Metabolite Biomarker Discovery For Metabolic Diseases By Flux Analysis

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***Abstract*—Metabolites can serve as biomarkers and their identification has significant importance in the study of biochemical reaction and signalling networks. Incorporating metabolic and gene expression data to reveal biochemical networks is a considerable challenge, which attracts a lot of attention in recent research. In this paper, we propose a promising approach to identify metabolic biomarkers through integrating available biomedical data and disease-specific gene expression data. A Linear Programming (LP) based method is then utilized to determine flux variability intervals, therefore enabling the analysis of significant metabolic reactions. A statistical approach is also presented to uncover these metabolites. The identified metabolites are then verified by comparing with the results in the existing literature. The proposed approach here can also be applied to the discovery of potential novel biomarkers.**

I. Introduction

Human diseases, in particular metabolic diseases, can be directly caused by the lack of essential metabolites. Metabolic diseases profiling is a promising technique to uncover the mechanism of disease-metabolite associations. Research in the past decades mainly focused on the analysis of metabolic networks [1], [2], [3]. Models for investigating large-scale metabolic networks with few parameters [4], [5] in constraint outperform other quantitative approaches. Three types of information have been used to describe a metabolic network. One of them is stoichiometry, which is used to depict the quantitative associations among reactants and products in all the involved reactions. Another part consists of enzymes corresponding to each reaction in the network. The last part is the flux capacity of each reaction. Here we employ Human Recon 1, one of the two independently developed human metabolic networks [6], [7] in our study. Within this genome-scale human metabolic network, there are in total 3742 reactions, involving 2766 metabolites and 1905 genes.

Flux balance analysis [8] is a traditional constraint-based approach to predict flux distribution. Taking into account the flux capacity, stoichiometry and thermodynamics, a number of constraints can be constructed so as to narrow down the range of flux. In [9], a list of important metabolic reactions was identified utilizing flux balance analysis. This demonstrates the importance of understanding metabolic reactions. Drug targets, in particular enzymes, are selected to reduce abnormal metabolites by formulating an optimal combination problem in enzyme combination on metabolic networks [10], [11]. In [12], a drug-reaction network was constructed to predict enzyme targets by profiling human metabolic reactions in NCI-60 cell lines. Here we develop a computational method to identify metabolic biomarkers by profiling human metabolic reactions with the integration of the disease-specific gene-expression data. We remark that metabolic biomarkers are metabolites demonstrating consistent variation in concentration in disease state and can be useful for diagnostic purpose. Metabolomics exhibits a series of superiorities for diagnostic purpose [13]. As a safe evaluator for drug candidates and an efficient diagnostic tool, metabolomics will therefore have an important role.

A Linear Programming (LP) based approach is efficient for determining the optimal flux distributions under a series of metabolic constraints. However, multiple solutions might exist which means that multiple optimal solutions may exist in the flux space with the same objective value. We address this issue by calculating both the lower and the upper bounds of fluxes in each reaction within the metabolic networks [17]. The obtained flux profiles are then used to determine the significance of metabolic reactions. The corresponding boundary metabolites involved in the highly significant reactions are identified as final metabolic biomarkers. In this paper, we propose simple statistical criteria to select significant metabolites. Furthermore, in our study, we consider two diseases: Diabetes and Obesity.

Diabetes Mellitus, Diabetes for simplicity, is a group of metabolic diseases which are among the major human malnutrition diseases. A possible way to prevent the disease is risk assessment. Metabolic profiling as an unbiased technique that can possibly contribute to the identification of high-risk candidates and reduce related costs [14]. Obesity increases the likelihood of various diseases, particularly heart disease and type II Diabetes. It is also our interest to understand the mechanism of Obesity so as to develop a potential medical treatment.

In this paper, we integrate disease-specific (two diseases:

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Diabetes and Obesity are considered) gene expression data with human metabolic network to analyze the flux profiles of each reaction within the network. Metabolic biomarkers are identified to hold potential applications for disease diagnosis. In the following section, we will introduce the framework of reaction profiling for metabolite biomarker discovery. Two diseases are considered where two sets of potential biomarkers are identified. The validity of our proposed approach will be further discussed in the conclusion of the paper.

II. Materials and Methodology

In this section, we introduce our proposed method for metabolite biomarker discovery which is different from the popular method for their discovery, see for instance [13].

A. Materials

Two types of data are adopted in this paper. The first type is gene expression data of two diseases: (i) Diabetes and (ii) Obesity. The gene expression data is publicly available in GEO datasets in the NCBI data bank [36]. In Diabetes gene expression data, there are 12558 genes involved. The expression value is measured as $\log_2$ transformed signal. The platform to process these data is Platform GPL8300. In Obesity gene expression data, there are 54675 genes involved. The expression value is measured as $\log_2$ transformed signal. The platform to process these data is Platform GPL570. The second type of data is genome-scale human metabolic network reconstructed by Duarte et al [7]. It can be downloaded from BiGG database [37], where 3742 reactions are included, with 2766 metabolites and 1905 corresponding genes.

B. Methodology

We introduce the procedures for integrating gene expression data and the human metabolic network. There are three major steps from the preparations to the LP model construction and then to the biomarker determination.

1) Expression Levels in Reactions: Gene expression data in disease/normal samples is used to determine the expression levels in reactions. Because for each reaction the related genes are involved, it is necessary to determine their expression levels. In the gene expression data, expression value is measured as $\log_2$ transformed signal value. Here we first transform them to binary variables with 0 indicating lowly expressed and 1 for highly expressed genes. The normalization scheme is given as follows:

$$\text{New\_Signal} = \frac{\text{signal} - \mu_{\text{signal}}}{\sigma_{\text{signal}}}.$$

Here μ_{signal} is the average signal value, σ_{signal} is the standard deviation of the signal vector. After the transformation, genes having negative New_Signal values are defined as lowly expressed, otherwise they are considered to be highly expressed.

We then use gene expression data to define gene expression level and determine also the expression levels for the reactions. In each reaction, there can be 0, 1 or a few genes participating in the reaction procedure. Those highly expressed reactions are defined if all the participating genes are highly expressed, otherwise, we define the reactions to be lowly expressed.

In this way, we make use of the gene expression values in the disease/normal sample to identify highly/lowly expressed reactions. Therefore, expression levels in some reactions in the disease sample will be different from those in the normal sample.

2) Flux Profiles with Linear Programming:

- **The Linear Programming (LP) Model:**
  Integration of tissue-specific gene and protein expression data with human metabolic network results in a Mixed Integer Linear Programming (MILP) model [15] to predict human tissue-specific metabolic behavior. The MILP model can be described in the following:

$$\max_{y_i^+, y_i^-, \mathbf{v}} \left\{ \sum_{i \in R_H} (y_i^+ + y_i^-) + \sum_{i \in R_L} y_i^+ \right\}$$
$$\begin{cases} \mathbf{S} \cdot \mathbf{v} = 0 \\ \mathbf{v}_{min} \le \mathbf{v} \le \mathbf{v}_{max} \\ v_i + y_i^+ (v_{min,i} - \epsilon) \ge v_{min,i}, \quad i \in R_H \\ v_i + y_i^- (v_{max,i} + \epsilon) \le v_{max,i}, \quad i \in R_H \\ v_{min,i}(1 - y_i^+) \le v_i \le v_{max,i}(1 - y_i^+), \quad i \in R_L \\ y_i^+, y_i^- \in \{0, 1\}. \end{cases}$$

  Here $\mathbf{v}$ is the flux of all the reactions, and $\mathbf{S}$ is the stoichiometric matrix describing the quantitative relationships among the products and the reactants in the reactions. The parameter ϵ is the flux threshold, and it is chosen to be 1, see for instance [12]. Here R_H and R_L are respectively the sets of highly and lowly expressed reactions. The binary variables y_i^+ and y_i^- represent if reaction i is active or inactive respectively. In our method, we relax the binary variables to continuous variables in $[0, 1]$, i.e., we replace the last constraint by $0 \le y_i^+ \le 1$ and $0 \le y_i^- \le 1$. Thus the MILP model is relaxed to a LP model. In the LP model, y_i^+ and y_i^- can also be interpreted as the likelihoods for reaction i to be active. More importantly, the LP model is much easier to handle when compared to the MILP model.

- **Flux Profiles in Disease / Normal Sample:**
  Multiple optimal solutions exist for the same objective function, meaning that we have multiple feasible solutions satisfying the same constraints in the model [16]. The emphasis of investigating the multiplicity of solutions is to find the lower bound and upper bound for each flux in the corresponding reactions satisfying the constraints and ensuring the optimal objective value. And it has been shown that one can determine all the flux ranges through solving a series of LP problems [17]. Hence one can use the obtained objective value to further explore the flux ranges in reactions through solving a list of LP problems.

- **Identification of Significant Reactions:**
  Investigation of alternate optimal solutions provides us

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TABLE I
SIGNIFICANT REACTIONS FOR DIABETES

Index	Reactions
1238	"[e]:ac $\rightleftharpoons$ ac"
1951	"gcald[c]+h2o[c]+nad[c] $\rightarrow$ glyclt[c]+(2)h[c]+nadh[c]"
2297	"eandrstrn[r]+h[r]+nadph[r]$\rightarrow$ andrstandn[r]+h2o[r]+nadp[r]"
2357	"atp[c]+xylu-D[c]$\rightarrow$ adp[c]+h[c]+xu1p-D[c]"
2700	"dcdp[c]+h2o[c] $\rightarrow$ dcmp[c]+h[c]+pi[c]"

with four flux profiles: lower bound of flux vector in disease sample, upper bound of flux vector in disease sample, lower bound of flux vector in the normal sample and upper bound of flux vector in normal sample. Alternatively, we obtain two vectors of intervals for both the disease and the normal samples respectively. We then identify and label those reactions as significant reactions when there is no overlapping between the two intervals.

- **Significant Metabolite Discovery:**
  For a specific disease, we consider both control and disease samples. This means that we can identify two sets of reaction markers. Finally reaction markers are determined if the corresponding flux ranges in disease and normal samples have no overlap. Metabolite Biomarkers are then selected as the boundary metabolites in the significant reactions.

Comparing to the well known model using the human metabolic network to predict metabolic biomarkers of human inborn errors of metabolism [13], our model takes into consideration more realistic constraints. Firstly, the genome-scale human metabolic network we utilize here, consists of 1905 boundary metabolites and 3742 reactions in total. Secondly, we integrate gene expression data in normal and disease state to mark highly and lowly expressed reactions. Without forcing the reactions to be active in normal state or inactive in disease state, we use a probability measure for the reaction to be active or inactive instead. We use two pairs of gene expression data in both healthy and disease status and consider the overlap of the discovered metabolic biomarkers. For solution of the LP problems involved we use a large-scale optimization method which is based on LIPSOL (Linear Interior Point Solver [35]) in MATLAB R2008a on a vista machine. These characteristics of our approach would contribute to discover metabolic biomarkers in a more significant way.

III. RESULTS AND DISCUSSIONS

In this section, we discuss some of our findings. In the case of Diabetes, after comparing the significant reactions in two pairs of samples in disease and normal state, we have filtered out five reactions, the indices of which are listed in Table I. Index i means the ith reaction in the metabolic network. On the right-hand side of the index, there is a description of the reaction. We also list all the participating genes in these five reactions in Table II.

TABLE II
SIGNIFICANT GENES FOR DIABETES

Reactions Index	Genes
1238	"NONE"
1951	"ALDH1A1" "ALDH1A2" "ALDH1A3" "ALDH3A1" "ALDH3A2" "ALDH3B1" "ALDH3B2" "ALDH7A1" "ALDH9A1"
2297	"HSD3B2"
2357	"KHK"
2700	"NONE"

TABLE III
SIGNIFICANT REACTIONS FOR OBESITY

Index	Reactions
158	"4abut[m]+akg[m]$\rightleftharpoons$glu-L[m]+sucsal[m]"
248	"ac[m]+atp[m]+coa[m]$\rightarrow$ accoa[m]+amp[m]+ppi[m]"
582	"apoC-Lys[c]+btamp[c]$\rightarrow$ amp[c]+apoC-Lys_btn[c]+h[c]"
1506	"[e]:pydam $\rightleftharpoons$pydam"
3682	"(2)na1[e]+uri[e]$\rightarrow$(2)na1[c]+uri[c]"

TABLE IV
SIGNIFICANT GENES FOR OBESITY

Reactions Index	Genes
158	"ABAT"
248	"ACAS2L"
582	"HLCS"
1506	"NONE"
3682	"SLC28A3"

For the case of Obesity, after comparing the significant reactions in two pairs of samples in disease and normal status, we obtained five significant reactions as well, with the index of these reactions listed in Table III. Index i means the ith reaction in the genome-scale human metabolic network. The right column of the table provides a description of the reactions. All the participating genes in these five reactions are listed in Table IV.

The symbol "$\rightleftharpoons$" means the reaction is reversible and "$\rightarrow$" means the reaction is one-way and irreversible. The number inside the parentheses $(.)$ is the quantity of the metabolite. For example, in Table II, Reaction 1951, we need "gcald[c]:h2o[c]:nad[c]=1:1:1" to produce "glyclt[c]:h[c]:nadh[c]=1:2:1" products. In the associated genes, 'NONE' means that no gene was involved in the reaction. Others like "SLC28A3" and "HLCS" are the gene symbols.

From the perspective of genes, we have identified 11 significant genes involved in Diabetes in Table II. Here the "ALDH" gene and its variants are Aldehyde dehydrogenase. In [18], experiments have shown that "ALDH" activity is related to the increasing risk of large vessel disease in Diabetes. And in a clinical study on Diabetes [19], researchers also have found that regardless of alcohol consumption, the ALDH2.487Lys allele was related to the decreasing prevalence odds of type II Diabetes. It has been shown that direct intra-pancreatic delivery of high aldehyde dehydrogenase (ALDH) [20] activity

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can be a potential viable strategy for Diabetes. "HSD3B2" is found highly expressed with regulation of FXR (farnesoid X receptor) where FXR agonists are emerging therapeutic treatment of Diabetes [21]. The KHK gene encodes two enzyme isoforms with distinctive substrate preferences. The value of KHK as a pharmacological target needs testing [22], but can be a potential biomarker in Diabetes treatment. For the case of Obesity, one of the identified genes is "ABAT". In Gastroesophageal reflux disease (GERD), Obesity patients have a high risk of acquiring the disease [23]. Results show that ABAT is a genetic risk factor for GERD, which strengthens the significance of this gene in Obesity [24]. "ACAS2L" is a known Obesity candidate gene [25] and Obesity is a proved cardiovascular disease risk factor [26]. As a nucleoside transporter regulating multiple cellular processes, "SLC28A3" was identified to have significant association with Anthracycline-induced cardiotoxicity (ACT) [27]. This strongly suggests the important role of "SLC28A3". For "HLCS", its official name is "holocarboxylase synthetase (biotin-(proprionyl-CoA-carboxylase (ATP-hydrolysing)) ligase)". A lack of holocarboxylase synthetase activity may result in breathing problems and other characteristic signs, influencing regulation of genes for normal development.

Regarding the metabolites related Diabetes, "ac[e]" as an inhibitor, is very useful for patients in clinical trials, see for instance [29]. Both "nadph" and "nadp" are important in l-xylulose which is intensively used in Diabetes diagnosis. And l-xylulose is obtained by "nadph" and 'nad' reduction with "d-xylulose" [28]. The name of Reaction 1951 is Glycolaldehyde dehydrogenase. It has been shown that glycolaldehyde has a significant role in the development of diabetic cardiomyopathy [30]. And "pi" in reaction 2700 is a key component in the disturbance of Diabetes [31]. While in Obesity, 4-aminobutyrate transaminase (ABAT) has demonstrated the importance in Obesity [24]. Reaction 248 has the name Acyl CoA synthetase. One of the related gene Acyl CoA synthetase5 (ACSL5) has an important role in fatty acid metabolism [32]. Reaction 582 has related gene HLCS, the role of which needs to be further investigated. And pyridoxamine in reaction 1506 potentially will be a new therapeutic target to improve insulin resistance in Diabetes with Obesity [33]. Uridine transport is the name of Reaction 3682. From the role of Uridine Adenosine Tetraphosphate in the Vascular System [34], one may find some new directions for the Obesity treatment.

IV. Concluding Remarks

In this paper, we proposed a computational method to select significant genes and metabolites involved in specific metabolic diseases. A Linear Programming (LP) based strategy is used to obtain flux profiles in both the disease and normal samples. Gene expression data of two pairs of samples in both disease and normal states helps in discovering genes and metabolites which can be potential biomarkers. The integration of gene expression levels with genome-scale human metabolic network data provides a new way for systematically analyzing potential biomarkers.

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