# Diabetic Complications Consortium

**Application Title:** Developing a metabolite biomarker model for nephropathy in T1D patients

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## 1. Project Accomplishments:

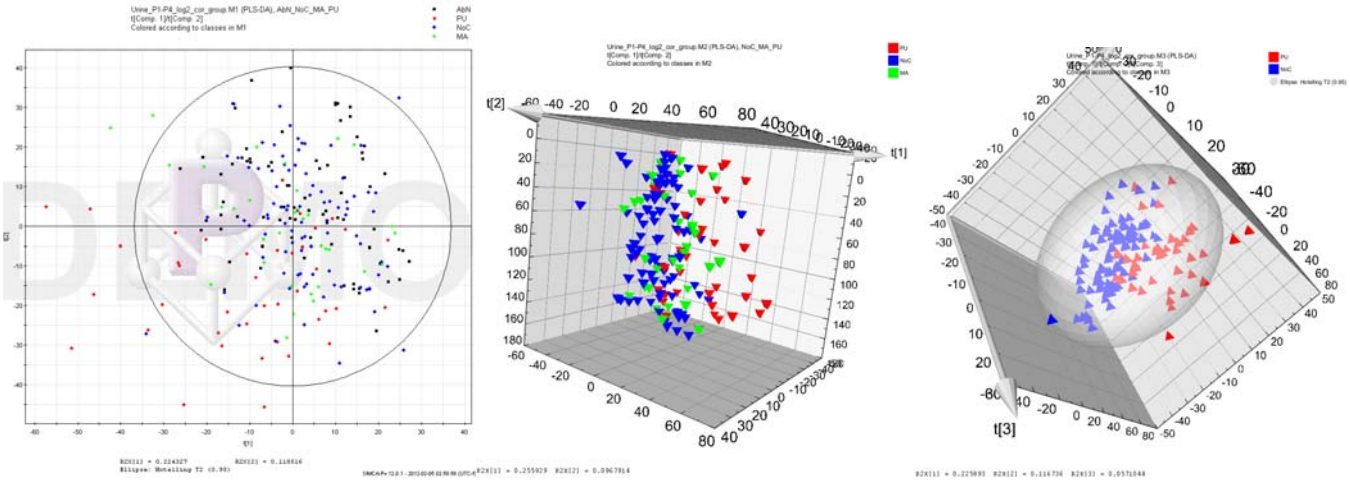
Urinary metabolome was successfully profiled for 320 subjects using an integrated LC-MS platform. 1,294 metabolite features were identified and among which, over 200 features have p values<0.05. Multiple ion monitoring (MIM) assays were then performed for confirmation purposes. The correlations between MIM assays and the extracted ion chromatograph from MS experiments were satisfactory with a range from 0.56 to 0.92 ($R^2$). Excellent separation was achieved between diabetic nephropathy group and T1D control group using MIM assays. Further work, including identification of lead metabolite candidates and mathematical model building will be performed during the no-cost extension period.

## 2. Specific Aims:

**Specific Aim 1**. We will profile the metabolome in 240 urine samples using our RP + HILIC LC-ESI MS metabolomic screening platform to generate the metabolite candidate list and identify the top metabolite candidates using MS;

**Results:**

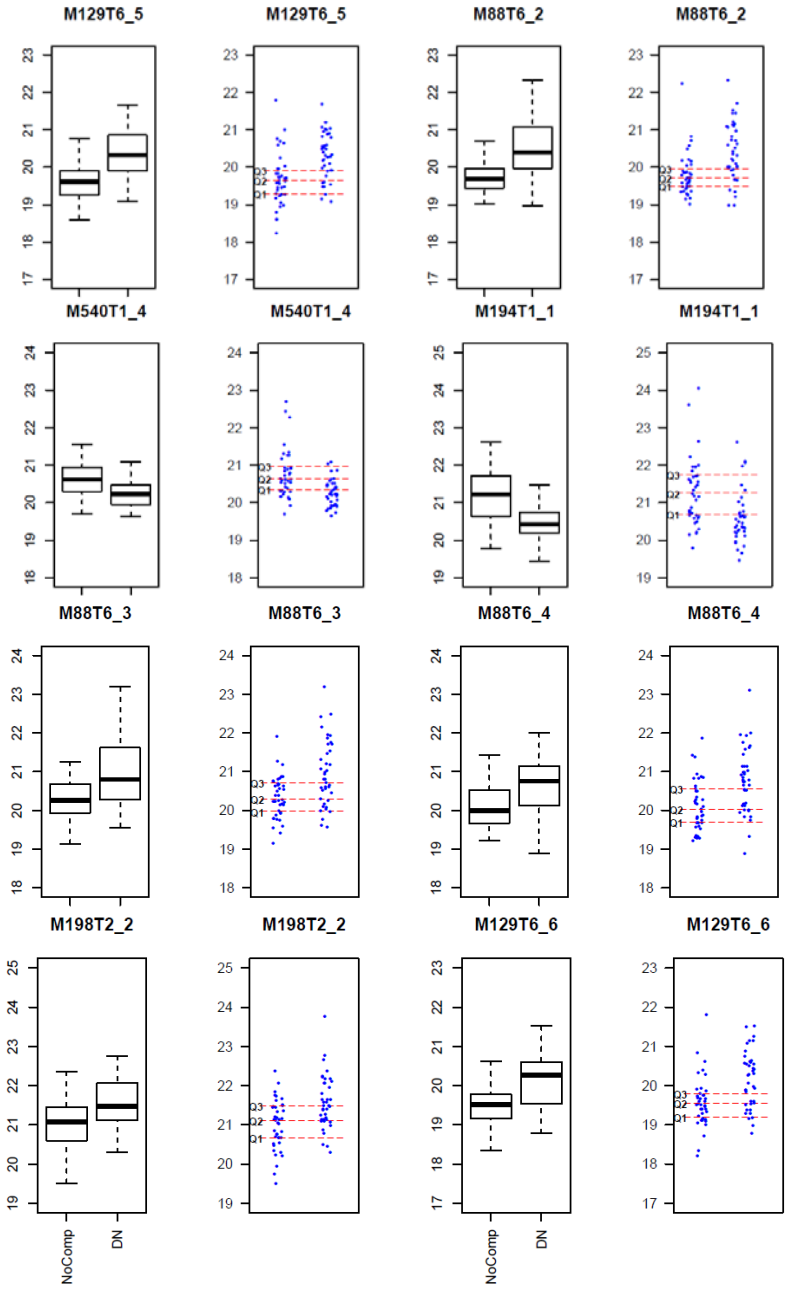
320 urine samples from antibody-negative controls (non-diabetic controls, 78 subjects), T1D patients with normal (<30 ug/mg, NoC, 132 subjects), medium (30-300 ug/mg, MA, 56 subjects) and high ACR values (>300 ug/mg, DN, 54 subjects) were profiled using LC-ESI MS metabolomic platform and totally 1,294 features (peaks) were identified. Partial least squares Discriminant Analysis (PLS-DA) was firstly performed to check the ability of these features to separate different phenotypic groups and the results were shown in the following figure.



A B C

Panel A shows the score scatter plot for all four groups. There are not much separation among AbN, NoC and MA groups, indicating the similarity of the metabolome of these groups. After removing AbN group from the plot, the separation gets better as shown in Panel B. When medium ACR group was further removed from the plot as shown in Panel C, the rest too groups (NoC and DN) have a good separation. All this may indicate that the metabolomic differences among the first three groups are too subtle for the current metabolomic platform to distinguish among them. One potential shortcoming of the metabolomics platform employed in this study is the relatively low mass accuracy.

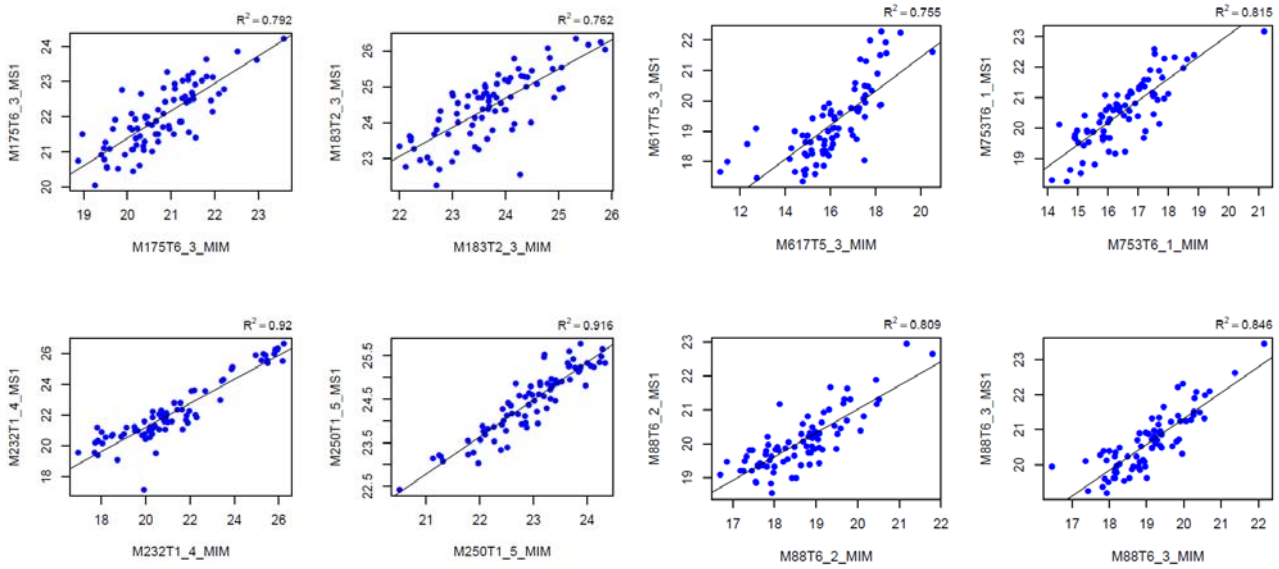
Based on the above results, only T1D control (NoC) group and DN group were used to select the metabolite candidates for further analysis. The ratios of the integrated peak areas between the T1D control group and DN group were calculated for all the features together with p values. There are over 200 features with significant p values (<0.05). Examples of the box and dot plots for a few of the top features were shown in the following figure.



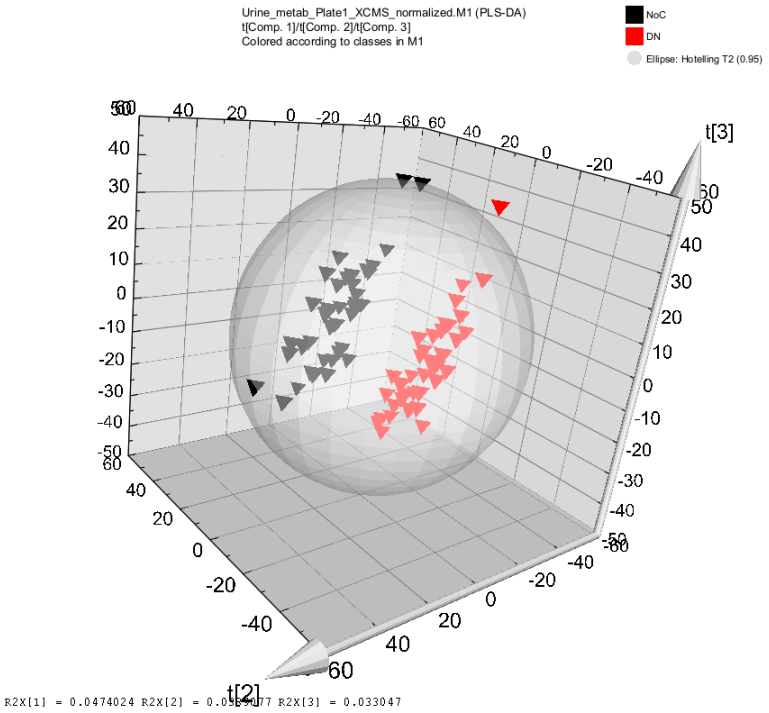
**Specific Aim 2**. We will develop multiple reaction monitoring (MRM) assay for the top 50 urine metabolite biomarker candidates and use it to analyze another 440 urine samples to generate quantitative information for metabolite panel/model building;

**Results:**

Due to the issue we have described in the no-cost extension request about the identification of the candidate metabolites that was seriously hampered by the relatively low resolution of our MS instrument and the extra time and cost to seek external collaboration for a high-resolution MS instrument (QTOF-type of MS) to increase the success rate of metabolite identification, we decided to use multiple ion monitoring (MIM) MS to perform quantification of the candidate features. MIM experiment only needs the m/z of the parent ion and does not require the identity/structure information of the targeted metabolites. It is a quick and dirty way to perform quantification study with limited information. Yet the specificity of the MIM assay is expected to be lower than a true MRM assay, due to the fact that no daughter ion information was used. To check this, we first performed MIM assay of the top 50 candidate metabolites and correlate them with the extracted ion chromatograph (XIC) data from the profiling MS study. The correlations, as exemplified in the following figure, are metabolite- and intensity- dependent and overall are very good with a range from 0.56 to 0.92 ($R^2$), indicating the efficacy of the MIM assays.




MIM assays were then performed for all the DN subjects and matched T1D controls. Then PLS-DA analysis was done for the MIM data and the score scatter plot was shown in the following figure. The two groups were very well separated, indicating the usefulness of the MIM assays as well as the selected candidate metabolites.




However, due to the two major issues we described in the no-cost extension (approved), including 1) the unexpected unavailability of the LC-MS instrument, caused by repair and maintain needed for several critical hardware failures of the LC-MS instrument and 2) The identification of the candidate metabolites were seriously hampered by the relatively low resolution of our MS instrument and we will seek external collaboration for a high-resolution MS instrument (QTOF-type of MS) to increase the success rate of metabolite identification, which is of great importance for further confirmation study and development of metabolite biomarkers, our second parts of this aim of identifying lead metabolite candidate cannot be finished as scheduled.

**Specific Aim 3**. We will develop and implement multivariate models for classifying and predicting DN and provide bioinformatics solutions to understand the biological significance of the metabolites profiles.
**Results:** Pending and to be finished during the no-cost extension.

## 3. Publications:

1. Identification and confirmation of urinary metabolomic changes in T1D patients with nephropathy. (in preparation)