4-minute method, negative mode

Sample Collection

All human subjects in this study were consented according to Colorado Multiple Institutional Review Board (COMIRB)-approved protocols. Written informed consent was obtained from parents or guardians of participants under the age of 18, and assent was obtained from participants over the age of 7 who were cognitively able to assent. Deidentified plasma samples for Cohort 1 were obtained from the Translational Nexus Clinical Data Registry and Biobank (University of Colorado Anschutz Medical Campus, COMIRB 08-1276). Additional plasma and WBC samples were obtained through the Crnic's Institute Human Trisome Project (University of Colorado Anschutz Medical Campus, COMIRB 08-1276). Additional plasma and WBC samples were obtained through the Crnic's Institute Human Trisome Project (University of Colorado Anschutz Medical Campus, COMIRB 15-2170, www.trisome.org). Plasma was collected in Vacutainer tubes (EDTA–purple capped or Lithium heparin–light green capped) and stored at -80°C. Participant medical history was collected by the respective biobanks.

Extraction

For plasma analyses, a volume of 20µL of was extracted in 480µL of ice-cold methanol:acetonitrile:water (5:3:2). Subsequently, these solutions were vortexed for 30 minutes at 4°C.Insoluble proteins were pelleted by centrifugation (10 minutes at 4°C and 12,000 g) and supernatants were collected and stored at -80°C until analysis. For quantitative analysis of kynurenine pathway (KP) metabolites, supernatants were spun in a Speedvac until dry and resuspended in 0.1% formic acid in water as previously described (PMID: 30213797, 30143553).

Chromatography

UHPLC-MS metabolomics analyses were performed using a Vanquish UHPLC system coupled online to a Q Exactive mass spectrometer (Thermo Fisher, Bremen, Germany). Samples were resolved over a Kinetex C18 column (2.1x150 mm, 1.7µm; Phenomenex, Torrance, CA, USA) at 45°C using a 4 minute gradient at 450µL/minute from 0-100% B (A: 95% water/5% acetonitrile, 5mM NH4OAc; B: 95% acetonitrile/5% water, 5mM NH4OAc) for negative mode. To monitor possible technical variability, aliquots of each of the individual samples were combined to make technical replicates, which were run after every 15 samples. Additionally, in each experiment, several lysis solution aliquots were run as blanks for artifact identification.

Mass Spectrometry

The Q Exactive mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) was operated in negative ion mode using electrospray ionization, scanning in Full MS mode (1µscan) from 65 to 975 m/z at 70,000 resolution, with 4 kV spray voltage, 45 sheath gas, 15 auxiliary gas. MS analysis and data elaboration were performed as described^{74,75}. Calibration was performed prior to analysis using the PierceTM Negative Ion Calibration Solution (Thermo Fisher Scientific).

Data Transformation

Acquired data was converted from .raw to .mzXML file format using RawConverter.⁷⁶

Metabolite Identification

Metabolite assignments to KEGG compounds, isotopologue distributions, and correction for expected natural abundances of ¹³C and ¹⁵N isotopes, were performed using MAVEN (Princeton, NJ, USA) on the basis of accurate intact mass, retention time and MS/MS^{77,78}. Isotopologue distributions in tracing experiments with ¹³C₁₁ ¹⁵N₂-tryptophan were also analyzed with MAVEN and Compound Discoverer (Thermo Fisher, Bremen, Germany). Peak areas were exported for further statistical analysis with R (R Foundation for Statistical Computing, Vienna, Austria). Data sets were log2 transformed. Putative metabolites were annotated using MAVEN. Subsequent data pre-processing included filtering out metabolites with low intensity values (permetabolite threshold informed by blanks) that were detected in less than 90% of the T21 samples or less than 90% of the D21 samples, leaving 91 metabolites for further analyses.