**Metabolomics involved in early life antibiotic exposures**

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 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 **DuraSTAT** sub-study, a total of 18 samples from 8 week old, female C57BL/6 mice; comprised of 6 urine samples, 6 cecal content samples and 6 liver tissue samples were analyzed. Three mice/matrix were given STAT penicillin and 3 mice/matrix were non-treated Controls. The mice were housed with conventional bedding and fed a high fat diet.

In the **TranSTAT** sub-study, a total of 18 samples from 8 week old, female Swiss webster mice; comprised of 6 serum samples, 6 cecal content samples and 6 liver tissue samples were analyzed. Three mice/matrix were inoculated with cecal contents from STAT mice and 3 mice/matrix were inoculated withcecal contents from Control mice. The mice were housed with conventional bedding and fed a high fat diet.

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.

In the **EstroSTAT** sub-study, a total of 18 samples from 23 week old, female C57BL/6 mice; comprised of 6 urine samples, 6 serum samples and 6 liver tissue samples were analyzed. Three mice/matrix were given STAT penicillin and 3 mice/matrix were non-treated Controls. The mice were housed with conventional bedding and fed a Low phyto-estrogen diet.

In the **VG STAT** sub-study, a total of 18 samples from 7 week old, male C57BL/6 mice; comprised of 9 cecal content samples and 9 liver tissue samples were analyzed. Three mice/matrix were given penicillin VK, 3 were given penicillin G, and 3 mice/matrix were non-treated Controls. The mice were housed with conventional bedding and fed a normal diet.

The data required for the metabolomics analysis can be found in the accompanying files and folders (hereafter cecal contents = cecal):

Procedures: 1. Blaser\_Metabolomics Procedure-Cecal

Blaser\_Metabolomics Procedure-Liver

Blaser\_Metabolomics Procedure-Serum

Blaser\_Metabolomics Procedure-Urine

Study Design Table: 2. Blaser\_Study Design Table-DuraSTAT

Blaser\_Study Design Table-TranSTAT

Blaser\_Study Design Table-NOD

Blaser\_Study Design Table-EstroSTAT

Blaser\_Study Design Table-VG STAT

Metadata: 3. Blaser\_MetaData and Analytical Metadata

Processed Data: 4. Blaser\_Normalized Binned Data-DuraSTAT-Cecal

Blaser\_Normalized Binned Data-DuraSTAT-Liver

Blaser\_Normalized Binned Data-DuraSTAT-Urine

Blaser\_Normalized Binned Data-TranSTAT-Cecal

Blaser\_Normalized Binned Data-TranSTAT-Liver

Blaser\_Normalized Binned Data-TranSTAT-Serum

Blaser\_Normalized Binned Data-NOD-Cecal

Blaser\_Normalized Binned Data-NOD-Liver

Blaser\_Normalized Binned Data-NOD-Serum

Blaser\_Normalized Binned Data-EstroSTAT-Liver

Blaser\_Normalized Binned Data-EstroSTAT-Serum

Blaser\_Normalized Binned Data-EstroSTAT-Urine

Blaser\_Normalized Binned Data-VG STAT-Cecal

Blaser\_Normalized Binned Data-VG STAT-Liver

Raw Data (folders): 5. Blaser\_Raw NMR Data-DuraSTAT-Cecal

Blaser\_Raw NMR Data-DuraSTAT-Liver

Blaser\_Raw NMR Data-DuraSTAT-Urine

Blaser\_Raw NMR Data-TranSTAT-Cecal

Blaser\_Raw NMR Data-TranSTAT-Liver

Blaser\_Raw NMR Data-TranSTAT-Serum

Blaser\_Raw NMR Data-NOD-Cecal

Blaser\_Raw NMR Data-NOD-Liver

Blaser\_Raw NMR Data-NOD-Serum

Blaser\_Raw NMR Data-EstroSTAT-Liver

Blaser\_Raw NMR Data-EstroSTAT-Serum

Blaser\_Raw NMR Data-EstroSTAT-Urine

Blaser\_Raw NMR Data-VG STAT-Cecal

Blaser\_Raw NMR Data-VG STAT-Liver

**Notes:**

Each of the bin integrals were normalized to the total integral of each of the NMR spectrum (for more details, see accompanying Procedures files, organized by matrix i.e. **1. Blaser\_Metabolomics Procedure-Urine.docx**).

Descriptions of abbreviations for factors are available in the Variable Dictionary in the accompanying Study Design Table files, organized by sub-study i.e. **2. Blaser\_Study Design Table-DuraSTAT.xlsx**.

The normalized binned NMR data are available in the accompanying Processed Data files for each matrix per sub-study (i.e. **4. Blaser\_Normalized Binned Data\_ DuraSTAT-Cecal.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.