**Metabolomics involved in early life antibiotic exposures (NOD)**

Metabolomic Analysis: RTI RCMRC

PI, RTI RCMRC P&F Study: Martin J. Blaser, MD, New York University

**Abstract:** Based on recent findings that the human the intestinal microbiota can alter host metabolism and contribute to obesity (1), the Blaser lab has developed animal models involving sub-therapeutic antibiotic treatment (STAT) and therapeutic dose-pulsed antibiotic treatment (PAT) of healthy young mice to further investigate these interactions to determine the impact of early-life exposure to antibiotic treatment. Under five sub-studies (DuraSTAT, TranSTAT, NOD, EstroSTAT and VG STAT) they have collected data that potentially support a hypothesis that early life exposure to antibiotics perturbs the intestinal microbiome, which alters metabolomics signatures. For example, previous studies performed in the Blaser lab have shown the development of increased adiposity with induction of hepatic gene expression, while results from the NOD mice have demonstrated intestinal immune (helper T cell) population shifts, and earlier development of Type 1 Diabetes.

This metabolomics pilot and feasibility (P & F) study was conducted to provide data to be used to confirm the hypothesis identifying differential metabolic markers, and gain a better understanding of whether these metabolic perturbations precede the phenotypic changes.

The project “Metabolomics Involved in Early Life Antibiotic Exposures” profiled a total of 90 samples from all five sub-studies which included a total of four sample matrices (urine, serum, liver tissue and cecal contents). Within each sub-study, there were three sample matrices except for VG STAT, for which there was only two. For each matrix type within each sub-study 6 samples were analyzed for a total of 18 samples per sub-study (9 of each in VG STAT), the samples were equally divided into STAT-treated (various time-points) versus untreated Controls for each matrix. The specific sample characteristics for each sub-study were as follows:

In the **NOD** sub-study, a total of 18 samples from 6 week old, male NOD/ShiLtj mice; comprised of 6 serum samples, 6 cecal content samples and 6 liver tissue samples were analyzed. Three mice/matrix were exposed to PAT and 3 mice/matrix were non-exposed Controls. The mice were housed with SPF (Helicobacter neg/MNV neg) bedding and fed a normal diet.

The data required for the metabolomics analysis can be found in the accompanying files and folders:

Procedures: 1. NOD\_Cecal Metabolomics Procedure

Study Design Table: 2. NOD\_Cecal Study Design Table

Metadata: 3. NOD\_Cecal METADATA

Processed Data: 4. NOD\_Cecal Normalized Binned Data

Raw Data (folders): 5. NOD\_Raw\_NMR\_Data

**Notes:**

Each of the bin integrals were normalized to the total integral of each of the NMR spectrum (for more details, see accompanying Procedures file, **1. NOD\_Cecal Metabolomics Procedure.docx**).

Descriptions of abbreviations for factors are available in the Variable Dictionary in the accompanying Study Design Table files, organized by sub-study ie. **2. NOD\_Cecal Study Design Table.xlsx**.

The normalized binned NMR data are available in the accompanying Processed Data files for each matrix per sub-study (ie. **4. NOD\_Cecal Normalized Binned Data.xlsx)**. Sample ID and factors can be found in the first 2 columns in the file no. 4. Other columns in the spreadsheet contain the normalized binned data.

If the statistical program does not allow variable names to begin with a number then add a prefix to the column names, for example, bin\_8.98 instead of 8.98.

Sample ID serves as the unique identifier of the individual samples and is used as the NMR folder name in the raw NMR data file.

**Reference:**

1. Cho I, Blaser MJ. The human microbiome: at the interface of health and disease. Nature Reviews. Genetics 2012; 13; 260-270.