An Untargeted Metabolomics Analysis of Antipsychotic Use in Bipolar Disorder

Kyle J. Burghardt, Pharm.D.[1], Simon J. Evans, Ph.D.[2], Kristen M. Wiese, Pharm.D.[3], and Vicki L. Ellingrod, Pharm.D.[2,3]

Abstract

Background: Second generation antipsychotic (SGA) use in bipolar disorder is common and has proven effective in short-term trials. There continues to be a lack of understanding of the mechanisms underlying many of their positive and negative effects in bipolar disorder. This study aimed to describe the metabolite profiles of bipolar subjects treated with SGAs by comparing to metabolite profiles of bipolar subjects treated with lithium, and schizophrenia subjects treated with SGAs.
Methods: Cross-sectional, fasting untargeted serum metabolomic profiling was conducted in 82 subjects diagnosed with bipolar I disorder ($n = 30$ on SGAs and $n = 32$ on lithium) or schizophrenia ($n = 20$). Metabolomic profiles of bipolar subjects treated with SGAs were compared to bipolar subjects treated with lithium and schizophrenia subjects treated with SGAs using multivariate methods.
Results: Partial lease square discriminant analysis (PLS-DA) plots showed separation between bipolar subjects treated with SGAs, bipolar subjects treated with lithium, or schizophrenia subjects treated with SGAs. Top influential metabolite features were associated with several pathways including that of polyunsaturated fatty acids, pyruvate, glucose, and branched chain amino acids.
Conclusions: The findings from this study require further validation in pre- and posttreated bipolar and schizophrenia subjects, but suggest that the pharmacometabolome may be diagnosis specific. Clin Trans Sci 2015; Volume 8: 432–440

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Background

Second generation antipsychotic (SGA) medications, commonly considered first-line therapy in schizophrenia medication management, have become an increasingly common pharmacotherapeutic choice in bipolar disorder.[1] Despite proven efficacy, and their arguable superiority to first generation antipsychotics in the treatment of schizophrenia, patient intolerance and discontinuation remain high.[2] This is likely due to a combination of factors including inefficacy and side effects. A possible major concern and contributor to this nonadherence are the cardiometabolic side effects that include weight gain, insulin resistance, dyslipidemia, and the metabolic syndrome. Although the contribution of antipsychotics to the overall high rates of cardiovascular disease in schizophrenia and bipolar disorder is not fully know, their impact is likely significant based on the side effect profile of this class of medications.[3,4]

Significant amounts of research have been conducted to better understand the pathophysiological mechanisms and processes that underlie the therapeutic efficacy and side effects of SGAs within schizophrenia, and numerous pathways have been implicated in antipsychotic outcomes.[5,6] Currently there is little evidence to show that SGAs have diagnosis-specific mechanisms, however, some antipsychotic-induced side effects may occur at different rates between bipolar and schizophrenia patients.[7,8] Also, these two populations may have different tolerance levels for antipsychotics and antipsychotics are not always used to treat the same psychiatric symptoms in both populations. Despite a lack of evidence for antipsychotics exerting their effects through diagnosis-specific pathways, the lifestyle, diet and genetics of bipolar and schizophrenia patients may be unique.[9,10] It is currently not known if these factors create a diagnosis-specific

"metabolomic environment" in which SGAs may affect their beneficial or adverse outcomes.

The field of pharmacometabolomics has emerged in the past 10 years to assist in the understanding of drug effects in psychiatric illness. One of the first reported pharmacometabolomic studies of antipsychotic use by Kaddurah-Daouk et al. utilized a lipidomic platform to investigate lipid changes after 2–3 weeks of olanzapine, risperidone, and aripiprazole treatment in schizophrenia.[11] Their work identified several classes of lipids, including phospholipids, which were increased by treatment with all three drugs. In a follow-up study, McEvoy et al. conducted another lipidomic study to characterize the effects of 2 weeks of SGAs (risperidone or aripiprazole) on the plasma lipidome in first episode and recurrent schizophrenia subjects.[12] Their findings showed again, in first episode patients, that antipsychotic treatment caused significant changes in phospholipids and polyunsaturated fatty acids. Within the McEvoy study, recurrent schizophrenia patient's phospholipids did not change, however there were changes in the polyunsaturated fatty acids of this patient subset.

Finally, work by Xuan et al. using an untargeted serum metabolomics strategy to characterize the treatment effects of 8-week risperidone monotherapy in schizophrenia found that myo-inositol, uric acid, tyrosine and tryptophan were most predictive as metabolite biomarkers in risperidone responders.[13]

Significant work has been conducted to look at the effects of antipsychotic treatment in the schizophrenia population however, to date, this has not been evaluated in the bipolar population and pharmacometabolomic profiles have not been compared between these two diagnostically distinct populations. This study aimed to take an unbiased metabolomics approach to investigate antipsychotic use in bipolar disorder. We utilized

a well-characterized set of bipolar and schizophrenia subjects on a narrowly defined set of psychopharmacology in order to describe the overall metabolite profiles of long-term antipsychotic use within bipolar disorder (SGA versus lithium) and across diagnoses (bipolar SGA versus schizophrenia SGA).

Methods

Subject population

Subjects were recruited from a cross-sectional parent study investigating the occurrence of antipsychotic-induced metabolic syndrome in the severely mentally ill.[14,15] Subjects were required to have a current diagnosis of bipolar I disorder or schizophrenia according to the Diagnostic and Statistics Manual for Mental Disorders, IV, Text Revision (DSM-IV-TR).[16] Diagnosis was confirmed by the Structured Clinical Interview for DSM-IV-TR Diagnoses (SCID) performed by a trained research assistant and confirmed via medical chart review. Subjects were included for metabolomic study if they (1) were stable on either a maintenance therapy of lithium or a second generation antipsychotic (SGA) for at least 6 months as determined by their primary physician or psychiatrist, (2) did not have metabolic abnormalities (e.g., diabetes, dyslipidemia, cardiovascular disease or obesity) prior to starting their SGA or lithium, and (3) were willing to participate. Potential subjects were excluded if they (1) were unable or unwilling to participate or (2) had an active substance abuse diagnosis (smoking was allowed).

Subjects meeting criteria were invited to participate at the Clinical Research Unit at the University of Michigan resulting in three groups for metabolomic study: (1) bipolar I subjects treated with SGA monotherapy, (2) bipolar I subjects treated with lithium monotherapy, and (3) schizophrenia subjects treated with SGA monotherapy. All study visits occurred within 2 hours of the subject's normal waking time and in a fasting state (no food consumption for at least 10 hours prior to visit). Fasting blood samples were collected and serum was obtained within 20 minutes, flash frozen and transferred to –80 °C until metabolomic assessment. The samples were never thawed between storage and metabolomic analysis. Metabolic syndrome was diagnosed by the National Cholesterol Education Program's Adult Treatment Panel III (NCEP/ATP III-a) criteria and smoking was defined as currently smoking one or more cigarettes per day.[17] Dietary data was collected using three separate, standardized 24-hour dietary recalls through the University of Michigan Dietetics Core.[18] The protocol was approved by the University of Michigan Medical Internal Review Board and was in accordance with the Decoration of Helsinki.

Untargeted metabolomics assessment

Serum samples were submitted for an untargeted metabolomics analysis through the NIH-supported Michigan Regional Comprehensive Metabolomics Resource Core (MRC²). The core's untargeted methodology has been previously published.[19] Briefly, Carbon-13 stable isotope internal standards, metabolite standards and all other reagents for the untargeted metabolomics assay were purchased from Sigma-Aldrich (St. Louis, MO, USA). Serum was extracted according to the methods of Bruce et al. utilizing a 1:1:1 methanol:acetonitrile:acetone extraction process.[20] A modified version of the mixed-mode Hydrophobic Interaction Liquid Chromatography from Bajad et al. was used for separation on a Phenomenex (Torrence, CA, USA) Luna NH$_2$ column where mobile Phase A was acetonitrile and mobile phase

B was 5-mM ammonium acetate in water adjusted to pH 9.9 with ammonium hydroxide.[21] The supernatant from the extraction, also containing a mixture of the Carbon-13 internal standards, was added directly to autosampler vials for separation followed by analysis on an Agilent 1200 LC/6520 Time of Flight MS system. Mass spectrometry was performed in both negative and positive ion mode with electrospray ionization.

Aiglent Masshunter Qualitative Analysis software (version 4.0) was used for untargeted metabolite profiling. Features were putatively identified with relative quantification using an in-house library that has been developed under the same LC-MS conditions and using the Metlin and the Human Metabolome Databases based on the m/z values with an accuracy of 20 ppm. The core visually inspected the peak shapes of all features and discarded any artifacts. Features unable to be identified via these methods were considered "unknowns" which were removed before statistical analysis. The core then exported the data to MZmine and returned a single preprocessed peak intensity table for both the positive and negative ion modes for downstream analysis by the investigators.

Statistical analyses

Demographic and clinical data are expressed as mean ± SD. Group comparison and statistical significance was determined between (1) the antipsychotic and lithium bipolar-treated groups or (2) the antipsychotic-treated groups using unpaired, two-tailed Student's t-test or chi-square test depending on the variable using R statistical software. A p value of 0.05 or below was considered significant for demographic analysis.

The peak intensity table containing the features identified from the untargeted analysis were analyzed using the freely available online metabolomics processing software Metaboanalyst 3.0.[22] Unknowns and features that had >70% missing data in the samples were removed from downstream analysis and remaining missing data was addressed by replacing with half of the minimum positive value in the data set.[23] In order to assist in removing noise and noninformative values, low-quality data was then filtered using the robust interquantile ranges (IQR) method.[24] Normalization of data was performed by internal controls, autoscaling, and log transformation. Multivariate analyses were conducted using the partial least discriminant analysis (PLS-DA) to visually inspect metabolite profile differences between the groups of interest. The PLS-DA model was validated by 100 random permutations of the Y variable and comparison of the goodness to fit (R2Y and Q2) to the original model in the validation plot.[25] Two-dimensional score plots were produced to visually assess separation between groups. Variable importance in the projection (VIP) scores were used to identify the most influential features in the PLS-DA model. In determining the most influential features multiple testing correction was made using the Metaboanalyst default of False Discovery Rate (FDR) method. Finally, the Metaboanalyst pathway module was used to further investigate the top influential features of the PLS-DA model.

Results

Group analysis

Serum samples from a total of 62 bipolar I subjects were included for untargeted analysis. The average age of the bipolar groups was 45.1 ± 11.5, 63% were female and 86% were Caucasian. Twenty schizophrenia patients treated with SGAs were chosen based on medication, age, race, and body mass index (BMI) to compare to

bipolar subjects on SGAs. The schizophrenia cohort had an average age of 45.9 ± 9.8, 50% were female and 90% were Caucasian.

In comparing the bipolar subjects on SGAs to those on lithium a significant difference was identified for the diagnosis of metabolic syndrome. Metabolic syndrome was more common for bipolar subjects on SGAs compared to the lithium-treated subjects (45.0 vs. 25.0, respectively, $p = 0.0470$). Bipolar subjects on SGAs also had a significantly lower ratio of dietary polyunsaturated fatty acids to saturated fatty acids (PUFA/SFA) compared to bipolar subjects on lithium, indicating an overall lower intake of PUFAs in the SGA-treated group. Additionally, systolic blood pressure was significantly higher in the bipolar patients taking SGAs as compared to those on lithium ($p = 0.05$), and differences in race/

ethnicity between these two groups approached significance as well ($p = 0.07$). The metabolic-related differences were expected given the known metabolic adverse effects of SGAs compared to lithium.

For demographic and clinical comparisons of the antipsychotic-treated bipolar and schizophrenia subjects, a significantly higher amount of schizophrenia subjects smoked ($p = 0.0383$). Diastolic blood pressure ($p = 0.07$) and HbA1C% ($p = 0.08$) approached, but did not reach, statistical significance. Of note, gender did not significantly differ between the two antipsychotic groups ($p = 0.30$). A full description of each group's demographic, clinical and medication characteristics can be found in *Tables 1* and *2*.

Treatment group	Bipolar I on SGAs	Bipolar I on lithium	Schizophrenia on SGAs
Sample size (*n*)	30	32	20
Age	45.4 ± 11.9	44.1 ± 12.2	45.9 ± 9.8
Gender (% female)	63.2	62.5	50.0
% Caucasian	87.7	84.4	90.0
% Currently smoking†	28.6	28.1	55.0
Body mass index (kg/m²; mean ± SD)	31.4 ± 8.5	32.2 ± 8.2	32.0 ± 6.83
Waist to hip ration (WHR; mean ± SD)	1.08 ± 0.10	1.10 ± 0.11	1.06 ± 0.08
Systolic blood pressure (mmHg; mean ± SD)*	125 ± 19.2	120 ± 13.0	118 ± 11.7
Diastolic blood pressure (mmHg; mean ± SD)	75.8 ± 11.7	70.7 ± 10.6	70.5 ± 8.35
Total cholesterol (mg/dL; mean ± SD)	196 ± 45.3	180 ± 37.5	177 ± 49.5
Triglycerides (mg/dL; mean ± SD)	154 ± 115	123 ± 72.0	167 ± 123
High-density lipoprotein (mg/dL; mean ± SD)	58.7 ± 18.2	55.5 ± 9.17	52.4 ± 15.3
Low-density lipoprotein (mg/dL; mean ± SD)	120 ± 37.5	110 ± 33.8	105 ± 33.3
HbA1C (%)	5.62 ± 0.64	5.79 ± 6.29	6.01 ± 1.14
Glucose (mg/dL; mean ± SD)	98.2 ± 15.0	99.5 ± 29.3	105 ± 17.6
% Metabolic syndrome*	46.0	25.0	55.0
Dietary caloric intake (kcal; mean ± SD)	1,833 ± 555	2,008 ± 605	1,853 ± 337
Dietary total fat intake (g; mean ± SD)	74.6 ± 31.0	76.6 ± 41.4	76.7 ± 16.4
Dietary total carbohydrate intake (g; mean ± SD)	220 ± 91.5	226 ± 108	218 ± 79.9
Dietary polyunsaturated to saturated fatty acid intake (mean ± SD)*	0.703 ± 0.070	1.06 ± 0.110	0.666 ± 0.202
Antidepressant use %	59	62	55

*Indicates statistically significant difference between bipolar I on SGA and bipolar I on lithium based on an indepedent *t*-test or a chi-square test.
†Indicates statistically significant difference between bipolar I on SGA and schizophrenia on SGA based on an indepedent *t*-test or a chi-square test.

Table 1. Demographic and clinical characteristics of the three study groups. Values expressed as ±SD or %.

Antipsychotic	Bipolar I on SGAs		Schizophrenia on SGAs	
	% on given antipsychotic	Average dose (mg)	% on given antipsychotic	Average dose (mg)
Olanzapine	16.7	12	20	16.7
Clozapine	16.7	150	20	300
Quetiapine	33	405	20	400
Risperidone	20	3	20	5
Aripiprazole	6.6	15	10	22
Ziprasidone	6.6	120	10	140

Table 2. Breakdown of antipsychotic frequency and dose for bipolar I subjects and schizophrenia subjects on second generation antipsychotics. No significant differences existed between the groups when comparing antipsychotic use.
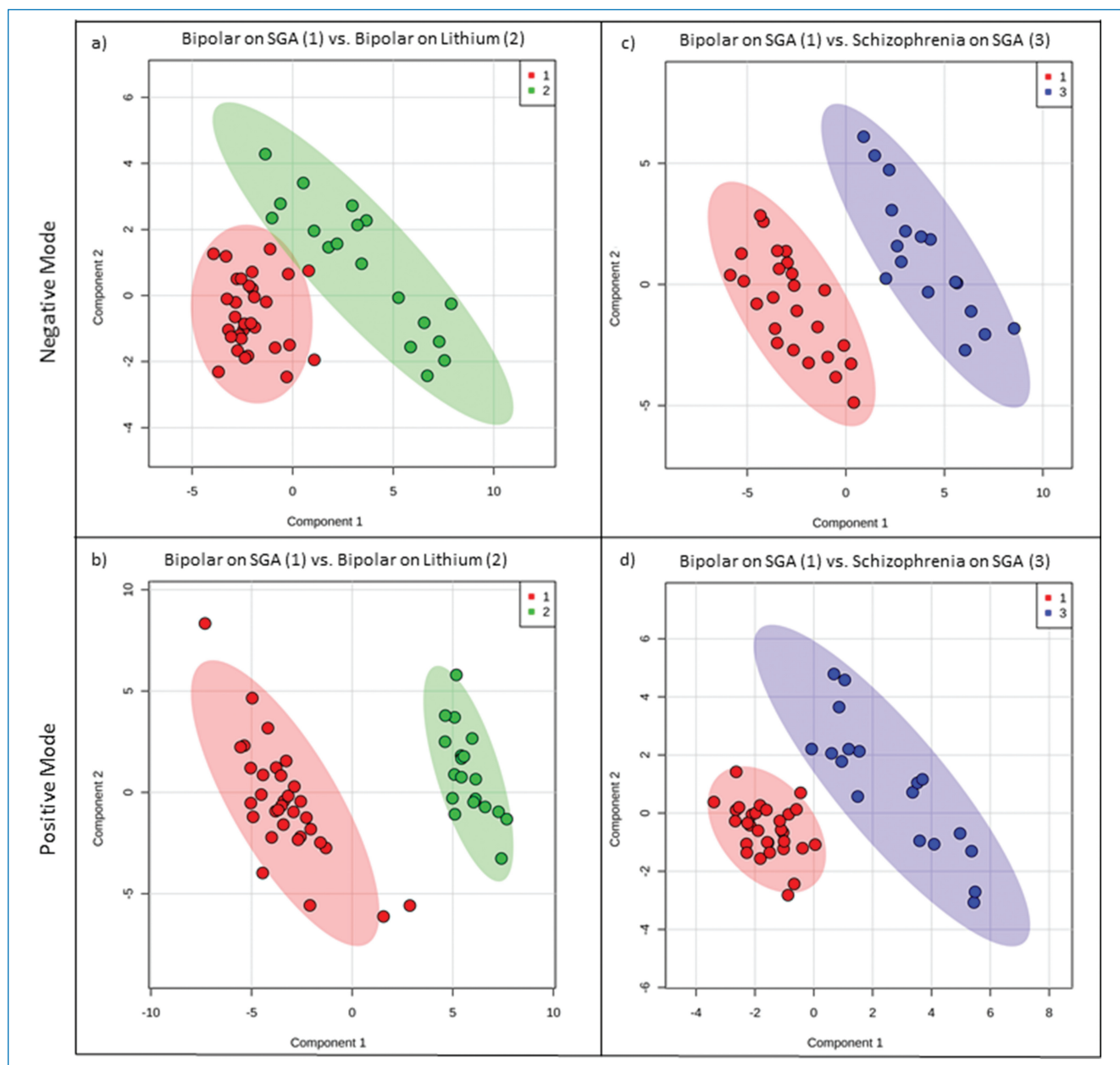
Figure 1. Partial least squares discriminant analysis (PLS-DA) of untargeted metabolomics data from bipolar and schizophrenia subjects treated with antipsychotics or lithium. The two-dimensional PLS-DA score plots reveal separation between bipolar subjects treated with SGAs (red dots) compared to bipolar subjects treated with lithium (green dots) in both (a) negative and (b) positive ion mode. Additionally, plots show separation between bipolar subjects treated with SGAs (red dots) and schizophrenia subjects treated with SGAs (blue dots) in both (c) negative and (d) positive ion mode. Colored shaded areas correspond to each group's.

Metabolome changes

Following preprocessing, 354 named features remained for analysis in negative mode and 480 named features were available for analysis from positive mode. PLS-DA modeling revealed divergent effects of current medication treatment and diagnosis on the serum metabolome (*Figure 1*). Each model was validated by random permutation of each group. Permutation tests showed that each model was outside the distribution of random class assignments based on each respective between-group sum of the squares and within-group sum of squares (B/W) ratio (all *p* < 0.05). Goodness of Fit analysis for score plots between bipolar subjects resulted in a R2Y = 0.88 and Q2 = 0.79 for positive mode and a R2Y = 0.83 and Q2 = 0.55 for negative mode. Goodness of Fit analyses for score plots between bipolar and schizophrenia subjects results in a R2Y = 0.95 and Q2 = 0.52 for positive mode and a R2Y = 0.76 and Q2 = 0.44 for negative mode.

Variable importance in projection (VIP) scores were computed in order to investigate the metabolite features that were most responsible for the observed differences in each of the PLS-DA score plots. The top five influential metabolites (with VIP > 1) listed as annotated features for each PLS-DA plot can be found in *Table 3*.

Putative HMDB match (ID)	Observed (m/z)	Retention time (min)	Mass accuracy (ppm)	VIP score	Relative abundance comparison between groups	Feature class
Bipolar on SGA (1) vs. bipolar on lithium (2)						
Negative mode score plot						
Linoleic acid (HMDB00673)	279.2328	3.448	8.61	4.96	1 > 2	Lipids—lineolic acids and derivatives
5-Hydroxyindoleacetic acid (HMDB00763)	190.05439	3.307	12.82	4.25	1 > 2	Indoles
Cyclamic acid (HMDB31340)	178.054338	2.571	11.33	3.96	1 > 2	Organic sulfuric acids and derivatives
Glyoxal*	56.998203	0.629	5.14	3.09	1 > 2	Aldehydes
L-Lactic acid (HMDB00190)	89.024418	0.52	7.38	2.74	2 > 1	Hydroxy acids and derivatives
Positive mode score plot						
3-Hydroxymethylglutaric acid (HMDB00355)	163.0601	1.659	2.92	3.2	2 > 1	Hydroxy acids and derivatives
Alpha-tocopherol (HMDB01893)	431.388357	6.774	4.35	2.8	1 > 2	Prenol lipids
Pantolactone (HMDB59876)	131.070271	2.801	10.59	2.8	2 > 1	Lactones
N-Acetylglutamic acid (HMDB01138)	190.070999	1.221	9.99	2.7	1 > 2	Amino acids and derivatives
Pyroglutamic acid (HMDB00267)	259.092463	1.069	2.19	2.7	1 > 2	Pyrrolidines
Bipolar on SGA (1) vs. schizophrenia on SGA (3)						
Negative mode score plot						
3Alpha,7alpha-dihydroxy-5beta-cholestanate*	433.33248	3.806	7.3	2.51	3 > 1	Lipids—sterol lipids
9,10-Epoxyoctadecenoic acid (HMDB04701)	295.22799	3.958	6.65	2.40	3 > 1	Lipids—linoleic acid and derivatives
Cyclamic acid (HMDB31340)	178.054338	2.571	11.33	2.25	1 > 3	Organic sulfuric acids and derivatives
Arachidonate (HMDB60102)	303.232954	4.104	7.11	2.24	3 > 1	Lipids—linoleic acid and derivatives
Pimelic acid (HMDB00857)	159.066282	0.5595	9.8	2.21	1 > 3	Carboxylic acids and derivatives
Positive mode score plot						
Oleoyl glycerol*	379.281881	8.16	4.542	2.94	3 > 1	Lipids—glycerolipids
LysoPC (20:3(5Z,8Z,11Z)) (HMDB10393)	506.26583	3.986	3.83	2.90	1 > 3	Lipids—glycerophospholipids
Adenosine (HMDB00050)	268.1025	2.299	4.92	2.76	1 > 3	Nucleosides, nucleotides, and analogues
3-Hydroxymethylglutaric acid (HMDB00355)	185.042044	1.648	12.46	2.65	3 > 1	Hydroxy acids and derivatives
L-leucine (HMDB00687)	132.101905	1.41	1.14	2.58	3 > 1	Amino acid

Table 3. Top differential metabolite features that influenced discrimination for PLS-DA score plots between medication groups. Variable importance in projection (VIP) scores were obtained with relative abundance differences from validated PLS-DA score plots.

*Indicates features without a HMDB entries. Alternate database entries are: Glyoxal (Kegg ID: C14448); 3alpha,7alpha-dihydroxy-5beta-cholestanate (Kegg ID: C04554); oleoyl glycerol (PubChemCID: 5283468).

Discussion

Discrimination of bipolar SGA use, bipolar lithium use, and schizophrenia SGA use

Bipolar disorder and schizophrenia pharmacotherapy can be difficult to study given the psychotropic polypharmacy seen within both populations.[26,27] We attempted to overcome this limitation by limiting metabolomics analysis to patients stable on a restricted set of psychopharmacotherapy. Specifically, bipolar subjects were maintained on either lithium or SGA mood stabilization monotherapy while schizophrenia subjects were maintained on SGA monotherapy. The only other psychotropic agent that was common amongst each group was antidepressant therapy, which was well matched between the groups (*Table 1*). PLS-DA score plots showed satisfactory visual separation and the models performed to varying degrees (see results) however all models achieved Q2 values >0.4 which has been considered as acceptable for certain human metabolomics studies.[28]

In this study, the comparison of metabolite features between bipolar subjects on differing psychiatric pharmacotherapy as well as comparisons to a group of schizophrenia subjects treated with SGAs did show differing profiles. This may suggest that the pharmacometabolome may have within diagnosis and between diagnosis effects. The implications of such preliminary findings and the hypotheses one can derive from them require further work and validation using specific targeted metabolomic approaches in pre- and postantipsychotic-treated populations in order to fully assess any diagnosis-specific effects on the pharmacometabolome. Several such studies have been completed in the schizophrenia population, but comparable data is lacking in the bipolar population.

Metabolite features characterizing distinction between antipsychotic- and lithium-treated bipolar subjects

Features that characterized the observed differences in the PLS-DA score plot of bipolar subjects treated with SGAs and bipolar subjects treated with lithium came from several different classes. Both the positive and negative ion modes identified metabolite features from lipid and hydroxy acid classes. Pathway analysis of the top metabolite features from both modes revealed several pathways of interest, including those involved in the metabolism of linoleic acid, pyruvate, and glucose. It is reasonable to expect that variation in each of these metabolic pathways would be significantly affected by antipsychotic use.

Pyruvate, a byproduct of glucose use in the TCA Cycle, is important in energy metabolism. Pyruvate metabolism has been investigated in the etiology of obesity, insulin resistance, and diabetes.[29] The effect of pyruvate administration and metabolism has also been investigated in SGA-induced metabolic disturbances.[30] Smith et al. found that pyruvate challenges in rats treated with olanzapine increased hepatic glucose output. This work suggested that the insulin resistance caused by SGAs may be due to indirect effects of antipsychotics to increase glucose output rather than directly inducing insulin resistance. Work has also shown that olanzapine administration increases gluconeogenesis by upregulation of several genes in the frontal cortex, including pyruvate kinase, the enzyme responsible for the production of a pyruvate from phosphoenolpyruvate.[31]

Lactate, a product of pyruvate metabolism, was found to influence the metabolomic feature of risperidone response in the metabolomics study by Xuan et al.[13] Their work showed an increase in lactate in both responders and nonresponders to risperidone, which may indicate a drug effect not necessarily related to efficacy. Within the current study, L-Lactic Acid, was higher in bipolar subjects treated with lithium compared to bipolar subjects treated with SGAs. Although contradictory to the findings of Xuan et al., other findings have suggested an increase in gray matter lactate of bipolar subjects treated with lithium which may be related to our peripheral finding.[32] Without a healthy control comparator or longitudinal design, we cannot draw firm conclusions from the results of this analysis and fully compare to our lactate result to that of Xuan et al. However, this finding, in addition to previous work, may indicate that lactic acid and pyruvate metabolism are important pathways for further study in the bipolar and SGA-treated populations.

In addition to pyruvate being a top metabolite feature, the finding of metabolite features that influence glucose metabolism is to be expected as it has been a known effect of SGAs for many years. Finally, work has shown linoleic acid, which will be further described below, was influenced by SGA treatment, particularly in bipolar disorder.

Metabolite features characterizing distinction between SGA-treated bipolar subjects and SGA-treated schizophrenia subjects

Comparisons of untargeted metabolite feature profiles between the bipolar and schizophrenia subjects treated with SGAs showed separation upon PLS-DA analysis. Influential metabolite features were associated with linoleic acid metabolism, biotin metabolism, valine, leucine, and isoleucine metabolism and degradation, and, glycerophospholipid metabolism.

Biotin is an important water-soluble B vitamin (B_7) used in two reactions involving amino acids: (1) leucine catabolism and (2) the use of methionine and threonine for the downstream production of succinyl CoA as it enters the TCA cycle.[33] Biotin also serves as the cofactor for the pyruvate and acetyl-CoA carboxylases in gluconeogenesis and fatty acid synthesis pathways.[34] The relative abundance of pimeleic acid, a precursor to biotin, was higher in the bipolar group compared to the schizophrenia group. Although pimelic acid has not been extensively studied in human disease, the supplementation and metabolism of biotin has been evaluated for therapeutic efficacy in improving metabolic control in diabetic patients.[35] It is not fully understood what role biotin metabolism may play in the SGA pharmacometabolome. Additional analyses of this pathway in SGA-induced insulin resistance may be warranted to further understand its importance, if any.

Another influential metabolite feature from our metabolomics comparison of a bipolar and a schizophrenia SGA-treated group was that of leucine. Leucine is one of several branched-chain amino acids (BCAA) known to serve as a precursor in the brain for the production of glutamate, which is essential for neuronal function in the brain.[36] In addition to this important function in the brain, the BCAAs have been implicated in both obesity and diabetes models[37] which may be of significance given SGAs metabolic side effects. A recent metabolomics investigation by Wang et al. found that six branched-chain amino acids, including leucine, were predictive of the development of diabetes.[38] Within our study L-leucine was higher in schizophrenia subjects on SGAs. Previously, elevated leucine levels were identified in the cerebral spinal fluid of schizophrenia subjects, but these findings were not replicated in the periphery of schizophrenia subjects treated with the SGA clozapine.[39] Amino acid supplementation has also been investigated in bipolar disorder and shown to improve manic

symptoms acutely.[40] Taken together, BCAA metabolism may be a feasible pathway for further study in both SGA efficacy and side effects. We believe this may be highlighted in the coming years with the advent of microbiome research within psychiatry.

Finally, another important pathway identified from our metabolomic comparisons of bipolar and schizophrenia subjects treated with SGAs was that of glycerophospholipids. These are important components of cell membranes (including neural membranes) and include plasmalogens, phosphatidates, and phosphatidylcholines. Various phospholipid species have long been thought to be dysregulated in schizophrenia however, investigation of the associated pathways in bipolar disorder is lacking. Work has shown that antipsychotics interact with lipids to cause cell membrane reorganization and they can have different affinities for particular lipid species, including phosphatidylcholine and plasmalogens.[41] This may suggest that antipsychotics are able to modulate receptor activity in the brain by altering the environment of the cell membrane. Our findings here may further validate the lipidomic work by Kaddurha-Doak and McEvoy in which they have identified, in two separate lipidomic studies, SGA-induced changes in phospholipid and plasmalogen metabolism in schizophrenia subjects at various states (e.g., first episode, recurrent) compared to healthy controls.[11,12] The particular lysophosphotidycholine identified in our study had a higher relative abundance in SGA-treated bipolar subjects however, we did not have a healthy control comparator to identify if the levels of this metabolite were overall elevated. Nevertheless, this work adds to a continued theme in the literature of phospholipids playing an important role in antipsychotic outcomes.

Potential role of linoleic acid metabolism in antipsychotic treatment

A common pathway that emerged from the PLS-DA score plots of the three treatment groups in this study was that of the linoleic acid. Linoleic acid is an omega-6 polyunsaturated essential fatty acid (PUFA) that is used as a constituent of cell membranes and in the biosynthesis of arachidonic acid, which mediates neuronal transduction and can induce neurotransmission.[42] As an essential fatty acid that must be obtained from the diet, linoleic acid has been the subject of a large amount of cardiometabolic research.

The role of linoleic acid in human health and disease processes continues to be debated. It is generally thought that omega-6 PUFAs are correlated with proinflammatory mediator production while the parallel pathway of omega-3 PUFAs are anti-inflammatory.[43] PUFAs have been found to be influential in the few metabolic and lipidomic studies involving antipsychotics to date.

In the schizophrenia lipidomic investigation by Kaddurah-Daouk et al., they found that linoleic acid increases following antipsychotic treatment and determined that a deficiency of linoleic acid was not present in SGA-treated patients.[11] Comparisons of the relative abundances of linoleic acid in our population showed similar results where bipolar subjects treated with SGAs had higher relative abundances of the metabolite compared to bipolar subjects treated with lithium. In the untargeted metabolomic study in schizophrenia by Xuan et al., they found increases in linoleic acid after risperidone treatment and particularly, in responders to risperidone.[13] They also showed that linoleic acid had a high statistical likelihood for both explaining metabolite profile differences and being predictive of risperidone response.

Although work has consistently ruled out linoleic acid deficiencies in antipsychotic-treated patients, there continues to be debate on the effect of antipsychotics on inflammatory status in patients.[44–47] One possible reason for these seemingly conflicting findings may be that linoleic acid plays different roles within diagnostic populations that have distinct dietary and environmental conditions. Furthermore, it could be that increases in linoleic acid leads to an imbalance between omega-6 and omega-3 PUFAs (or between particular species in each pathway) further leading to altered cell membrane stability and downstream negative effects. It is not known what this correct "balance" may be, if there is indeed one. One study did identify differing ratios of proinflammatory to antiinflammatory mediators when comparing bipolar disorder patients to schizophrenia patients[48] however they did not investigate PUFA profiles. Finally, other factors, including genetic polymorphisms of the Fatty Acid Desaturase (FAD) genes, which are responsible for PUFA metabolism, have been shown to play a role in antipsychotic outcomes.[49]

A highly targeted approach to investigating PUFA metabolism may help to further clarify the interaction of inflammation and SGAs and whether it plays a role in both bipolar disorder and schizophrenia or rather a particular population. Pre and postmeasures in antipsychotic-treated populations are needed and should include metabolite and inflammatory levels, genotyping and gene expression and possibly epigenetic work. Indeed, work on this pathway has already started within bipolar disorder disease risk with significant outcomes.[50] Evans et al. demonstrated linoleic acid disturbances in bipolar subjects compared to healthy controls, which may be partly attributable to dietary and psychiatric medication use. Thus it can be seen that that this pathway may have a pivotal role in both bipolar disorder disease burden as well as SGA outcomes.

Limitations

The major limitation of the current study is its cross-sectional design and lack of healthy control group that limits our ability to draw causal conclusions from the relationships identified between SGA use and diagnosis. As mentioned, prospective studies assessing metabolome changes before and after SGA treatment in bipolar disorder is needed. Furthermore, although the use of an untargeted metabolomic platform enables us to better assess the global pharmacometabolome of the subject populations in this work, targeted and quantitative work must follow to fully assess the usefulness of the identified pathways as biomarkers for treatment outcome.

Conclusions

The purpose of this work was to describe the outcomes of an untargeted metabolomic analysis of SGA use in bipolar disorder by conducting a within diagnosis comparison and across diagnosis comparison. The analyses presented in this study are essential to enable scientists, and ultimately clinicians, to better understand the input of disease-specific processes on the metabolome versus the effects environmental effects such as medications, diet and lifestyle. Distinct metabolite features were identified between SGA-treated bipolar subjects, lithium-treated bipolar subjects and SGA-treated schizophrenia subjects. Pathways of interest were identified for further study including biotin, pyruvate, and linoleic acid metabolism. Many of these findings support other pharmacometabolomic work conducted in the schizophrenia

population treated with antipsychotics. This study's findings add to a growing body of literature showing PUFAs like linoleic acid may play an important role in both disease specific processes and SGA outcomes in bipolar disorder.

Financial Disclosures

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