pharmacogenomics, specifically, in genome-wide association studies; however, understandably, the limitations are thoroughly acknowledged. In reference to patient sample sizes, 9 out of the 28 patients who received lithium and optimal therapy were classified as lithium treatment responders. Further, 30% of men and 33% of women, who were treated with lithium, were found to be responders at the respective gender categories (Beech *et al.*, 2014). However, the strengths of our findings are in the gender-gene screening capability for lithium treatment-responders in the general population of 60 patients at baseline, minus the tested responder group. Opportunities exist for any further clinical studies, prospective clinical trials, and application of the methods outlined in this work for other therapeutic agents across several medical specialties and other disciplines.

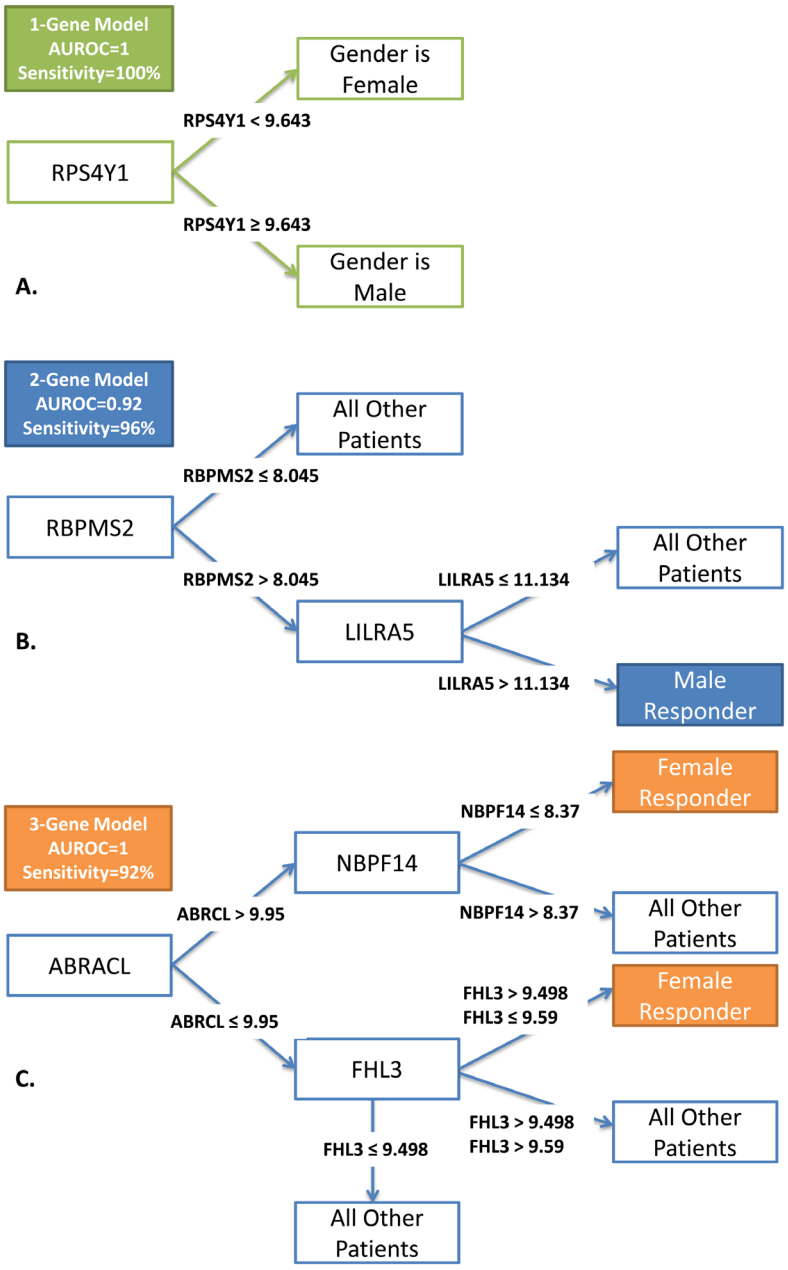

Figure 5. Machine learning classification results of gene expression levels that determine (**a**) gene expression sample gender using a 1-gene (RPS4Y1) model with a sensitivity of 100% and an area under the receiver operator curve (AUROC) of 1, (**b**) male lithium treatment responders using a 2-gene (RBPMS2 and LILRA5) model with an AUROC of 0.92, and (**c**) female lithium treatment responders using a 3-gene (ABRACL, NBPF14, and FHL3) model with an AUROC of 1.

Conclusion

We explored the Lithium Treatment-Moderate dose Use Study clinical trial gene expression data with the aim of identifying gender-specific transcriptome-level regulators of lithium treatment response. We found that male and female labeled patients were misclassified and used the following Decision Tree rule for accurate classifying of gender: if RPS4Y1 < 9.643, then patient is female with a probability of 100%. Further, using machine learning, we successfully developed a pre-treatment gender- and gene-expression-specific predictive model selective for lithium responders with an AUROC of 0.92 for male lithium responders (sensitivity=96%) and an AUROC of 1 for female lithium responders (sensitivity=92%). Moreover, by using well-established Bayesian statistical methods, to identify differentially

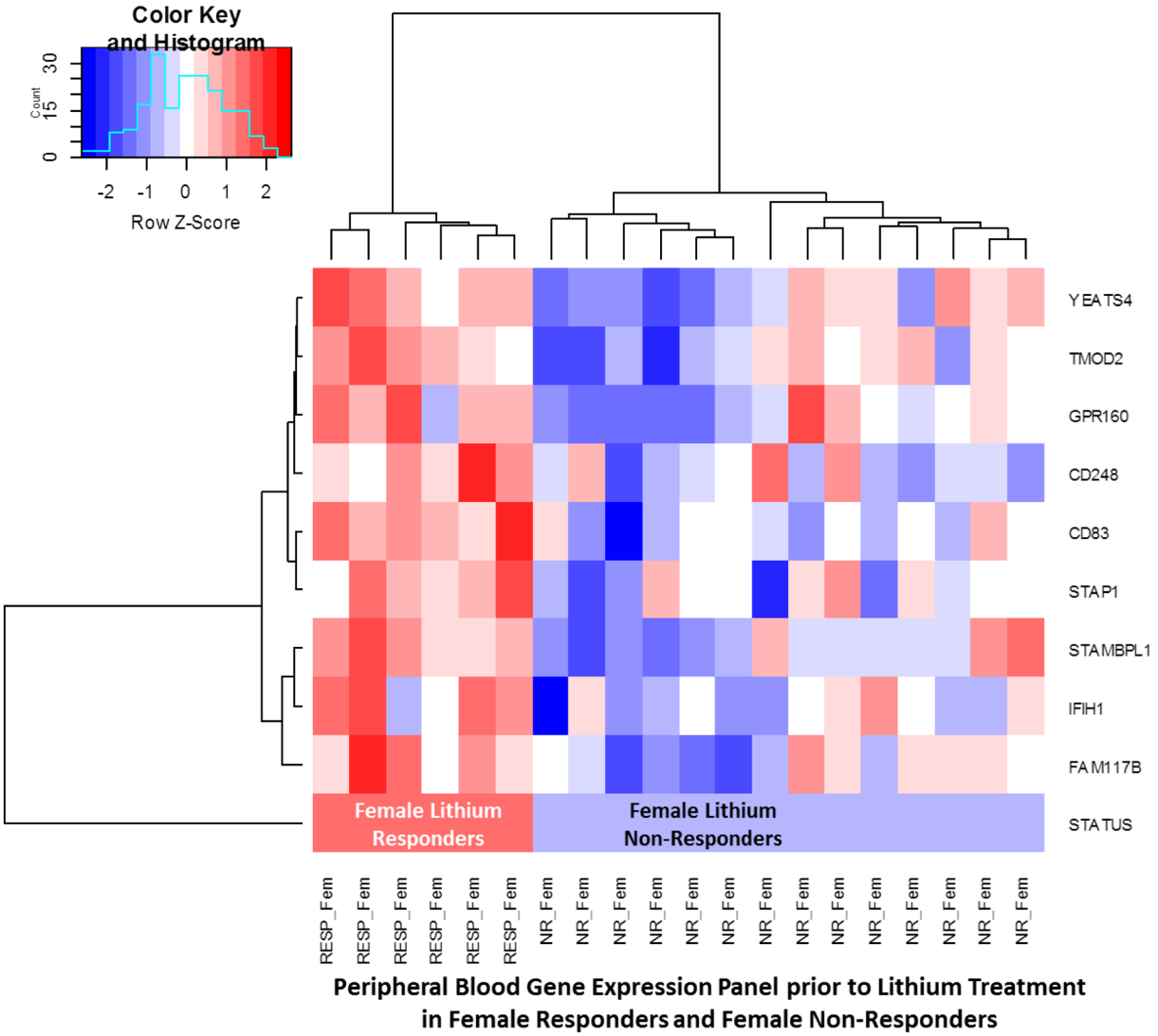

Figure 6. Heat-map and dendrogram overview of the two-way unsupervised hierarchical cluster analysis of differentially expressed genes prior to lithium treatment in female lithium responders (n=6, RESP_Fem) and female non-responders (n=14, NR_Fem).

expressed genes and then machine learning, we discovered 2-genes (RBPMS2 and LILRA5) selective for male lithium responders and 3-genes (ABRACL, FHL3, and NBPF14) selective for female lithium responders that will inform physicians and the medical staff of whether the patient will respond to lithium prior to being prescribed the mood stabilizer. Further, due to the small number of patients classified as responders from the clinical trial, our results should be confirmed. Lastly, in an overall context, our results suggest that the methodology used in this analysis may be extended to other therapeutic drug classes and provides insight to the gender-based gene transcriptome differences influencing lithium pharmacodynamics.

Data availability

Data used in this study are available from https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE45484

Grant information

The author(s) declared that no grants were involved in supporting this work.

Acknowledgements

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Supplementary material

Supplementary File 1: Supplementary methods

Click here to access the data

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Open Peer Review

Current Referee Status: ✔ ? ?

Version 3

Referee Report 18 February 2019

https://doi.org/10.5256/f1000research.18909.r43262

? **Duan Liu**
Division of Clinical Pharmacology, Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, MN, USA

Using a published data set, the authors built a model based on gender-specific gene expression to predict lithium treatment response in bipolar disorder patients. Here are my questions and concerns:

1. The authors realized that the sample size is small and the results will need further confirm. Is there any other similar (lithium treatment response) clinical trial/data available to test the predicting model?
2. After the Step 1 analysis, why not exclude the four samples that their genders had been mis-classified? (Since the clinical data for those four patients may be also mis-labelled.) Data accuracy is critical for building models, especially when dealing with small sample size.
3. Those three genes, the *ABRACL*, *FHL3*, and *NBPF14*, which classified female responders seem not differentially expressed between female responders and female non-responders (Table 5). Please explain why this might be happened.
4. What is the known function of those 5 genes that chosen for classification of male and female responders? The authors might want to discuss the possible molecular mechanism of those gene function related to lithium treatment response in bipolar disorders.
5. Since the authors re-analyzed data from a previously published study, a comparison of the findings from this study to the original one may be necessary. I understood that the original study looked at differences between responder and non-responders, regardless of genders. Is there any gene, of which its expression differentiates responder and non-responder, overlapped with what the authors found in this study? What will be found if apply a machine learning approach to this data set without classifying patients by genders?

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pharmacogenomics

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Referee Report 09 January 2019

https://doi.org/10.5256/f1000research.18909.r42767

? **Sunil V. Kalmady** [1,2]
[1] Department of Psychiatry, University of Alberta, Alberta, Canada
[2] Alberta Machine Intelligence Institute, Alberta, Canada

Authors have made improvements to the manuscript in terms of clarity of methodology. Hence, now I can comment on their methods.
Biostatistical methods are mostly sound.

However, machine learning part of study seems have major issues:

1. Authors haven't specified number of responders in their test sets or whether split is class-balanced. But based on their description of 70 – 30 train -test split, sample size is severely limiting for model evaluation, with mere one or two examples of positive class (responders) in test set (30% of 3 male ~ 1 ; 30% of 6 female ~ 2). As a fellow researcher, I completely respect the motivation and effort behind the efforts here, but we as scientific community should understand that the real danger of generalizing observations based on handful of cases is not so much of being underpowered to detect real effect, but of generating false positives results that add to prevailing burden of irreproducible results.
2. It seems that features (250 control gene selection, 2-gene model, 3-gene model etc..) were selected using analyses of both training and testing data partitions. This is called double-dipping and leads to invalid or over-optimistic estimates of model performance.

While above two points are deal breakers, I will also mention following points for sake of completion.

1. Hyper-parameters should also be selected '*in fold*' or their choice should be explained.
2. Baseline performance (chance level accuracy) is rather high due to class imbalance – eg: 25/28 = 89% for male responders. Reporting the confusion matrix will be more useful than sensitivity, AUC etc in such cases.
3. For small samples, consider simple linear models than complex non-linear ones such as random forest to avoid over-fitting. Also, consider leave-one-out or k-fold cross validation instead of single test-train split for better estimate of performance.

Hence, in my humble opinion, the manuscript in its current form doesn't meet the necessary scientific rigor. That is at least without a major revision in machine learning methods, such as learning models to

predict treatment response in larger undivided dataset of 60 subjects, appropriate use of feature selection etc.

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Version 2

Referee Report 12 November 2018

https://doi.org/10.5256/f1000research.16407.r39873

Sunil V. Kalmady [1,2]

[1] Department of Psychiatry, University of Alberta, Alberta, Canada

[2] Alberta Machine Intelligence Institute, Alberta, Canada

Eugene et al. tackles the hard question on the transcriptome predictors of clinical outcome in lithium treatment of Bipolar disorder. This research question is of clinical relevance and importance, however, there might be some issues with how the data was analyzed and presented in the manuscript.

- The study design and methods do not seem to be coherent to a single unifying goal. Whether the goal is to predict the responder using the 'gender' and 'transcriptome' data as features? If so, such a model can be learned using standard machine learning methods. On the other hand, whether the goal is to identify are genes with statistically significant group differences in their expression? If so, this can be achieved by biostatistical inference tests of association.

Please note that task of 'association' and 'prediction' are quite distinct in their formulation and desired objective. Authors have to be really careful about that they trying to test and claim while using both of approaches in conjunction.

- Study design is a bit unconventional, and hence needs to be motivated and explained better. For example: Performing successive sub-group analyses partitioned on factors like gender have less power, and should be generally restricted to post-hoc tests. Why not simply use standard biostatistical tools such as factorial ANOVA with 'sex' and 'response' as between-subjects factors of interest?

I agree with Reviewer #1 that flowchart of analysis pipeline will help the understanding. Steps of sub-group selection and variable/feature selection can be indicated in this flowchart. Care should be taken to avoid the circularity that can arise from selection because statistical inference can be invalid whenever the results statistics are not inherently independent of the selection criteria under the null hypothesis.

- Authors might be asking too many questions with limited data in hand. Sample size might not enough to study individual effects of multiple factors - such as treatment-type, response and then, the gender. Cell-wise sample sizes resulting from this 8-way split is less than 10 for all but two cells (less than 5 for 3 cells). Suitability of applied statistical tests and generalizability of their claims are questionable here. Authors should also think about 1:2 skew in male:female ratio, which makes this issue worse. Study can greatly benefit from asking specific and limited hypothesized questions.

Also, since multiple objectives are stated, methods and results section can describe each objective separately for sake of better clarity.

- Machine learning methods are not described. The methods used for learning of model and its evaluation process needs be specified. Example: How was training and test splits performed? How was feature selection performed? How were the hyper-parameters optimized? Whether the reported performance metrics are for training or testing sets? Whether the discovery dataset used for identifying '250 genes' disjoint from validation set? etc. Without these details, it is hard to comment on validity of a predictive study.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
No

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Psychiatric research, Biostatistics, Machine learning.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 27 Nov 2018

Andy Eugene, Independent Researcher, USA

Please see our responses in bold-face to the comments from Referee #2. We thank you for reviewing this article.

Eugene et al. tackles the hard question on the transcriptome predictors of clinical outcome in lithium treatment of Bipolar disorder. This research question is of clinical relevance and importance, however, there might be some issues with how the data was analyzed and presented in the manuscript.

- The study design and methods do not seem to be coherent to a single unifying goal. Whether the goal is to predict the responder using the 'gender' and 'transcriptome' data as features? If so, such a model can be learned using standard machine learning methods. On

the other hand, whether the goal is to identify are genes with statistically significant group differences in their expression? If so, this can be achieved by biostatistical inference tests of association.

- Response: **First and foremost, I sincerely appreciate you taking time out to review this article and provide constructive feedback. In answering these points, this paper addressed questions that were not addressed in the original clinical study and attempted not to duplicate work previously published. Hence, is why the methods are creative and not employ standard biostatistical inference tests of association. For example, we aimed to test the hypothesis if gender influenced selecting transcriptome signatures associated with lithium efficacy. Therefore, in order to accomplish this task, we conducted a quality control test that identifies known gender-biased transcriptome signatures. This proved to be essential and is why we identified that several patients were misclassified as male/female. Results are shown, documented, and corroborated with other studies identifying the same genes as being gender-specific. Next, standard statistical tests were indeed used, however, we chose to use those adapted for Gene Expression analysis as has been developed using the "limma" package and for machine learning methods, we used the Random Forest algorithm. We then aimed to develop a predictive model to see if this may be a first-step for other medical research teams to validate with further clinical studies and for Clinical Pharmacology laboratories to pursue and develop novel experiments to determine lithium's effect in cells, tissues, laboratory animals, and later in humans.**

Please note that task of 'association' and 'prediction' are quite distinct in their formulation and desired objective. Authors have to be really careful about that they trying to test and claim while using both of approaches in conjunction.

Response: **Well said and this is duly noted. We first aimed to identify genes associated with responders and hoped to identify if using those genes would help in creating hypothesis-generating predictors treatment response, only to be later validated by other studies. Thank you for the comment.**

- Study design is a bit unconventional, and hence needs to be motivated and explained better. For example: Performing successive sub-group analyses partitioned on factors like gender have less power, and should be generally restricted to post-hoc tests. Why not simply use standard biostatistical tools such as factorial ANOVA with 'sex' and 'response' as between-subjects factors of interest?
- Response: **While your point with "gender" should be restricted to post-hoc analysis, we are looking to make gender the primary point of our analysis in patients responding to lithium treatment and specifically not repeat the analysis from the original publication in Nature. This was attempted on the original article, however, we clearly identified that there was patient-gender misclassification in the original study. So, we sought another route of analysis to ensure that gender was a primary point of analysis and not side-lined to the post-hoc analyses. However, we do understand that this compromises statistical power and therefore, sought not to analyze the data with ANOVA, because we are addressing minor gene expression level changes that might have real-world clinical insight. This is a hypothesis-generating analysis, however, we do thank you as well for this comment.**

I agree with Reviewer #1 that flowchart of analysis pipeline will help the understanding. Steps of sub-group selection and variable/feature selection can be indicated in this flowchart. Care should be taken to avoid the circularity that can arise from selection because statistical inference can be invalid whenever the results statistics are not inherently independent of the selection criteria under the null hypothesis.

Response: **We appreciate the request for having a graphical flowchart depicting the analysis pipeline and have included the figure in the updated version of the manuscript. We are confident that circularity is not an issue, given the specific aims of our analysis. These methods are determined to seeking to identify the influence of linked gender-drug-response to genes at baseline and not after treating with the mood stabilizer. The gender differences in clinical practice are a well-documented reality and we literally sought to identify any signal, on the gene expression level, to address the clinical question rather than entirely use traditional statistical methods which did not necessarily translate to clinical translation. With our results, we are expecting laboratories having strengths in gene knock-down/out and gene over-expression experiments to identify the mechanisms to lithium's efficacy. This is an old drug and until this day, most textbooks lack knowledge of the drug's mechanism. These mechanisms may be attributable to biological, biochemical, gene expression, hormonal, and proteomic differences that we are aiming to identify here in this article. Please see the new figure showing the data analysis pipeline used in this paper.**

- Authors might be asking too many questions with limited data in hand. Sample size might not enough to study individual effects of multiple factors - such as treatment-type, response and then, the gender. Cell-wise sample sizes resulting from this 8-way split is less than 10 for all but two cells (less than 5 for 3 cells). Suitability of applied statistical tests and generalizability of their claims are questionable here. Authors should also think about 1:2 skew in male:female ratio, which makes this issue worse. Study can greatly benefit from asking specific and limited hypothesized questions.
- Response: **We do appreciate your robustness in identifying the obvious study limitations due to sample-size, however, we are limited to the feasibility, cost of research, patient population, and all of the work accomplished from the original study team stemming from Case Western Reserve University, Massachusetts General Hospital (Harvard University), Stanford University, Yale University, the University of Pittsburgh, Texas Health Science Center at San Antonio, and the University of Pennsylvania that uploaded this data into the Gene Expression Omnibus (GEO) database maintained by the National Institutes of Health. This was a massive undertaking in a multi-site trial. The sample-size limitations are classically used to not have results generalized, however, we ask you realize that this work not necessarily straight-forward to accomplish with in the real-world of medical care in mental health. Nevertheless, your points are well noted.**
- Response: **The 1:2 male:female skew you are referring to is exactly what is seen in clinical medicine. Females tend to respond more so than males and I clearly stated this in the introduction of the paper. We are working with the data that has been uploaded and have not found any other datasets in GEO. However, please understand that our efforts are indeed, as you clearly pointed in the beginning of this review, that this is a difficult clinical question to answer. Rather than saying we do not have enough samples, we aimed to do 'something' rather than let the dataset sit in GEO while patients are in need and laboratories have the capability and funding to seek follow-up studies. We appreciate your clear expertise and concern.**

However, we are working to create hypothesis-generating results to be later confirmed, expanded-upon, and validated for sick patients. Therefore, the generalizability of our claims are clearly limited to the dataset we obtained from clinical study of the aforementioned university hospitals. Of the 60 (sixty) patients treated with Lithium, literally only 9 responded in follow-up my expert medical teams and high quality care. Hence the need to find 'some signal' with the data at-hand in the form of expression patterns of lithium treatment responders. Thank you for the statement and again these are well-noted points you stated here.

Also, since multiple objectives are stated, methods and results section can describe each objective separately for sake of better clarity.

- Machine learning methods are not described. The methods used for learning of model and its evaluation process needs be specified. Example: How was training and test splits performed? How was feature selection performed? How were the hyper-parameters optimized? Whether the reported performance metrics are for training or testing sets? Whether the discovery dataset used for identifying '250 genes' disjoint from validation set? etc. Without these details, it is hard to comment on validity of a predictive study.
- Response: **Thank you for these questions and comments. We have updated the methods to better explain the approach used in the study. The new graphical analysis pipeline will help in explaining the approach. We also added the final Decision Tree diagrams to identify male- and female-treatment responders. Thank you for the review and we have made considerable updates to this version of the paper to address these concerns and improve this research manuscript.**

Competing Interests: None.

Referee Report 27 June 2018

https://doi.org/10.5256/f1000research.16407.r34437

Ming-Fen Ho
Division of Clinical Pharmacology, Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, MN, USA

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Referee Report 21 May 2018

https://doi.org/10.5256/f1000research.15730.r33941

Ming-Fen Ho
Division of Clinical Pharmacology, Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, MN, USA

The authors demonstrated that sex-differences gene expression might contribute to lithium treatment response using microarray expression data.

Major comments:

1. A samples size of 60 might be too small to determine the sex effects. Can the sample size n=60 provide adequate power for data interpretation, especially separated men and women for study sex-effect on gene expression?

2. The authors stated that their predictive model for lithium responders with an ROC AUC 0.92 for men, and 1 for women. If the prediction accuracy is so significant, what are the potential biological mechanisms beyond these genes? More discussion regarding the biology of those genes should be included in the paper. Once again, if the prediction accuracy is so significant, it is needed a replication study using different data sets? In summary, the authors claimed the prediction model with very high accuracy; it should be included either functional validation of those genes or a replication study population.

Specific comments:

1. Methods - study design, it might be better to use a flow chart to demonstrate the study design.

2. Methods - study design, please clarify the rationale of filtering out “250” genes.

3. Table 1 shows total study population n=60, but figure 1 legend shows male: n=41, female: n=39?

4. Figure 2: please elaborate the data presented in Figure 2. The key results for each of the four panels should be summarized in Results.

5. Table 2 and Table 4, the log FC threshold of 0.5 or 0.3 might be too low. The changes in gene expression are very subtle in Table 4.

6. Table 2, are there any gene up-regulated in males? /downregulated in females?

7. Limitations of the study should be addressed in Discussion.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 21 May 2018

Andy Eugene, Independent Researcher, USA

Major Comment Responses:
Response 1:
This point is well noted; however, it is important to realize that our gender-effects of gene expression is consistent with other studies noted within the paper and shown below:

Jansen, Rick, et al. "Sex differences in the human peripheral blood transcriptome." *BMC genomics* 15.1 (2014): 33.

Mayne, Benjamin T., et al. "Large scale gene expression meta-analysis reveals tissue-specific, sex-biased gene expression in humans." *Frontiers in genetics* 7 (2016): 183.
Further, our gender-specific results met the Benjamini-Hochberg multiple comparisons criteria adjustment due to multiple comparisons.

Comment Response 2:
We welcome and thank the reviewer's comments on the biological mechanisms beyond these genes. Clearly, it is well noted and cited in the paper that in clinical practice there is a wide inter-individual variability in the treatment and response to treatments of biplolar disorder. Moreover, these patients were not treated with lithium monotherapy, alone, and therefore further insight into the biological mechanisms were left out due to these patients were treated with an "Optimal Therapy" that includes a variety of other FDA-approved mood stabilizers.

In reference to the comment regarding the prediction accuracy, we agree that the study may warrant functional validation in a laboratory; however, it is beyond the scope of our computational psychiatry study and we will leave the functional genomics characterization of the genes to investigators seeking to pursue the findings from our results.

Competing Interests: No competing interests were disclosed.

Author Response 21 May 2018

Andy Eugene, Independent Researcher, USA

Specific Comment Responses:
We thank you for your specific comments and have addressed several of the pertinent points in your review. For all differentially expressed results reported throughout tables within the manuscript, we changed the wording from genes up-regulated or down-regulated in males or females to a clearer description statement that of genes-associated with males or females. However, we thought not necessary to include an extra figure, but rather encourage the reader to (1) review the study design section within the methods to better understand the computational approach used in our analysis and (2) read the systematic tabular reporting of the results in the manuscript text as well to understand that study approach.

For the caption in Figure 1, we thank you for the comment and have updated the sample sizes for males and female patients. The updated Figure 1 text reads: Males (n=20; with 40 pre- and post-treatment samples) and Females (n=40; with 80 pre- and post-treatment samples).

The comments regarding: (1) the fold-change of 0.5 and 0.3 being subtle and (2) the study limitations, are already specifically addressed within the original version of the manuscript. Again, it is well established and referenced within the text that small changes in gene expression have already been reported to result in major functional outcomes in human physiology.

We will update the variable importance illustration shown in Figure 2 and that will be added to the updated version of the manuscript.

Competing Interests: No competing interests were disclosed.