

Supplemental Information

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

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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 |
| Porphyrim 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.

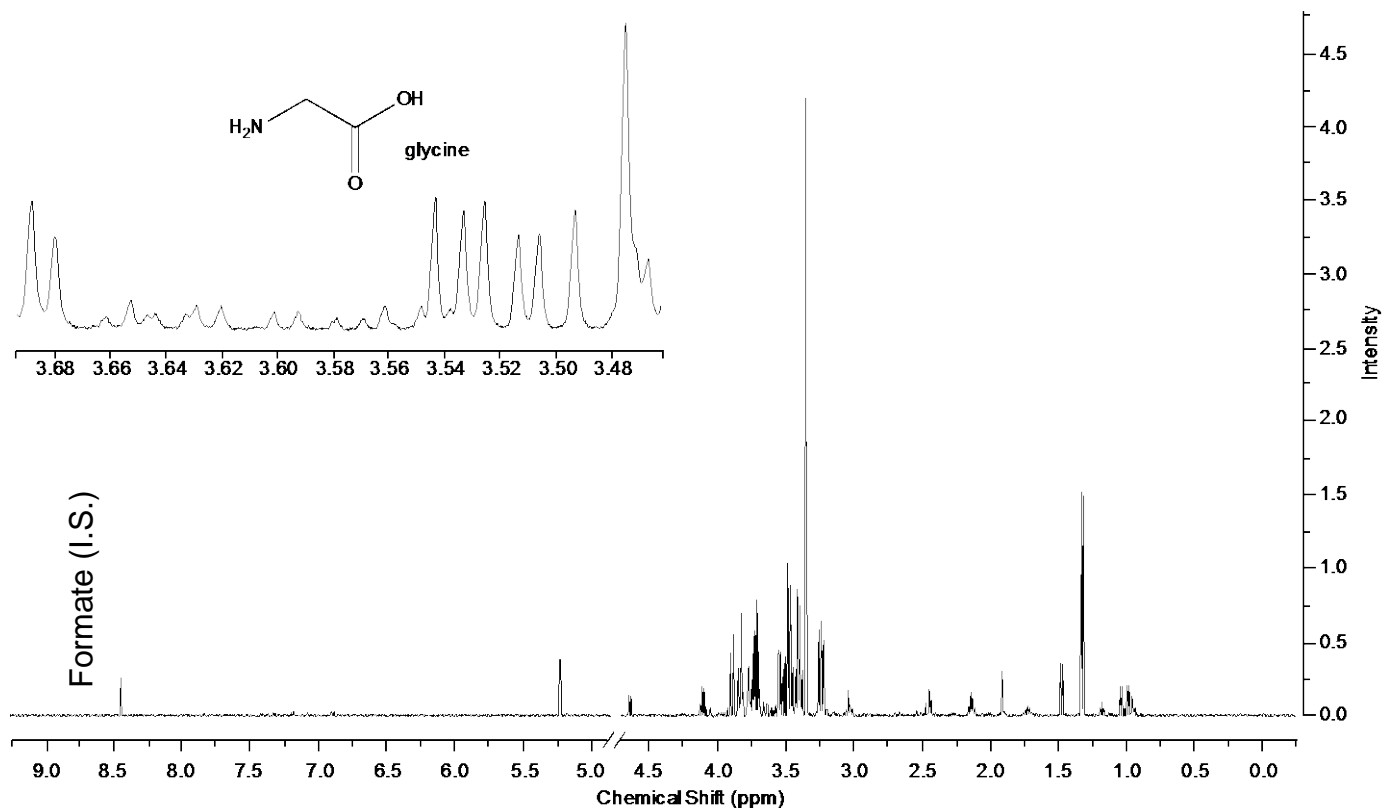
[†]Corresponds to the number of compounds differentiating the schizophrenia quartiles that are mapped in the listed pathway. [‡]Metabolites involved in the Metscape pathway that differentiate the schizophrenia quartiles (as separated by log transformed fasting insulin concentration), as determined by 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 |

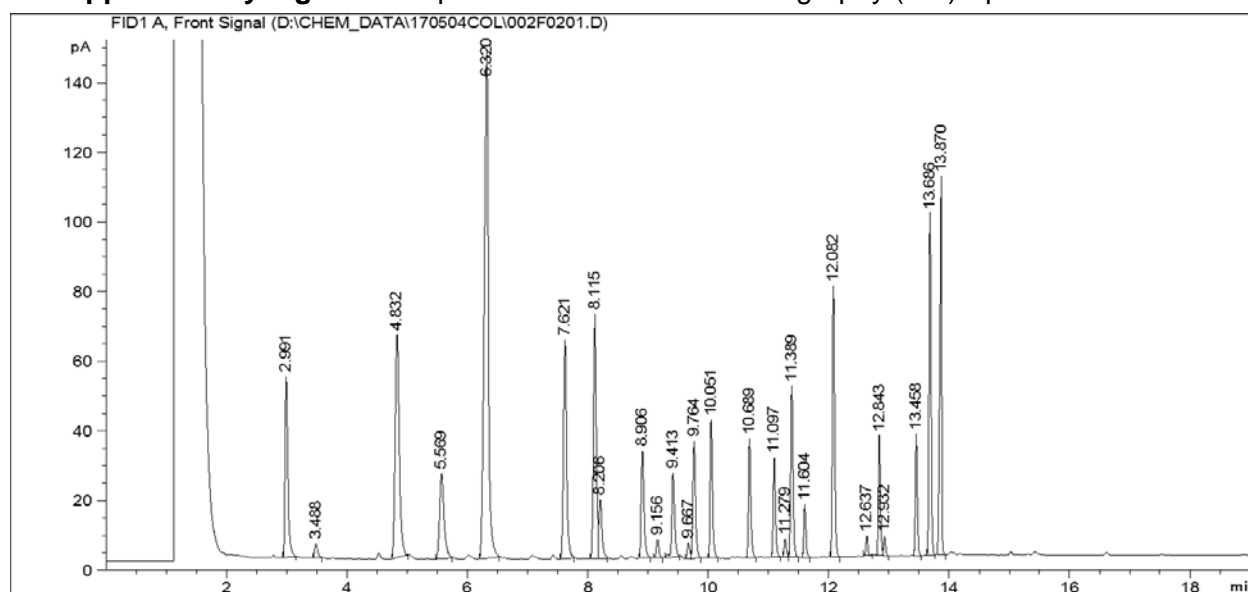
Metabolites identified as significantly different between the schizophrenia participants when grouped by BMI (≥ 30 or < 30 kg/m²), and compared using a Student's t-test with a False Discovery Rate (FDR) of $< 25\%$ to correct for multiple comparisons.

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 | Myristeloic 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

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2. Mangold, H. K. *Thin-Layer Chromatography*. (Springer New York, 1969).