

Supplemental Information

Atypical Antipsychotic Exposure May Not Explain the Broad Range of Metabolic Phenotypes of Schizophrenia Patients

Kristen M. Ward¹, Larisa Yeomans², Cora McHugh², A. Zarina Kraal³, Stephanie A. Flowers⁴,
Alla Karnovsky⁵, Arun Das^{6,9}, Vicki L. Ellingrod^{1,7}, Kathleen A. Stringer^{1,2,8}

¹Department of Clinical Pharmacy, and the ²NMR Metabolomics Laboratory, College of Pharmacy; ³Department of Psychology, College of Literature, Science and the Arts; ⁴Department of Pharmacy Practice, University of Illinois College of Pharmacy; ⁵Department of Bioinformatics and Computational Medicine, ⁶Department of Internal Medicine, Division of Metabolism, Endocrinology and Diabetes, ⁷Department of Psychiatry, and ⁸Division of Pulmonary and Critical Care Medicine, School of Medicine; and the ⁹Michigan Regional Comprehensive Metabolomics Resource Core, University of Michigan, Ann Arbor, MI, USA

Corresponding Author:

Kathleen A. Stringer, PharmD

Department of Clinical Pharmacy, College of Pharmacy, University of Michigan

428 Church Street, Ann Arbor, MI, 48109

stringek@umich.edu

Phone: (734) 647-4775

Fax: (734) 763-4480

TABLE OF CONTENTS

SUPPLEMENTARY METHODS: METABOLOMICS ANALYSES.....	3
SUPPLEMENTARY TABLE 1: METABOLITES DIFFERENTIATING BMI CONTROL GROUPS.....	4
SUPPLEMENTARY TABLE 2: METSCAPE PATHWAY MAPPING RESULTS	5
SUPPLEMENTARY TABLE 3: METABOLITES DIFFERENTIATING SCHIZOPHRENIA PARTICIPANTS GROUPED BY OBESITY STATUS	6
SUPPLEMENTARY FIGURE 1: REPRESENTATIVE NMR SPECTRA.....	7
SUPPLEMENTARY FIGURE 2: REPRESENTATIVE GAS CHROMATOGRAPHY (GC) SPECTRUM	8
REFERENCES	10

Supplementary Methods: Metabolomics Analyses

Quantitative 1D-1H-NMR Metabolomics Sample Preparation, Spectral Analysis, and Profiling:

Following extraction with H₂O/methanol (MeOH):chloroform (CHCl₃), the H₂O/MeOH fractions were each re-suspended in 0.5 mL of deuterated water, formate (5.387 mM) was added as an internal standard, and each sample was pH-adjusted to within 6.5 to 7.5. Samples were transferred to NMR tubes (5mm; Wilmad rated for 500 MHz) for assay. NMR spectral analysis was performed using Chenomx NMR Suite 8.1 software (Chenomx, Inc., Edmonton, Alberta, Canada; chenomx.com) to manually phase shift and correct spectra baselines, then identify and quantify metabolite concentrations relative to the internal standard. The 336 compound Chenomx Library was used for compound identification. Metabolite profiling was completed by a group of users who were unaware of participant group allocation, and a NMR core member cross-checked all profiling results.

Gas Chromatography (GC) to detect Free Fatty Acids (FA):

Briefly, preprocessing steps of the CHCl₃ serum fractions included addition of known amount of heptadecanoic acid (C17:0), as an internal standard, followed by methyl ester derivation of the FA,¹ then thin layer chromatographic purification of the FA methyl esters.² An Agilent 5890 gas chromatograph with an Agilent HP 88 column was used to detect and quantify FA components. Additional GC specifications include: sample injection volume of 1-2 μL, hydrogen carrier gas with nitrogen makeup gas, a run time of 20 minutes per sample, and flame ionization detection. ChemStation software (Agilent) was employed to identify FA methyl esters based on retention time comparison with respect to authentic standards run side by side, and to perform quantification relative to the internal standard.

Supplementary Table 1: Metabolites Differentiating BMI Control groups

¹H-NMR Metabolite	P value	FDR (%)
Valine	0.0080	8.46
Isoleucine	0.0120	8.46
Leucine	0.0130	8.46
Ornithine	0.0137	8.46
Proline	0.0179	8.46
Lysine	0.0194	8.46
Alanine	0.0218	8.46
Tyrosine	0.0232	8.46
Glucose	0.0238	8.46
Glutamine	0.0272	8.46
Histidine	0.0291	8.46
Threonine	0.0297	8.46
Carnitine	0.0327	8.46
Betaine	0.0329	8.46
Citrate	0.0396	9.26
Taurine	0.0412	9.26
Serine	0.0438	9.27
3-Hydroxybutyrate	0.0482	9.64
Glycine	0.0545	9.94
2-Hydroxybutyrate	0.0552	9.94
Creatinine	0.0588	10.08
Choline	0.0736	12.05
Phenylalanine	0.0822	12.55
Lactate	0.0867	12.55
O-Acetylcarnitine	0.0871	12.55
Creatine	0.1146	15.86
Glutamate	0.1325	17.66
Methionine	0.1916	24.64
Fatty Acid	P value	FDR (%)
20:0	<0.0001	0.00
22:1	0.0001	0.13
14:0	0.0018	1.43
18:3 n-3	0.0038	2.27
20:3	0.0080	3.82
24:1	0.0181	7.23
18:1 n-7	0.0240	8.23
16:1	0.0323	9.68

Metabolites identified as significantly different between the BMI control groups (BMI ≥ 30 or < 30 kg/m²) when compared using a Student's t-test with a False Discovery Rate (FDR) of $< 25\%$ to correct for multiple comparisons.

Supplementary Table 2: Metscape Pathway Mapping Results

Pathway	Number of Hits[†]	Compounds[‡]
Bile acid biosynthesis	2	glycine, taurine
Butanoate metabolism	2	3-hydroxybutyrate
Glycerophospholipid metabolism	3	acetate, serine, creatine phosphate
Glycine, serine, alanine and threonine metabolism	2	glycine, serine
Glycolysis and Gluconeogenesis	1	acetate
Glycosphingolipid metabolism	1	serine
Leukotriene metabolism	1	glycine
Lysine metabolism	2	glycine, carnitine
Methionine and cysteine metabolism	2	serine, taurine
Porphyrin metabolism	1	glycine
TCA cycle	1	acetate
Urea cycle and metabolism of arginine, proline, glutamate, aspartate and asparagine	2	glycine, carnitine
Vitamin B9 (folate) metabolism	2	serine, glycine

Results of pathway analysis of the metabolites differentiating schizophrenia quartiles.

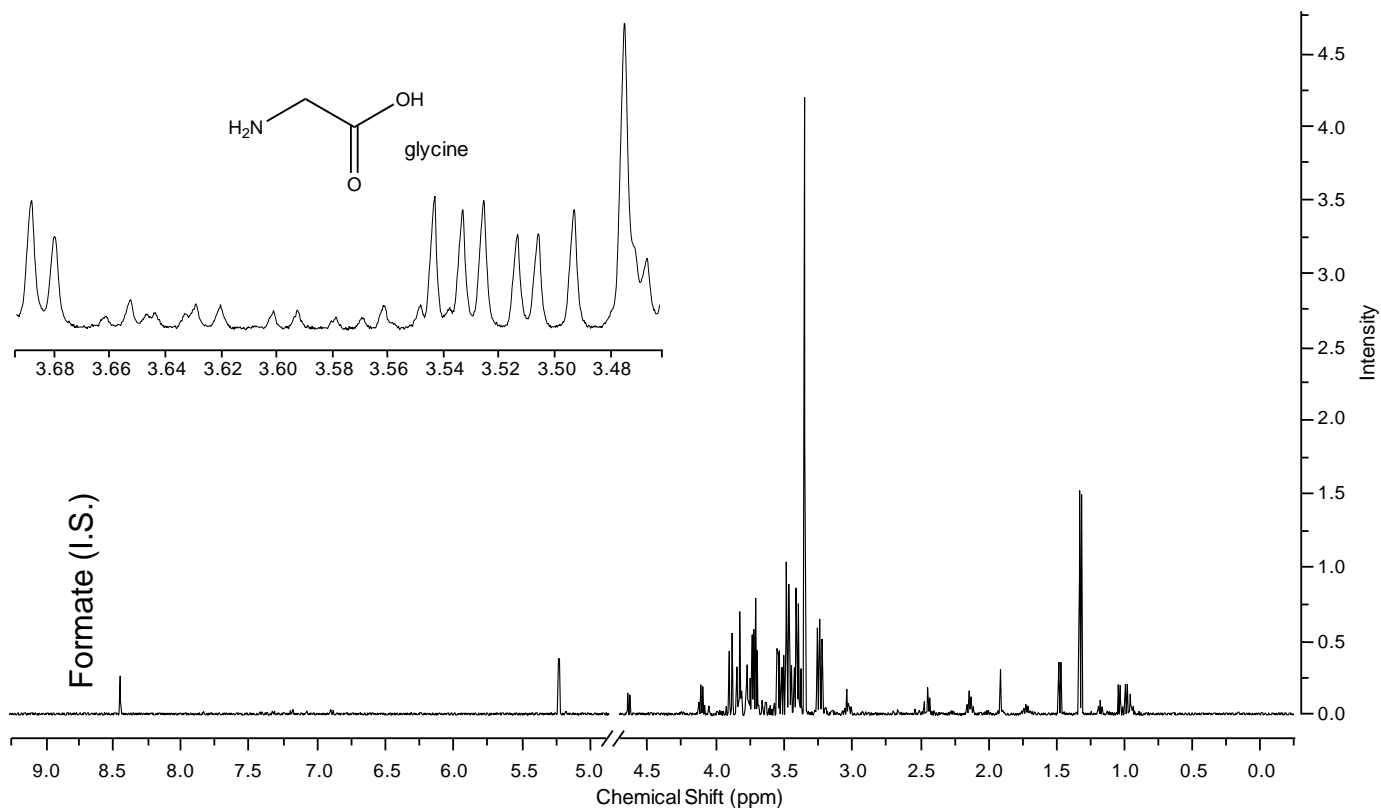
[†]Corresponds to the number of compounds differentiating the schizophrenia quartiles that are mapped in the listed pathway. [‡]Metabolites involved in the Metscape pathway that differentiate the schizophrenia quartiles (as separated by log transformed fasting insulin concentration), as determined by an ANOVA analysis with an FDR of <25%. As discussed in the text, the metabolites included in the Metscape analysis do not include those potentially associated with BMI variability.

Supplementary Table 3: Metabolites Differentiating Schizophrenia Participants Grouped by Obesity Status

¹H-NMR Metabolite	<i>P</i> value	FDR (%)
Tyrosine	0.0001	0.22
Isoleucine	0.0030	4.10
Alanine	0.0042	4.10
Glycerol	0.0046	4.10
Leucine	0.0065	4.59
Valine	0.0076	4.59
Glutamate	0.0158	7.81
O-Acetylcholine	0.0207	7.81
Phenylalanine	0.0211	7.81
Proline	0.0220	7.81
Lysine	0.0239	7.81
Lactate	0.0470	14.09
Glucose	0.0613	16.98
Fatty Acid	<i>P</i> value	FDR (%)
14:0	0.0077	10.76
16:1	0.0094	10.76
18:2	0.0427	20.65
24:0	0.0449	20.65
18:1 n-7	0.0476	20.65
18:1 n-9	0.0539	20.65

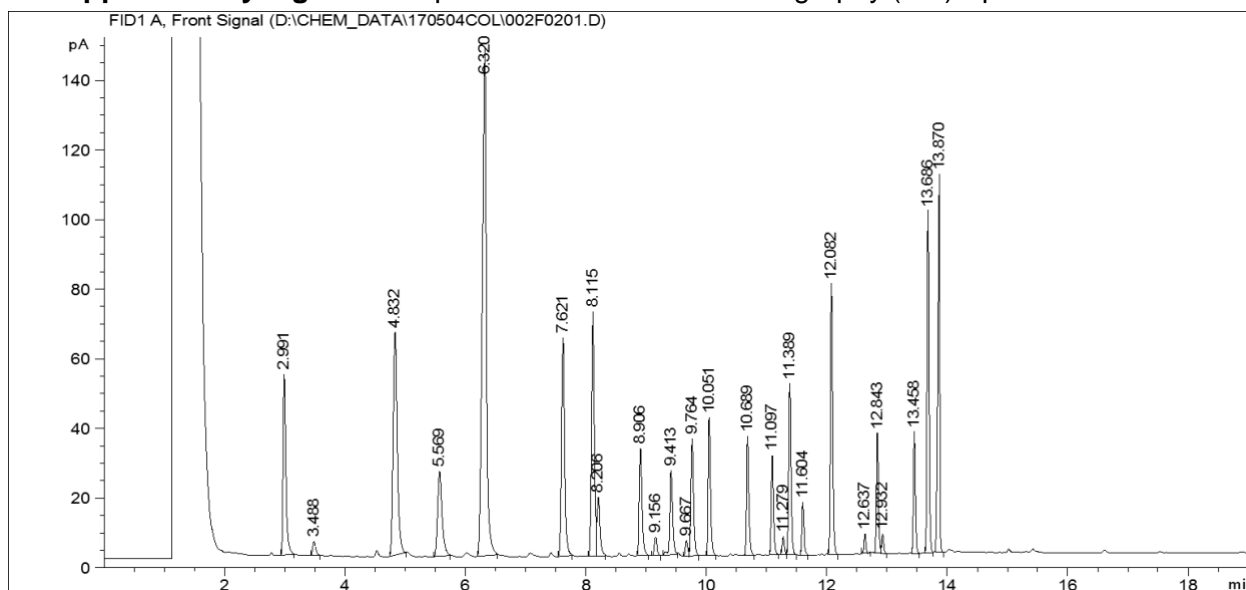
Metabolites identified as significantly different between the schizophrenia participants when grouped by BMI (≥ 30 or < 30 kg/m²), and compared using a Student's t-test with a False Discovery Rate (FDR) of $< 25\%$ to correct for multiple comparisons.

Supplementary Figure 1: Representative NMR Spectra



A representative ¹H-NMR spectrum of the methanol:chloroform fraction of a serum sample illustrating the profiling (identification and quantification) of glycine. Formate was added as an internal standard (I.S.). The x-axis represents the chemical shift in parts per million (ppm) and the y-axis corresponds to signal intensity.

Supplementary Figure 2: Representative Gas Chromatography (GC) Spectrum



A representative GC spectrum of standard fatty acid methyl esters containing saturated and unsaturated components generated from the hydrophobic fraction of a serum sample following methanol:chloroform extraction. The GC parameters and the running procedures have been described in the text. The x-axis is given in minutes, and the y-axis is relative signal in picoAmps. The list of retention times used to identify fatty acids are provided below. GC parameters and the running procedures have been described in the text.

Retention Time (minutes)	Fatty acid
2.991	Myristic acid (14:0)
3.488	Myristoleic acid (14:1, n-5)
4.832	Palmitic acid (16:0)
5.569	Palmitoleic acid (16:1, n-7)
6.32	Heptadecanoic/Margaric acid (17:0)
7.621	Stearic acid (18:0)
8.115	Oleic acid (18:1, n-9)
8.206	Vaccenic acid (18:1, n-7)
8.906	Linoleic acid (18:2, n-6)
9.156	Nonadecanoic acid (19:0)
9.413	Alpha-linolenic acid (18:3, n-3)
9.667	Eicosanoic/Arachidic Acid (20:0)
9.764	Gamma-linolenic acid (18:3, n-6)
10.051	Eicosenoic acid (20:1, n-9)
10.689	Eicosadienoic acid (20:2, n-6)
11.097	Eicosatrienoic acid (20:3, n-6)
11.279	Docosanoic/Behenic acid (22:0)
11.389	Arachidonic acid (20:4, n-6)

11.604	Docosenoic/Erucic acid (22:1, n-9)
12.082	Eicosapentaenoic Acid (20:5, n-3)
12.637	Lignoceric acid (24:0)
12.843	Docosatetraenoic acid (22:4, n-6)
12.932	Tetracosenoic acid (24:1, n-9)
13.458	Docosapentaenoic acid (22:5, n-3)
13.686	Docosahexaenoic/Cervonic acid (22:6, n-3)
13.87	Hexacosanoic/Cerotic acid (26:0)

References

1. MORRISON, W. R. & SMITH, L. M. PREPARATION OF FATTY ACID METHYL ESTERS AND DIMETHYLACETALS FROM LIPIDS WITH BORON FLUORIDE--METHANOL. *J. Lipid Res.* **5**, 600–8 (1964).
2. Mangold, H. K. *Thin-Layer Chromatography*. (Springer New York, 1969).