Supplemental Methods I

Brain imaging. Functional resonance imaging (fMRI) data was acquired using a 3.0T

MRI scanner (Siemens Trio; Siemens, Erlangen, Germany). A high resolution structural

image was acquired from each subject for registration purposes with a magnetization-

prepared rapid acquisition gradient-echo sequence, repetition time = 2200ms, echo time

= 3.26ms, structural acquisition time =5m 12s, slice thickness = 1mm, 176 slices,

256*256 voxel matrix, 1mm voxel size. Resting state scans were acquired using the

following parameters: 40-slice whole brain volumes, slice thickness = 4mm, repetition

time = 2000ms, echo time= 28ms, resting acquisition time = 10m6s, flip angle = 77°,

field of view = 220, 2×2×2 mm voxel size. Noise reducing headphones were used.

Subjects rested with eyes closed while functional blood oxygen-level dependent

images.

Image quality control and pre-processing

Imaging data were processed using SPM12. Preprocessing for quality control included

bias-field correction, co-registration, motion correction, spatial normalization, tissue

segmentation, and Fourier transformation. Structural images were included in

subsequent analyses based on compliance with acquisition protocol, full brain

coverage, minimal motion, Gibbs ringing, absence of flow/zipper and minor

atrophy/vascular degeneration. Functional imaging pre-processing included

transformation from DICOM into NIFTI, slice-time correction, co-registration with high-

resolution structural images, spatial normalization into MNI space, realignment to

correct for subject movement and unwarping to correct for the movement-by-distortion

interaction (using Unwrap in SPM12). Functional images were included in subsequent analyses based on compliance with acquisition protocol, full brain coverage, motion estimate of <2 mm in the three directions of translation and three directions of rotation, <.25mm average framewise displacement.[49]

Functional network construction

Normalizes resting-state functional images were processed using the CONN 17 toolbox in MATLAB.[50] A band-pass filter (0.001 Hz/<f < 0.01 Hz) was applied to reduce low and high frequency noise. A component-based noise correction method, CompCor, was used to remove motion artifacts - including six motion realignment parameters, as well as confounds for white matter and cerebrospinal fluid (CSF). Gray matter images for each subject were segmented using the Destrieux (cortical)[41] and Harvard-Oxford Subcortical Atlases[42] and parceled into 165 cortical and subcortical regions.

Linear measures of region-to-region functional connectivity were computed using the CONN toolbox [50]. The connectivity between the 165 brain regions was indexed by a matrix of Fisher z transform correlation coefficients reflecting the association between average BOLD time series signals across all voxels in each brain region. The connectivity matrix was then smoothed with a 4 mm isotropic Gaussian kernel. Functional connections were retained at z>.3 and all other values were set to 0. The magnitude of the z-score represented the weights in the functional network. An absolute threshold of z=.30 was chosen as we consider this the minimum effect size of interest. Connectivity less than Z=.30 less was considered to be a random connection or noise. The end result was a subject-specific undirected connectivity matrix or functional network.

Computing Network Metrics. Using in-house matlab workflows, the Graph Theory GLM toolbox (GTG) (www.nitrc.org/projects/metalab_gtg) we calculated the strength for each region of interest using each subject-specific functional brain networks [51-54] *Strength* represents the sum of connections (number of connections with Z>=.30) at a given brain region, factoring in the "weight" (magnitude of Z score) of each connection (e.g. correlation). Regions with high values for strength provide a strong indicator of influence or centrality. Regions of interest included subcortical [basal ganglia, nucleus accumbens, caudate nucleus, globus pallidus putamen)thalamus and brainstem] and cortical (Precentral gyrus , inferior part of the precentral sulcus, superior part of the precentral sulcus, paracentral lobule and sulcus, subcentral sulci and gyrus (central operculum) and sulci, central sulcus, postcentral sulcus, postcentral gyrus , long insular gyrus and central sulcus of the insula, and the inferior segment of the circular sulcus of the insula.

Supplemental Materials Methods II

Metabolon Platform

Sample Accessioning: Following receipt, samples were inventoried and immediately stored at -80ºC. Each sample received was accessioned into the Metabolon LIMS system and was assigned by the LIMS a unique identifier that was associated with the original source identifier only. This identifier was used to track all sample handling, tasks, results, etc. The samples (and all derived aliquots) were tracked by the LIMS system. All portions of any sample were automatically assigned their own unique identifiers by the LIMS when

a new task was created; the relationship of these samples was also tracked. All samples were maintained at -80ºC until processed.

Sample Preparation: Samples were prepared using the automated MicroLab STAR® system from Hamilton Company. Several recovery standards were added prior to the first step in the extraction process for QC purposes. To remove protein, dissociate small molecules bound to protein or trapped in the precipitated protein matrix, and to recover chemically diverse metabolites, proteins were precipitated with methanol under vigorous shaking for 2 min (Glen Mills GenoGrinder 2000) followed by centrifugation. The resulting extract was divided into five fractions: two for analysis by two separate reverse phase (RP)/UPLC-MS/MS methods with positive ion mode electrospray ionization (ESI), one for analysis by RP/UPLC-MS/MS with negative ion mode ESI, one for analysis by HILIC/UPLC-MS/MS with negative ion mode ESI, and one sample was reserved for backup. Samples were placed briefly on a TurboVap® (Zymark) to remove the organic solvent. The sample extracts were stored overnight under nitrogen before preparation for analysis.

QA/QC: Several types of controls were analyzed in concert with the experimental samples: a pooled matrix sample generated by taking a small volume of each experimental sample (or alternatively, use of a pool of well-characterized human plasma) served as a technical replicate throughout the data set; extracted water samples served as process blanks; and a cocktail of QC standards that were carefully chosen not to interfere with the measurement of endogenous compounds were spiked into every analyzed sample, allowed instrument performance monitoring and aided chromatographic alignment. Tables 1 and 2 describe these QC samples and standards.

Instrument variability was determined by calculating the median relative standard deviation (RSD) for the standards that were added to each sample prior to injection into the mass spectrometers. Overall process variability was determined by calculating the median RSD for all endogenous metabolites (i.e., non-instrument standards) present in 100% of the pooled matrix samples. Experimental samples were randomized across the platform run with QC samples spaced evenly among the injections.

Table 1: Description of Metabolon QC Samples

Type	Description	Purpose
MTRX	Large pool of human plasma maintained by Metabolon that has been characterized extensively.	Assure that all aspects of the Metabolon process are operating within specifications.
CMTRX	Pool created by taking a small aliquot from every customer sample.	Assess the effect of a non-plasma matrix on the Metabolon process and distinguish biological variability from process variability.
PRCS	Aliquot of ultra-pure water	Process Blank used to assess the contribution to compound signals from the process.
SOLV	Aliquot of solvents used in extraction.	Solvent Blank used to segregate contamination sources in the extraction.

Table 2: Metabolon QC Standards

Type	Description	Purpose
RS	Recovery Standard	Assess variability and verify performance of extraction and instrumentation.
IS	Internal Standard	Assess variability and performance of instrument.

Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy (UPLC-MS/MS): All methods utilized a Waters ACQUITY ultra-performance liquid chromatography (UPLC) and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35,000 mass resolution. The sample extract was dried then reconstituted in solvents compatible to each of the four methods. Each reconstitution solvent contained a series of standards at fixed concentrations to ensure injection and chromatographic consistency. One aliquot was analyzed using acidic positive ion conditions, chromatographically optimized for more hydrophilic compounds. In this method, the extract was gradient eluted from a C18 column (Waters UPLC BEH C18-2.1x100 mm, 1.7 µm) using water and methanol, containing 0.05% perfluoropentanoic acid (PFPA) and 0.1% formic acid (FA). Another aliquot was also analyzed using acidic positive ion conditions, however it was chromatographically optimized for more hydrophobic compounds. In this method, the extract was gradient eluted from the same afore mentioned C18 column using methanol, acetonitrile, water, 0.05% PFPA and 0.01% FA and was operated at an overall higher organic content. Another aliquot was analyzed using basic negative ion optimized conditions using a separate dedicated C18 column. The basic extracts were gradient eluted from the column using methanol and water, however with 6.5mM Ammonium Bicarbonate at pH 8. The fourth aliquot was analyzed via negative ionization following elution from a HILIC column (Waters UPLC BEH Amide 2.1x150 mm, 1.7 µm) using a gradient consisting of water and acetonitrile with 10mM Ammonium Formate, pH 10.8. The MS analysis alternated between MS and data-dependent MS^n scans using dynamic exclusion. The

scan range varied slighted between methods but covered 70-1000 m/z. Raw data files are archived and extracted as described below.

Bioinformatics: The informatics system consisted of four major components, the Laboratory Information Management System (LIMS), the data extraction and peak-identification software, data processing tools for QC and compound identification, and a collection of information interpretation and visualization tools for use by data analysts. The hardware and software foundations for these informatics components were the LAN backbone, and a database server running Oracle 10.2.0.1 Enterprise Edition.

LIMS: The purpose of the Metabolon LIMS system was to enable fully auditable laboratory automation through a secure, easy to use, and highly specialized system. The scope of the Metabolon LIMS system encompasses sample accessioning, sample preparation and instrumental analysis and reporting and advanced data analysis. All of the subsequent software systems are grounded in the LIMS data structures. It has been modified to leverage and interface with the in-house information extraction and data visualization systems, as well as third party instrumentation and data analysis software.

Data Extraction and Compound Identification: Raw data was extracted, peak-identified and QC processed using Metabolon's hardware and software. These systems are built on a web-service platform utilizing Microsoft's .NET technologies, which run on high-performance application servers and fiber-channel storage arrays in clusters to provide active failover and load-balancing. Compounds were identified by comparison to library entries of purified standards or recurrent unknown entities. Metabolon maintains a library based on authenticated standards that contains the retention time/index (RI),

mass to charge ratio (*m/z),* and chromatographic data (including MS/MS spectral data) on all molecules present in the library. Furthermore, biochemical identifications are based on three criteria: retention index within a narrow RI 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 biochemicals. More than 3300 commercially available purified standard compounds have been acquired and registered into LIMS for analysis on all platforms for determination of their analytical characteristics. Additional mass spectral entries have been created for structurally unnamed biochemicals, which have been identified by virtue of their recurrent nature (both chromatographic and mass spectral). These compounds have the potential to be identified by future acquisition of a matching purified standard or by classical structural analysis.

Curation: A variety of curation procedures were carried out to ensure that a high quality data set was made available for statistical analysis and data interpretation. The QC and curation processes were designed to ensure accurate and consistent identification of true chemical entities, and to remove those representing system artifacts, mis-assignments, and background noise. Metabolon data analysts use proprietary visualization and interpretation software to confirm the consistency of peak identification among the various samples. Library matches for each compound were checked for each sample and corrected if necessary.

Metabolite Quantification and Data Normalization: Peaks were quantified using area-

under-the-curve.