Comprehensive Human Fecal Metabolome Analysis Using Chemical Isotope Labeling LC-MS

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Introduction:

Human fecal samples contain endogenous human metabolites, gut microbiota metabolites, and other components. Quantitative fecal metabolome analysis can provide information which may suggest direct structural or functional evidences about the relationship between gut microbiome and human health. However, fecal samples are more difficult to deal with compared to other biofluids for metabolome profiling. The sample collection and storage conditions are also critical for conducting valid biological discovery work. Analytical tools such as NMR, GC-MS and LC-MS have been applied in fecal metabolomics. However, due to the complexity of the fecal metabolome, more sensitive technique is needed to improve the coverage of metabolites and gauge the sampling conditions in a more comprehensive manner. In our work, we develop and apply a chemical isotope labeling LC-MS metabolomics platform for comprehensive and quantitative analysis of the amine-, phenol, and carboxylic acid-containing metabolites in fecal samples. We study the effects of various sample extraction and storage conditions on metabolome profiling. We will report our study of the effect of diet on human fecal metabolome profiling and discuss the implication of diet effect on future work of applying human fecal metabolomics for disease biomarker discovery.

Methods:

For fecal metabolite extraction optimization, aliquots of fecal samples were dried and subjected to solvent extraction by water, methanol (MeOH), acetonitrile (ACN) and the combination of them, then followed by ultrasonication, vortexing and spinning down. The supernatants were collected for downstream chemical labeling reactions. For storage condition studies, stool samples were stored in several different ways including samples being stored immediately or after being left outside under different conditions for different time intervals. Metabolites from

these samples were extracted using the method optimized above. 12C/13C dansylation and p-dimethylaminophenacyl (DmPA) labeling were then performed separately. The total amount of labeled metabolites in each sample were measured by an LC-UV system and samples were analyzed afterwards on an Agilent 6230 ESI-TOF system equipped with an Agilent 1290 UHPLC.

Preliminary results:

For metabolite extraction method optimization, a previously reported method of differential $^{13}C_2/^{12}C_2$-dansyl labeling of the amines and phenols was used to improve LC separation efficiency and MS detection sensitivity. Water, methanol and acetonitrile were used as an extraction solvent. We found that more unique peak pairs were detected in water and ACN extracts, compared to MeOH extract. For example, 1037, 940 and 978 peak pairs were detected in the water, methanol and ACN extracts, respectively, for a combined total of 1184 pairs. However, only 7 pairs were detected uniquely from the MeOH extract, representing less than 0.6% of the total number. Thus, the amine/phenol metabolites extracted from a fecal sample using MeOH could be mostly covered by the water and ACN extracts. We also evaluated a sequential solvent extraction method in the order of water and ACN to see if we could extract more metabolites than using either water or ACN alone. An average of 1234 ± 25 (n=9) pairs were detected per run from the H_2O/ACN extract, compared to 989 ± 6 (n=9) from the water extract and 942 ± 14 (n=6) from the ACN extract. Thus, the sequential water-acetonitrile extraction method was found to be optimal. Many of these peak pairs could be putatively identified based on mass match using MyCompoundID against a Human Metabolome Database and an Evidence-based Metabolome Library, respectively.

In addition to using dansylation labeling LC-MS for profiling the amine/phenol submetabolome, we also applied the DmPA labeling for profiling the acid-containing submetabolome. The comparison of the two submetabolome datasets in terms of metabolome overlap and overall coverage is current underway. The combined results offer the possibility of generating more comprehensive profile of the human fecal metabolome.

For the study of fecal sample storage conditions, fecal samples from 4 volunteers were collected daily for 7 days. Each fecal sample was aliquotted and stored in several different ways: stored immediately after collection in -20 ℃ or -80 ℃ freezer; left on ice or room temperature for different time intervals and then stored in -20 ℃ or -80 ℃ freezer; stored immediately after

collection for 24 hrs, left on ice for different time intervals and then stored in -20℃ or -80℃ freezer. We are currently investigating the effects of the above storage conditions on metabolome profiling.

Finally, we plan to apply this method to study the fecal metabolome changes related to diet with an objective of understanding how diet may affect the fecal metabolome profiles, which is important for our future work in applying fecal metabolomics for disease biomarker discovery.