Supplementary methods

Liquid chromatography/Mass Spectrometry (LC/MS)

The LC/MS portion of the platform was based on a Surveyor HPLC and a Thermo-Finnigan LTQ mass spectrometer, which consisted of an electrospray ionization source and linear ion-trap mass analyzer. Positive and negative ions were monitored within a single analysis by consecutively alternating the ionization polarity of adjacent scans. The vacuum-dried sample was dissolved in 100 µl of injection solvent that contained five or more injection standards at fixed concentrations. The internal standards were used both to assure injection and chromatographic consistency. The chromatographic system used a binary solvent system delivered as a gradient. Solvent A was water and solvent B was methanol. Both were high purity grade and both contained 0.1% formic acid as a pH stabilizer. The HPLC columns were washed and reconditioned after every injection. For ions with counts greater than 2 million, an accurate mass measurement could be performed. Accurate mass measurements could be made on the parent ion as well as fragments. The typical mass error was less than 5 ppm. Ions with less than two million counts require a greater amount of effort to characterize. Fragmentation spectra (MS/MS) were typically generated in data dependent manner, but if necessary, targeted MS/MS could be employed, such as in the case of lower level signals. The information output from the raw data files was extracted as discussed below.

Gas chromatography/Mass Spectrometry (GC/MS)

Samples for GC/MS analysis were re-dried under vacuum desiccation for a minimum of 24 hours prior to being derivatized under dried nitrogen using bistrimethyl-silyl-triflouroacetamide (BSTFA). The GC column was 5% phenyl and the temperature ramp is from 40° to 300° C in a 16 min period. Samples were analyzed on a Thermo-Finnigan Trace DSQ fast- scanning single-quadrupole mass spectrometer using electron impact ionization. The instrument was tuned and calibrated for mass resolution and mass accuracy on a daily basis.

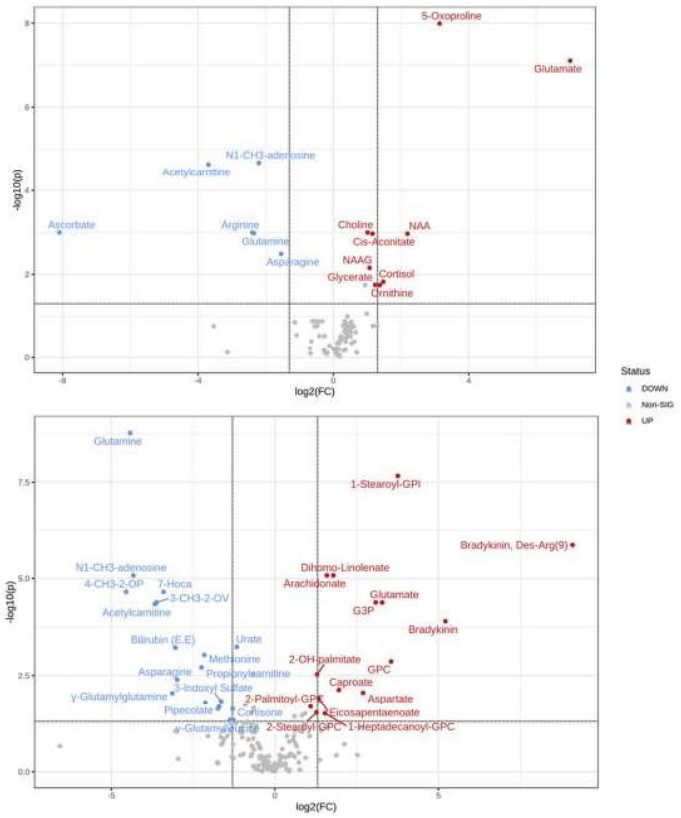
Bioinformatics

The bioinformatics workflow consisted of four major components, the Laboratory Information Management System (LIMS, Metabolon), the data extraction and peak-identification software, the data processing tools

for QC and compound identification, and a collection of tools for visualization and data interpretation, to be used by data analysts. The hardware and software infrastructures for these bioinformatics components were the LAN backbone, and a database server running Oracle 10.2.0.1 Enterprise Edition. Compounds were identified by comparison to library entries of purified standards or recurrent unknown entities. Identification of known chemical entities was based on comparison to metabolomic library entries of purified standards. The combination of chromatographic properties and mass spectra gave an indication of a match to the specific compound or an isobaric entity. Additional entities could be identified by virtue of their recurrent nature (both chromatographic and mass spectral).

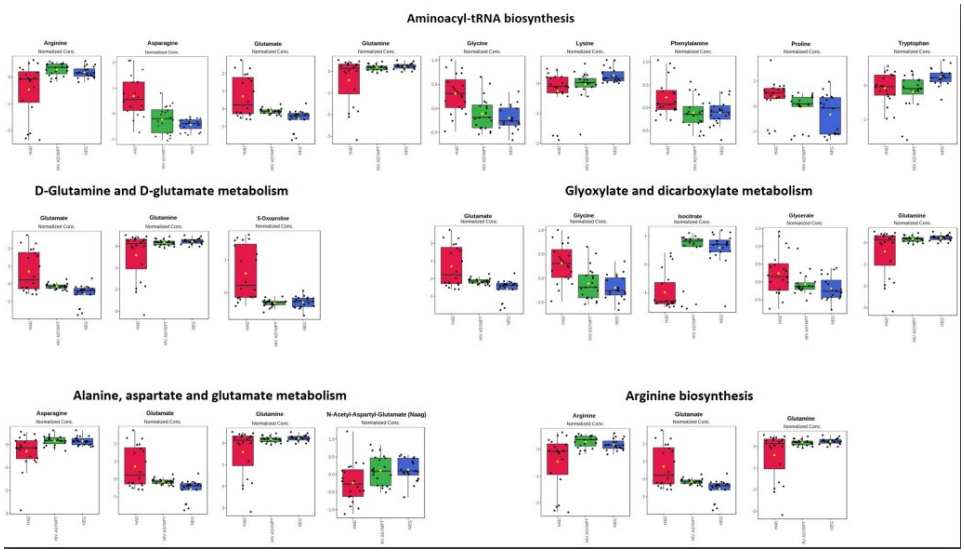
Supplementary Figure 1. HAD-1 vs. HAD-2 comparison

Top panel: volcano plot of CSF metabolomic profile in HAD-1 vs. HAD-2 comparison. Bottom panel: volcano plot of plasma metabolomic profile in HAD-1 vs. HAD-2 comparison. Compounds with fold-change values >2 and FDR-corrected p-value <0.1 are individually annotated.



Supplementary figure 2. Box-plots of differentially expressed metabolites included in altered CSF pathways

The normalized concentration of differentially expressed compounds identified by pathway analysis is shown. HAD, ASYM and NEG box-plots are pictured in red, green and blue, respectively; each individual participant is shown as a black dot. Significantly different values are marked by a yellow diamond.



Supplementary figure 3. Box-plot of differentially expressed metabolites included in altered plasmatic pathways

The normalized concentration of differentially expressed compounds identified by pathway analysis is shown. HAD, ASYM and NEG box-plots are pictured in red, green and blue, respectively; each individual paerticipant is shown as a black dot. Significantly different values are marked by a yellow diamond.