Gastroenterology and Hepatology From Bed to Bench.

REVIEW ARTICLE

Metabonomics exposes metabolic biomarkers of Crohn's disease by ^{1}HNMR

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ABSTRACT

Metabonomics and other "omics" fields are essential science in analytical chemistry. Modern analytical instruments such as proton nuclear magnetic resonance (^{1}H NMR) can be provided the great quantity of analytical information. In order to assign unknown samples, chemometrics methods recognition build classification model based on experimental data. Firstly some current strategies for regarding disease diagnosis are exhibited in metabonomics studies. Some disease such as crohn's disease can be difficult to diagnosis since its signs and symptoms may be similar to other medical problems or often mimic other symptoms. Applications of NMR and supervised pattern recognition in the field of metabonomics are also reviewed in recent years. The aim of present study was to review the assessment of ^{1}HNMR to investigate a metabolic profile of crohn's disease.

Keywords: Metabonomics, ^{1}HNMR, Crohn disease, Chemometrics.

(Please cite as: **Fathi F, Ektefa F, Hagh-Azali M, Asadzadeh Aghdaie H. Metabonomics exposes metabolic biomarkers of Crohn's disease by ^{1}HNMR. Gastroenterol Hepatol Bed Bench 2013;6(Suppl.1):S19-S22).**

Introduction

A revolution has created in the practice of biological research in the last decade (1). These changes can develop of techniques that are valuable to profile levels of molecular organization at body. 'Omics' informally refers to this wide coverage of techniques that can perform metabolic profiling studies such as metabonomics or metabolomics (2, 3). In this viewpoint, there is a particular emphasis on how the levels of metabolites change in response to pathophysiological stimuli or genetic modification in living systems because of many molecules with small molecular weight involved in metabolism (4). Crohn's disease and ulcerative colitis are the two main types of chronic inflammatory bowel disease. Crohn's disease is called regional enteritis that affects the gastrointestinal tract. The disorder usually affects the colon and rectum, however, may influence in each area of the mouth to the anus. In some cases the correct diagnosis of crohn's disease may be difficult because this disease often imitates other symptoms. To make correct diagnosis in many areas, metabonomics field is to achieve improvements in understanding of metabolic processes that occur while a person is

Received: 11 July 2013 *Accepted*: 5 September 2013
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suffering from a disease (5). There are several available methods for metabolite profiling. One of them is proton nuclear magnetic resonance (^{1}H-NMR) that has been utilized as an analytical tool in studies of metabolism for many years (6-8). NMR spectroscopy technique is non-selective in the analytical studied and obtained vital information in a short time. Also it is non-destructive and highly reproducible with minimal sample preparation.

Statistical modeling in metabolic profiling has goals; one of them is to visualize the overall similarities and differences between samples and variables. The main focus in this review is to provide general knowledge of data preprocessing in ^{1}HNMR approach and the use of chemometrics techniques. For reach to this purpose, we have focused on studies on crohn's disease using a metabonomics.

Sample and data preprocessing

To convert the NMR data into a form that can be useable in statistical procedures firstly the FIDs - already have been fourier transformed to the frequency domain- manually phased and baseline-corrected applying XWINNMR or other software(1). Then the NMR data are usually transformed into a matrix in which a sample and a metabolic signal pertain to a row and a column, respectively. The spectral intensity at a particular chemical shift corresponds to the estimated level of a particular metabolite. The Carr–Pucell–Meiboom–Gill (CPMG) spin-echo pulse sequence, $\pi/2\text{-}t_D\text{-}\pi\text{-}t_D$, was used for serum samples (9). CPMG experiment can enhances visualization of the low molecular weight metabolites and to suppress the broad signal from the protein (9). Spectral regions within the range of 0.2 to 10 ppm were used after deleting the region containing residual water and urea signals (4.5 to 6.0 ppm) (10). Biological fluids such as serum, plasma and urine have been used for studies of crohn's disease. Serum and plasma are the most common fluids in metabonomics surveys. These samples are also relatively easy to obtain.

The human metabolome database (HMDB) is a freely available electronic database containing detailed information about metabolites found in the body. Urine is one of the simplest fluids physical that frequently used as a bio-fluid for human and animal metabolic studies. Schicho and co-authors performed quantitative metabolomic profiling of serum, plasma and urine by ^{1}HNMR spectroscopy on patients with inflammatory bowel disease (crohn's disease and active ulcerative colitis) (11). In other survey, Stephens et al. highlighted the potential for metabolomics to distinguish inflammatory bowel disease from the healthy state in urine sample (12). They build a metabolic profile applying NMR spectroscopy to distinguish patients with inflammatory bowel disease from healthy individuals (12). Fathi and colleagues reveal new differentiating metabolites for crohn's disease by examining on serum of Iranian samples (13). Murdoch and colleagues correlated the progress of inflammatory bowel disease with specific changes in a mouse urinary metabolic fingerprint applying metabolomics (14). Marchesi and colleagues displayed the first classification of fecal extracts acquired from patients with ulcerative colitis and crohn's disease using a metabonomics technique. This approach combines multivariate pattern recognition analysis and high resolution ^{1}HNMR spectroscopy (15).

Scaling in metabonomics

In some researches, for each samples 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) was added as internal reference substance (δ*=0 ppm*). Also peak of the lactate methyl doublet (δ=1.33 ppm) or other metabolites are referenced within XWINNMR for spectra (16). Scaling is a column- each spectral intensity across all samples- operation that including mean center, auto scaling, pareto scaling etc. Mean centering includes subtracting the column mean from each value in the column and given a mean of zero. Second scaling is auto scaling also called unit or unit variance scaling; in

which each value in the column is divided by the standard deviation of the column (17). Pareto scaling is other forms of scaling that is very similar to auto scaling. Here, each variable is divided by the square root of the standard deviation of the column (18).

Rezaei-Tavirani et al. stated that preprocessing the spectroscopic data is essential (19). They showed scaling effects in metabonomics investigation of patients diagnosed with crohn's disease by applying two techniques of scaling including: mean centering and auto scaling (19). Results of recent study reveal that the mean centering is more useful to segregate patients from healthy subjects in the data set of crohn's disease. They showed that the mean centering option is more effective at eliminating correlation from the principal component analysis (PCA) residuals than the auto scaling in this situation. In survey of Fathi et al. the data were scaled to pareto scaling preceding multivariate analysis (13).

Regarding the published studies in this field, it can be suggested that the goal of scaling is to regulate for the distinctions in fold differences between the different metabolites and improving their biological interpretability.

Classification methods

Classification methods are widely used in order to diagnosis crohn's disease from healthy subjects and to seek the metabolic biomarkers causes of crohn's disease compare to control group. Methods applied in crohn's literatures include the following: classification and regression tree (CART) and orthogonal projections to latent structures discriminated analysis (OPLS-DA).

Breiman et al. in 1984 develop CART as non-parametric statistical technique (20). This method is able both classification and regression. A decision tree is built in both cases and describes a response *Y* by selecting some independent variables *X* from a larger set of *X* values. Fathi and colleagues utilized CART in order to classification of crohn's disease and healthy subjects (13). They obtained classification model showed 89% correct classification of crohn's disease and healthy subject for the external test set. According to CART diagram, they concluded that just using one descriptor (lipid) crohn's disease and control group could be classified separately. Based on CART diagram, lipid level reduces in blood serum of patients compared to healthy individuals. The parameters of classification in last research showed that CART classification model has great chance in diagnosis of crohn's disease.

OPLS-DA as regression technique is frequently used for classification method (11). Using this method, those metabolites which show a discrepancy between diagnostic groups can be identified. In order to divide the systematic variation into two model parts in the metabolite levels, OPLS-DA is used (21). These two parts are including co-variation between metabolite levels and the class of each sample and captures systematic variation in metabolite levels orthogonal to the class of each sample (11).

Schicho et al. used hierarchical OPLS-DA for comparing several blocks derived from the same subject to examine differences in metabolites. Based on recent research, methanol, mannose, formate, 3-methyl-2-oxovalerate, and amino acids increased in serum and plasma and mannitol, allantoin, xylose, and carnitine increase in urine of IBD patients (11). Quantitative metabolomic profiling of serum, plasma and urine discriminated healthy subjects and IBD patients. However, they showed that the metabolic differences between the ulcerative colitis and crohn's disease cohorts are less pronounced (11). Stephens et al. performed OPLS-DA in order to examine which metabolites were contributing to the separation crohn's disease and healthy cohorts (12). In this study IBD patients could be differentiated from healthy according to urinary metabolomics. TCA cycle intermediates, amino acids and gut microflora metabolites have

main differences between inflammatory bowel disease patients and healthy cohorts. (12).

Conclusions and future prospects

In this review, we evaluated the use of classification method to build an easily interpretable predictive model to distinguish between crohn's disease and healthy controls. It is clear that statistical model and bioinformatics methods are valuable in metabonomics investigation. The aim of these "omics" science is to enable the maximum useful information to be gleaned from this rich and complex data. We suggest using metabonomics to identify other patients in the future.

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