

## **Metabolomics of World Trade Center-Lung Injury: a Machine Learning Approach**

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### **Supplemental Methods**

#### **Metabolite Quantification and Identification**

Aliquots of serum dedicated for metabolomics were maintained at -80°C until processing and quantification using the automated MicroLab STAR® (Hamilton).

Proteins were precipitated with methanol and the extract was divided into 5 fractions, each of which was used to identify different subtypes of metabolites: 2 for reverse phase (*RP*)/*Ultra-high Performance Liquid Chromatography-Tandem Mass Spectroscopy (UPLC-MS/MS)* methods with *positive ion mode electrospray ionization (ESI)*, one for *RP/UPLC-MS/MS with negative ion mode ESI*, one for *HILIC/UPLC-MS/MS with negative ion mode ESI*, and one backup sample. Controls were analyzed with the samples: a technical replicate, blanks, and a cocktail of quality check standards to allow instrument performance monitoring and aid chromatographic alignment. All methods used a Waters ACQUITY ultra-performance liquid chromatography (UPLC), a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionization (HESI-II) source, and Orbitrap mass analyzer.

Furthermore, metabolite identifications are based on three criteria: retention index within a narrow retention index window of the proposed identification, accurate mass match to the library +/- 10 ppm, and the MS/MS forward and reverse scores between the experimental data and authentic standards. The MS/MS scores are based on a comparison of the ions present in the experimental spectrum to the ions present in the library spectrum. While there may be similarities between these molecules based on one of these factors, the use of all three data points can be utilized to distinguish and differentiate metabolites.

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### **Supplemental Discussion**

Metabolites in C1 were strongly negatively correlated with the metabolites in C5, suggesting interplay between C1 and C5. Docosahexaenoylcholine is a metabolite of docosahexaenoate (DHA), alterations of which have been observed in pathological and physiological conditions in the lung.<sup>1,2</sup>

Another notable amino acid in C2 is n-acetylglutamine, a product of protein degradation in the glutamate subpathway. Glutamate is a metabolite of glutamine, a pervasive protein with a key role in immune cell recruitment and important in lung diseases, including ARDS and ALI.<sup>3</sup> Lastly, two ascorbate (vitamin C) and aldarate metabolites, gulonate and threonate, were found in C2. Recent clinical investigation reveals promise in high-dose vitamin C as a treatment for lung cancer.<sup>4,5</sup>

C2 was strongly correlated with C3, both containing BCAA metabolites, which, as previously mentioned, are thought to be bioactive in lung disease. In C3, the BCAA metabolites propionylcarnitine and isobutyrylcarnitine clustered with the FA, dicarboxylate metabolite azelate (nonanedioate). Propionylcarnitine is both a FA and BCAA metabolite and suggests why these metabolites may have clustered together.

Also a member of C3, dimethylglycine, a glycine precursor, played a role in class differentiation. Glycine is a known anti-inflammatory mediator and decreased expression of dimethylglycine in cases of WTC-LI may be indicative of decreased availability and therefore anti-inflammatory regulation of glycine.<sup>6</sup>

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- 6 Razak, M. A., Begum, P. S., Viswanath, B. & Rajagopal, S. Multifarious Beneficial Effect of Nonessential Amino Acid, Glycine: A Review. *Oxidative medicine and cellular longevity* **2017**, 1716701, doi:10.1155/2017/1716701 (2017).