

Metabolomic differences and interactions between coronary heart disease and diabetes mellitus: Pathogenesis understanding and auxiliary diagnosis

Wuping Liu

Xiamen University

Pengfei Guo

Xiamen University

Tao Dai

Henan Institute of Science and Technology

Xiulin Shi

First Affiliated Hospital of Xiamen University

Guiping Shen

Xiamen University

Jianghua Feng (✉ jianghua.feng@xmu.edu.cn)

Xiamen University <https://orcid.org/0000-0001-8899-2750>

Original investigation

Keywords: Diabetes, Coronary heart disease, Metabolomics, Pathogenic pathways

Posted Date: August 19th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-47559/v1>

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Abstract

Background: Comprehensive understanding of plasma metabotype of diabetes (DB), coronary heart disease (CHD) and especially diabetes with coronary heart disease (CHDDB) has kept lacking. The metabolic fingerprints of CHD, DB and CHDDB were investigated to reveal their pathogenic mechanisms and interactions and identify their specific biomarkers.

Methods: The plasma metabolomic differences and links of 15 DB, 15 CHD and 30 CHDDB patients and 15 matched healthy controls were investigated by NMR-based strategy. The univariate and multivariate statistical analyses-based pattern recognition was combined with network analysis to determine disease specific biomarkers and understand the corresponding pathogenic pathways.

Results: A total of 17 metabolites related to the development of disease were identified by comparative metabolomic analysis, and 6 of them were shared by the three diseases. The metabolites involved in amino acid synthesis (valine, alanine, leucine, isoleucine, and N-acetyl-glycoprotein) were positively associated with CHD and CHDDB (Odds Ratios (OR) >1); The trimethylamine oxide, glycerol, lactose, indoleacetate and *scyllo*-inositol are closely related to the development of DB to CHDDB (OR>1), and indoleactate (OR: 1.06, 95% confidence interval (CI): 1.01-1.12) and lactose (OR: 2.46, 95% CI: 1.67-3.25) are particularly prominent in CHDDB. The three different multi-biomarker signatures could serve to distinguish between CHDDB, DB, and CHD. All diseases demonstrated the disturbed glycolysis/gluconeogenesis and amino acid biosynthesis pathway. Furthermore, inositol phosphate metabolism, tryptophan metabolism and microbiota metabolism play a major role in the development of CHDDB from DB.

Conclusions: The disease-specific multi-biomarkers provide promising capability in auxiliary diagnosis of DB-related disease and follow-up of disease progression and treatment. The comparative metabolomics strategy of multi-diseases offers a comprehensive perspective in disease-specific markers and pathogenic pathways.

Background

At present, the patients with diabetes mellitus (DB) and/or coronary heart disease (CHD) account for a significant proportion of the world's population. Many studies reveal that DB is associated with a 2- to 4-fold greater risk of developing CHD than nondiabetic populations, and it is also an independent risk factor of heart failure and even death developing from CHD ^(1, 2). Furthermore, the DB patients with CHD (CHDDB) have a much poorer prognosis of survival than those without CHD ⁽³⁾. DB complications comprise heart disease, stroke, blindness, and kidney failure, which are mostly due to the microvascular and microvascular bed impairments ⁽⁴⁾. These major complications related to vasculature are usually determined by the metabolic, hemodynamic and inflammatory factors and further exacerbated by the presence of common risk factor in DB such as obesity, insulin resistance, hypertension, and states of inflammation and oxidation. However, not all of DB patients will develop into vascular complications,

indicating the related but different biological mechanisms between DB and CHD. Therefore, the potential factor and links in the development and progression of patients with CHD related to diabetes need to be well elucidated. Based on it, effective early diagnosis strategies would play an important role in clinical treatment of CHD and CHD with diabetes.

The metabolic links and differences in metabolites variations and pathway between DB and CHD have not been discovered although they are of great importance to understand the interaction between the two diseases. With the improvement of analytical techniques and the continuous development of related bioinformatics, metabolomics can not only detect the composition of the metabolomes and the changing trends of the disease-related metabolites, but also integrate metabolites information and pathological information to provide relevant metabolic and signaling pathways and their interaction ⁽⁵⁾. Metabolomic analysis of human plasma is very useful to understand dysfunctions and pathological status since plasma is a primary carrier of small molecular in the body and contains a range of metabolites ⁽⁶⁾. In recent years, non-targeted metabolomic researches on DB and CHD have identified a large number of endogenous characteristic metabolites as potential biomarkers ⁽⁷⁾, but no specific metabolic differences, especially their metabolic interactions and links, were clarified and identified between DB and CHD patients.

Therefore, the scope of this work was to explore the differences and links in metabolic phenotype and pathogenic pathway between DB, CHD and CHDDB. Furthermore, the purpose of this study was to identify potential biomarkers for auxiliary diagnoses of the three diseases as well as to offer a theoretical basis for the prevention and treatment of CHD population.

Methods

Study population

A case-control study of chronic disease risk factors was conducted. The population was involved in four groups: (i) DB patients (n=15); (ii) CHD patients (n=15); (iii) CHDDB patients (n=30); (iv) healthy controls (HC, n=15). These patients were mainly from Zhongshan Hospital Affiliated to Xiamen University and the First Affiliated Hospital of Xiamen University (Xiamen, China), and the healthy controls were from the First Affiliated Hospital of Xiamen University in the same period of time and matched with diabetic patients for demographic characteristics.

We consecutively recruited patients who had been diagnosed with diabetes in the two hospitals according to American Diabetes Association criteria. All of the selected CHD patients were diagnosed and confirmed by coronary angiography. The diagnosis criteria for CHD patients refer to “Treatment guide of stable angina” (ACC/AHA/ACP-ASIM, 1999) and “Diagnosis and treatment recommendations of unstable angina” (Chinese Society of Cardiology, 2000). The inclusion, exclusion and rejection criteria of CHD refer to previous research ⁽⁸⁾. The diagnosis of CHDDB is determined by combining the above DB and CHD criteria.

Collections of clinical data and plasma sample

The clinical and demographic characteristics were collected within 24 hours after the patients were admitted. These diagnostic data were acquired by professional and experienced physicians who have the occupation qualification, attending physician or above, and have a more-than-two-year relevant clinical experience.

The samples of peripheral venous blood were taken in morning after fasting overnight. The plasma (with heparin sodium) was immediately separated from the peripheral venous blood by centrifugation at 3000 g at 4 °C for 20 min. Then, the supernatant was carefully aspirated into a 2-mL EP tube and stored at -80 °C until analysis. Plasma samples were thawed at 4 °C and centrifuged at 13000 g at 4 °C for 20 min. And 200 μ L supernatant was mixed with 400 μ L of phosphate buffer solution (0.2 M, pH 7.4, 99.9% D₂O) and then moved to 1.5 mL EP tube for centrifugation under the same conditions above. Then, 550 μ L supernatant was transferred into a 5 mm NMR tube for ¹H NMR spectral acquisition.

¹H NMR spectroscopy and spectral preprocessing

The ¹H NMR spectra of plasma samples were recorded on a 600 MHz Varian NMR spectrometer (Varian INOVA; Varian Medical Systems, Palo Alto, USA) equipped with a triple resonance probe at 600.04 MHz and 298 K. The ¹H NMR experiments were conducted with presaturation of water resonance using a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence. Spectra were acquired with a spin-spin relaxation delay, 2 τ , of 700 ms, and water signal irradiation was applied during the relaxation delay. The 90 °C pulse length was adjusted individually for each sample. Typically, 64 scans were collected into 32 K sampling points over a spectral width 8012.8 Hz with a relaxation delay of 2.0 s and an acquisition time of 4.0 s.

For spectral processing, the free induction decays (FIDs) were multiplied by an exponential function it a 0.5 Hz line. All spectra were manually phased, baseline-corrected and referenced to the endogenous lactate at δ 1.33. The bucketing algorithm was applied to the spectra in the range between δ 0.50 and δ 9.50 with a bucket width of δ 0.005 on MestReNova software (Version 12.0, Mestrelab Research S. L., Spain). The spectral region δ 5.05-4.55 was excluded from the dataset to avoid spectral interference from residual water.

Data analysis

All data analysis was processed using SIMCA 14.0 software (Umetrics, Umea, Sweden) and R language (version 3.5.3). The dataset was normalized to the sum of the spectral integrals and pareto-scaled. Orthogonal signal correction partial least squares discriminant analysis (OPLS-DA) model was applied to achieve differences in metabolite profiling between disease and healthy people, identifying the relevant variables responsible for the clusters. The R²Y describes the proportion of variance in the data explained by the models and indicates goodness of fit, and the Q² is defined as the predictive ability of the model. The performance of the models was evaluated by the leave-one-out cross validation (LOOCV) and 500 random permutations for quality-of-fit parameter and the predictive-ability parameter (R² and Q²). The

specific variables between classes were interpreted using variable importance in the projects (VIP) and correlation coefficients (Cor). The NMR signals were assigned to metabolites with the reference proton NMR peaks from Chenomx NMR Suite 8.1 (Chenomx Inc., EDBonton, AB, Canada) and previous study⁽⁹⁾ and confirmed by the public HMDB (Human Metabolome Database) NMR database.

The batman R package was used to automatic metabolite quantification⁽¹⁰⁾. The files required by batman were displayed Tables S1 and S2. The differences in metabolite between the HC and patients were tested with T-test and kruskal-wallis test. Adjustment of multiple comparison was performed for false discovery rate (FDR) with Benjamin-Hochberg method⁽¹¹⁾. The enhanced volcano plots were generated based on the concentration ratio (fold change), VIP, Cor and adjusted p-value of the metabolites. The metabolites with $VIP > 1$, $abs(Cor) > 0.50$ and adjusted p-value < 0.05 were considered to contribute significantly to the diagnosis of disease. To identify metabolites predictors, odds ratios (ORs) of developing different disease with each metabolite were calculated by conditional logistic regression models, after adjusting for age, gender, smoking status, drinking status, body mass index (BMI), systolic blood pressure (SBP) and diastolic blood pressure (DBP).

The pathway over-representation analysis (ORA) of metabolites was conducted by the IMPaLA web tool⁽¹²⁾ (<http://impala.molgen.mpg.de/>). The scoring algorithm based on heat diffusion model was performed using the FELLA R package to obtain network-based pathway enrichment⁽¹³⁾. Accordingly, the network was constructed using the igraph R packages. The hierarchical agglomeration algorithm was performed by the igraph R packages for find the dense subgraph⁽¹⁴⁾. Sensitivity and specificity of every metabolite in the disease were respectively analyzed by the receiver operating characteristic curve (ROC) analyses, and the area under ROC (AUC) and confidence interval (CI) were determined by the pROC R package⁽¹⁵⁾.

Results

Baseline characteristics of study population

The baseline characteristics of the study groups are summarized in Table 1. All groups were very similar with regard to most clinical biological characteristics such as sex, age, body mass index (BMI), blood pressure, low density lipoprotein (LDL), high density lipoprotein (HDL), uric acid (UA) and lifestyle factors (smoking and drinking) except those associated with DB and CHD. Fasting glucose and blood urea nitrogen were significantly higher in the DB and CHDDB groups than the HC group, but no significant difference between the CHD and HC groups. Moreover, all patients demonstrated significantly higher glycated hemoglobin (HbA1c) and triglycerides (TG) levels than the HC subjects.

Metabolomic analysis determines potential disease biomarkers

An NMR-based strategy was applied to obtain the plasma metabolomes and identify 42 distinct metabolites from all disease groups and the healthy controls. The mean ¹H-NMR spectra from the

different biological regime are shown in Figure S1, and the detailed assignment data for metabolites are included in Tables S3. We observed a clear separation between the patients and HC in the OPLS-DA scores plot (Figures 1A, 1B, and 1C). The R^2Y and Q^2 values were greater than 0.80 and 0.50, respectively and the p-values from CV-ANOVA (cross validated analysis of variance) in the models were less than 0.0001 (Table S4), suggesting that the OPLS-DA models were effective and reliable for discriminating the patients. The models were further validated by the permutations tests (Figure S2), where all permutation R^2 and Q^2 values were lower than the original values, and intercepts of Q^2 linear regression were below zero. According to the enhanced volcano plots (Figures 1A, 1B and 1C), 10, 12, and 12 disease-related potential biomarkers were identified from the disease of DB, CHDDB and CHD, respectively. Among these metabolites, 6 common potential biomarkers, N-acetyl glycoprotein (NAG), α - β -glucose, glutamine, ethanol, and lactate, were shared by the three diseases; 2 metabolites were common to groups CHDDB and CHD, namely leucine and valine; and 3 metabolites were common to groups CHDDB and DB, namely lactose, glycerol and trimethylamine N-oxide (TMAO) (Figure 1D). In addition, indoleacetate was unique to CHDDB; methanol, alanine and methylamine were unique to CHD, and *scyllo*-inositol was unique to DB (Figure 1D). These results illustrated the characteristic metabolic changes corresponding to the specific diseases.

Metabolic pathway and network analysis between diseases

A comprehensive analysis of the disturbed metabolic pathways (Figures 1E, 1F, and 1G) revealed several significantly changed pathways shared by all disease groups, such as AGE-RAGE signaling pathway, protein digestion and absorption, glycolysis/gluconeogenesis, cAMP signaling pathway and pyruvate metabolism. Amino acid biosynthesis pathway (alanine, aspartate and glutamate metabolism and valine leucine and isoleucine biosynthesis), membrane transport (ABC transporters) and signal transduction (mTOR signaling pathway) were disturbed in the CHDDB group as well as CHD group, while glycerolipid metabolism was aberrant in the CHDDB and CHD groups. In addition, tryptophan metabolism was aberrant and unique in the CHDDB group, and inositol phosphate metabolism was prominent in the DB group.

The network of the metabolites-pathway-reaction-enzyme-module associated with progression of DB to CHDDB was constructed (Figure 2), which facilitates subsequent targeted omics and genetic analyses. The hierarchical agglomeration algorithm of network was combined with the properties of pathways and metabolites from each disease to determine metabolic interactions and links among the diseases. In our study, four notable subgraphs (Figure S3) were identified from the global networks (Figure 2) via hierarchical agglomeration analysis. Subgraph I matches those metabolites common to the three diseases, including lactate, glucose, glutamine, and ethanol, which mainly involved in energy metabolism and amino acid synthesis. Subgraph II comprises indoleacetate, which are involve in tryptophan metabolism and subgraph III comprises DB specific *scyllo*-inositol, which involved in inositol phosphate metabolism, whereas subgraph IV constitutes TMAO, methylamine and methanol that has been shown to be closely related to microbial metabolism. Combined the results of metabolite pathway analysis, global networks revealed that the abnormal energy metabolism and amino acid synthesis make CHD

development more seriously, and persistent abnormalities in tryptophan metabolism, microbial metabolism and inositol phosphate metabolism may be pathogenic pathway for DB developing into CHDDB.

Determination of disease-specific biomarkers

The logistic regression analysis among the baseline data and metabolites provided a descriptive measure of effects of the potential biomarkers on the risk of progression to each disease (Figure S4A). It is noted that the OR of HbA1c for the three disease groups was 2.11 (95% CI: 1.59-2.89, DB), 7.09 (95% CI: 2.49-9.56, CHD) and 1.29 (95% CI: 1.15-1.42, CHDDB), respectively, suggesting that HbA1c has a strongly connection with the occurrence and development of the three diseases. Additionally, the glucose was also an important factor related to the development of DB and CHDDB, with an OR 1.22 (95% CI: 1.03-1.43) and 1.07 (1.00-1.14), respectively (Figure S4A). Taken together, glucose levels were not significantly associated with incident CHD, while it was positively associated with incident DB and CHDDB, and HbA1c was positively associated with both the three diseases.

The metabolites involved in amino acid synthesis (valine, alanine, leucine, isoleucine, and NAG) were positively associated with CHD and CHDDB (OR>1), but slightly associated with incident DB (OR<1). TMAO, glycerol and *scyllo*-inositol were closely related to the development of CHD with diabetes patients because the OR value of these metabolites in DB and CHDDB was higher than 1, among which TMAO is the most significant, with an OR 1.38 (95% CI: 1.14-1.68, DB) and 1.16 (95% CI: 1.06-1.28, CHDDB), respectively (Figure S4A). It was observed that the ORs of lactose and indoleacetate are particularly prominent in the incident CHDDB, with a value of 2.46 (95% CI: 1.67-3.25) and 1.06 (95% CI: 1.01-1.12), respectively (Figure S4A). The OR values of the remaining metabolites were below 1.0, suggesting their infeasibility to specifically diagnose the disease as potential biomarkers.

Based on metabolomics analysis and regression analysis of the plasma metabolites, we selected three sets of specific biomarkers for auxiliary diagnosis of each disease, namely *scyllo*-inositol, TMAO and glucose for DB, indoleacetate, lactose and glucose for CHDDB, and NAG and valine for CHD. In term of specific biomarkers, NAG and HbA1c demonstrate significant difference among all of the groups. The levels of *scyllo*-inositol in DB group were significantly decreased but not significantly different in CHDDB and CHD patients compared to the healthy controls (Figure S4B), while valine levels were elevated in CHDDB and CHD patients but kept indifferent in DB patients compared the controls. TMAO and glucose, the most sensitive markers of CHDDB and DB patients, however, were not significantly increased in CHD patients comparing to HC patients (Figure S4B). In addition, compared to HC, lactose and indoleacetate were significantly elevated in CHDDB patients but not in CHD patients (Figure S4B), indicating that the two metabolites may be promising to monitor DB progresses to CHDDB as important biomarkers.

Multi-biomarkers for auxiliary diagnose

The performance of disease specific biomarkers was then determined by the ROC curve analysis (Figure 3). In regard to DB, TMAO had the highest AUC 0.91 (95% CI: 0.884-1), followed by glucose 0.91 (95% CI:

0.80-1) and *scyllo*-inositol 0.77 (95% CI: 0.60-0.95); In regard to CHDDB, the AUC of indoleacetate, lactose and glucose was 0.85 (95% CI: 0.73-0.97), 0.78 (95% CI: 0.63-0.93), and 0.89 (95% CI: 0.77-1) respectively; For NAG and valine in CHD, the AUC was 0.93 (95% CI: 0.87-1) and 0.86 (95% CI: 0.73-0.99), respectively (Figures 3A&3B). According to the results, these disease-specific biomarkers displayed high AUC and provided better discrimination in disease diagnosis (Figure 3B). In order to take advantage of the every biomarker, the disease-specific biomarkers responsible for each disease were combined to determine their synergistic power. The results indicate the AUC of multi-biomarker raised to 0.97 (95% CI: 0.73-0.99) in DB, 0.91 (95% CI: 0.82-1) in CHDDB and 0.98 (95% CI: 0.95-1) in CHD, respectively (Figure 3B). Importantly, the each disease-related multi-biomarker displayed the strongest diagnostic capability compared with the other diseases (Figures 3B&3C), reflecting the specificity of the multi-biomarkers. In fact, the multi-biomarker demonstrated the superior specificity and sensitivity to the individual biomarker (except the specificity of TMAO and glucose) in the diagnosis of each disease (Table S5). It has been confirmed that HbA1c is a reliable clinical biomarker to monitor glycemic control in patients with both DB and CHD^(16, 17). In our study, it has been observed that TMAO, indoleacetate, lactose, valine, NAG and glucose were positively correlated with HbA1c, while *scyllo*-inositol was negatively correlated with HbA1c (Figure 3D), further indicating the potential values of these metabolites in the disease diagnosis.

Discussion

In order to gain a deeper understanding into the metabolic differences and interactions between CHD and DB, disease-specific comprehensive metabolomic fingerprints in plasma were detected, and especially focusing on the different molecular changes and the related biological pathways of each disease. The systems biological results were derived from *in situ* metabolomics data from healthy humans and patients with DB, CHDDB and CHD and *in vitro* functional studies. (I) All the patients show specific plasma metabolite characteristics that served to understand the disease-specific metabolic phenotypes and pathogenic mechanisms; (II) Abnormal energy metabolism and amino acid synthesis are universal metabolic characteristics in the three diseases, furthermore, the continuous abnormalities in tryptophan metabolism, microbial metabolism and inositol phosphate metabolism were key factors for developing into CHDDB from DB; (III) our multi-diseases omics approach confirmed the known diagnostic markers (*scyllo*-inositol and TMAO for DB and HbA1c for CHDDB, CHD and DB) and identified some new potential biomarkers for DB developing into CHDDB (indoleacetate). Furthermore, the disease-related multi-biomarkers demonstrated the great sensitivity and specificity, and thus providing promising capability in auxiliary diagnosis and follow-up of disease progression and treatment.

Seventy six percent (76%) of diabetic patients attending outpatient department were reported significant gastrointestinal symptoms⁽¹⁸⁾. Lactose is composed of equimolar amounts of glucose and galactose, and its accumulation may cause some gastrointestinal symptoms include abdominal pain, diarrhea, flatulence and bloating. In this study, the lactose was significantly accumulated in CHDDB patients. Hence, we suggested that test of lactose intolerance is essential for DB patients, thus potentially preventing the diabetic patients to suffer from other complications. *Scyllo*-inositol is the most common

inositol in body, and it not only acts as an osmolyte, but also plays key roles in intracellular signaling and making membrane phosphatidylinositol. *Scyllo*-inositol is derived from the intestinal bacteria fermentation of *myo*-inositol and may be involved in glucose homeostasis since its metabolic abnormalities were associated to insulin resistance and long-term diabetes microvascular complications in diabetic subjects ⁽¹⁹⁾. Furthermore, the increased excretion of uremic toxins TMAO in the disease-associated to DB, and the enhanced excretion of the glucogenic amino-acid (valine) in the disease-associated to CHD were observed in our study, which corroborates other metabolomics studies ^(20–22). Studies have shown that elevated TMAO levels in DB patients were associated with a risk of DB developing into CHD ^(23, 24), while the levels of branched-chain amino-acid metabolites (valine, leucine, isoleucine) have also been associated with risk of CHD ^(25, 26). Moreover, valine abnormality is regarded as a metabolic risk factor in insulin resistance incident type 2 diabetes and future cardiovascular events ⁽²⁷⁾.

The recent study reported several alterations of CHD and DB in metabolic pathway, including cAMP signaling pathway ⁽²⁸⁾, glycolysis/gluconeogenesis and pyruvate metabolism ⁽²⁹⁾, which keep consistent with our results (Fig. 1). Additionally, a prominent metabolic abnormality pattern of inositol phosphate metabolism was observed in DB in our case (Fig. 2, subgraph III), which has also been involved in the development of several other disease states (e.g. obsessive compulsive disorders, depression, and Alzheimer's disease) and in particular in the development of insulin resistance and diabetic complications ⁽¹⁷⁾. The most prominent hits in CHDDB pointed to aberrant tryptophan metabolism, which contains three major metabolic pathways, kynurenine pathway, serotonin pathway and indole/AhR pathway ⁽³⁰⁾. In the host tryptophan metabolism, intestinal flora can not only directly convert tryptophan into bioactive molecules, but also control the metabolism of tryptophan through intestinal flora. In these metabolisms, kynurenine pathway is related to several pathologic conditions, including inflammatory disease and psychiatric disorders ⁽³¹⁾. In addition, indole/AhR pathway (Fig. 3E) is closely related to the flora metabolism, which is able to control aspects of bacterial physiology such as antibiotic resistance, sporulation, and biofilm formation ⁽³⁰⁾. A growing evidence has suggested that gut microbiota is a major role in the development of obesity, type 2 diabetes mellitus and their complications ⁽³²⁾.

The present study identified several metabolites association with microbial metabolism, including TMAO and indoleacetate, as also observed in the other study ⁽³³⁾. TMAO is the stepping stone metabolite linking microbial metabolites to physiological status of host. It has been found that the elevated TMAO plasma level is associated with *prevotella* enterotype, as opposed to the *bacteroides* enterotype ⁽³⁴⁾. In blood, TMAO accumulation can cause a number of adverse effects, including altered steroid and biliary metabolism, vascular and endothelial cell dysfunction, increased risk of atherosclerosis and major cardiovascular events. The recent study reveals that the detrimental role of TMAO in glucose metabolism could potentially attribute to activation the endoplasmic reticulum stress kinase that was identified as a direct host target of TMAO ⁽³⁵⁾. Indoleacetate is tryptophan-derived microbial metabolites, and is known to activate the nuclear receptor AhR. It is usually involved in indole/AhR pathway (Fig. 3E), a key

component of the immune response at barrier sites by acting on epithelial renewal and barrier integrity, and thus it is quite crucial for intestinal homeostasis⁽³⁰⁾. In the indole/AhR pathway, indoleacetate is a ligand for AhR and known to affect the intestinal permeability and host immunity⁽³⁶⁾. The animal experiment has revealed that AhR activation generally reduces inflammation and thus maintains gut homeostasis⁽³⁷⁾. In this study, it was observed that TMAO and indoleacetate were significantly elevated in DB and CHDDB patients, suggesting that the metabolites related to microbial metabolism have a strong relationship with development of DB to CHDDB and are the key markers for the follow-up individualized treatment and diagnosis.

Several limitations about the multi-biomarkers exist in the current study. First, the metabolite panel and the model served to the screening of the biomarkers related to DB and CHD were obtained from a small sample size and some specific regions, which may lead to the potential lack of diagnostic power. A measurement in a large prospective cohort for replication will determine the universally applicable weights. Second, the metabolite panel should be tested in patients with different grades of DB and CHDDB to determine its metabolomic dynamics during disease progression, and thus facilitate their early diagnosis. Third, the microbes may influence the baseline levels of metabolites and prevent correlations with identified metabolites because the microbial composition from the participants is unknown.

Conclusions

Our foregoing data reveal three multi-biomarkers modules for CHD, CHDDB and DB diagnosis, comprising clinical biochemical indices and six metabolites, which are related mainly to glycolysis/gluconeogenesis, amino acid biosynthesis pathway, inositol phosphate metabolism, tryptophan metabolism and changes in gut microbiota metabolism. Among them, inositol phosphate metabolism, tryptophan metabolism and microbiota metabolism play a major role in the development of DB to CHDDB. This is also the first study to comprehensively identify the metabolotypes between DB, CHDDB and CHD, and further find that TMAO, *scyllo*-inositol and indoleacetate, lactose, HbA1c and glucose and some amino acids are related to high risks of coronary heart disease with diabetes events.

Declarations

Acknowledgments

We thank all the research subjects for their participation and acknowledge the medical staff of Xiamen Diabetes Institute and Department of Endocrinology and Diabetes, the First Affiliated Hospital of Xiamen University.

Authors' Contributions

W. L. conducted the experiments, analyzed the data, and wrote the manuscript; P. G. conducted the experiments and reviewed the manuscript; T. D. contributed to the discussion and reviewed the manuscript; X. S. collected the samples and reviewed the manuscript; G. S. contributed to the discussion

and reviewed the manuscript; J. F. conceived and designed the study, analyzed the data, contributed to the discussion, and wrote the manuscript.

Funding

This work is financially supported by the National Natural Science Foundation of China (Nos. 31671920) and the United Fujian Provincial Health and Education Project for Tackling the Key Research (No. 2019-WJ-07).

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study protocol was approved by ethics committee of each institution, and informed consent for the procedure was obtained from each participant.

Consent for publication

Not applicable

Competing interests

No potential conflicts of interest relevant to this article were reported.

Authors' information

¹ Department of Electronic Science, Fujian Provincial Key Laboratory of Plasma and Magnetic Resonance, Xiamen University, Xiamen, 361005, China

² Third Affiliated Hospital, Henan University of Science and Technology, Luoyang, 471003, China

³ The Xiamen Diabetes Institute and Department of Endocrinology and Diabetes, the First Affiliated Hospital of Xiamen University, Xiamen, 361003, China

Abbreviations

DB: Diabetes; CHD: Coronary heart disease; CHDDB: Diabetes with coronary heart disease; NMR: Nuclear magnetic resonance; OR: Odds ratios; HC: Healthy controls; ACC/AHA: The American College of Cardiology/American Heart Association; ACP-ASIM: The American College of Physicians–American Society of Internal Medicine; FIDs: Free induction decays; OPLS-DA: Orthogonal signal correction partial least squares discriminant analysis; VIP: Variable importance in the projects; Cor: Correlation coefficients; FDR: False discovery rate; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood

pressure; ROC: Operating characteristic curve; CI: Confidence interval; AUC: Area under ROC; LDL: Low density lipoprotein; HDL: High density lipoprotein; HbA1C: Glycated hemoglobin; TG: Triglycerides; NAG: N-acetyl glycoprotein; TMAO: Trimethylamine N-oxide.

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Table

Table 1 Baseline biological characteristics of the patients and healthy controls

	HC ^a (n=15)	DB(n=15)	CHD(n=15)	CHDDB(n=30)
Age (years)	56±6 ^c	51±5	59±8	59±7
Gender (male/female)	7/8	9/6	7/8	12/18
Current smoking [n(%)]	5(33)	6(40)	7(47)	14(47)
Current alcohol use [n(%)]	4(27)	5(33)	4(27)	12(30)
BMI ^b (Kg/m ²)	25.41±2.19	25.71±2.51	26.02±2.63	25.92±3.46
Systolic blood pressure (mmHg)	120.6±15.8	127.1±16.5	122.1±16.5	126.2±12.0
Diastolic blood pressure (mmHg)	78±7.7	79.3±10.8	75.7±8.6	77.5±10.7
BUN (mmol/L)	4.26±1.09	5.67±1.55 ^{*, d}	4.98±0.89	5.72±1.21 [*]
UA (µmol/L)	270.07±77.85	315.39±81.24	345.52±91.91	311.01±74.27
TG (mmol/L)	1.01±0.39	1.52±0.65 ^{**}	1.95±1.42 ^{**}	2.15±1.48 ^{**}
HDL (mmol/L)	1.12±0.32	1.15±0.27	1.09±0.31	1.1±0.25
LDL (mmol/L)	2.62±0.24	2.85±0.73	2.75±0.83	2.71±0.73
Fasting glucose (mmol/L)	5.57±0.28	8.06±2.32 ^{***}	5.26±0.59	7.98±3.17 ^{***}
HbA1c (%)	4.82±0.39	7.25±0.68 ^{***}	5.57±0.30 ^{***}	7.79±1.22 ^{***}

^a HC, healthy controls; DB, the diabetes only patients; CHD, the patients only with coronary heart disease; CHDDB, the DB patients with CHD

^b BMI: body mass index; BUN: blood urea nitrogen, UA: uric acid; TG: triglycerides; HDL/LDL: high-density/low-density lipoprotein; HbA1c: glycated hemoglobin.

^c Data are represented as mean ± SD for continuous variables and n (%) for categorical variables.

^d T-test and nonparametric Kruskal-Wallis test were used for comparisons of continuous variables. *, p<0.05; **, p < 0.01; and ***, p<0.001 for HC vs. patients group.

Figures

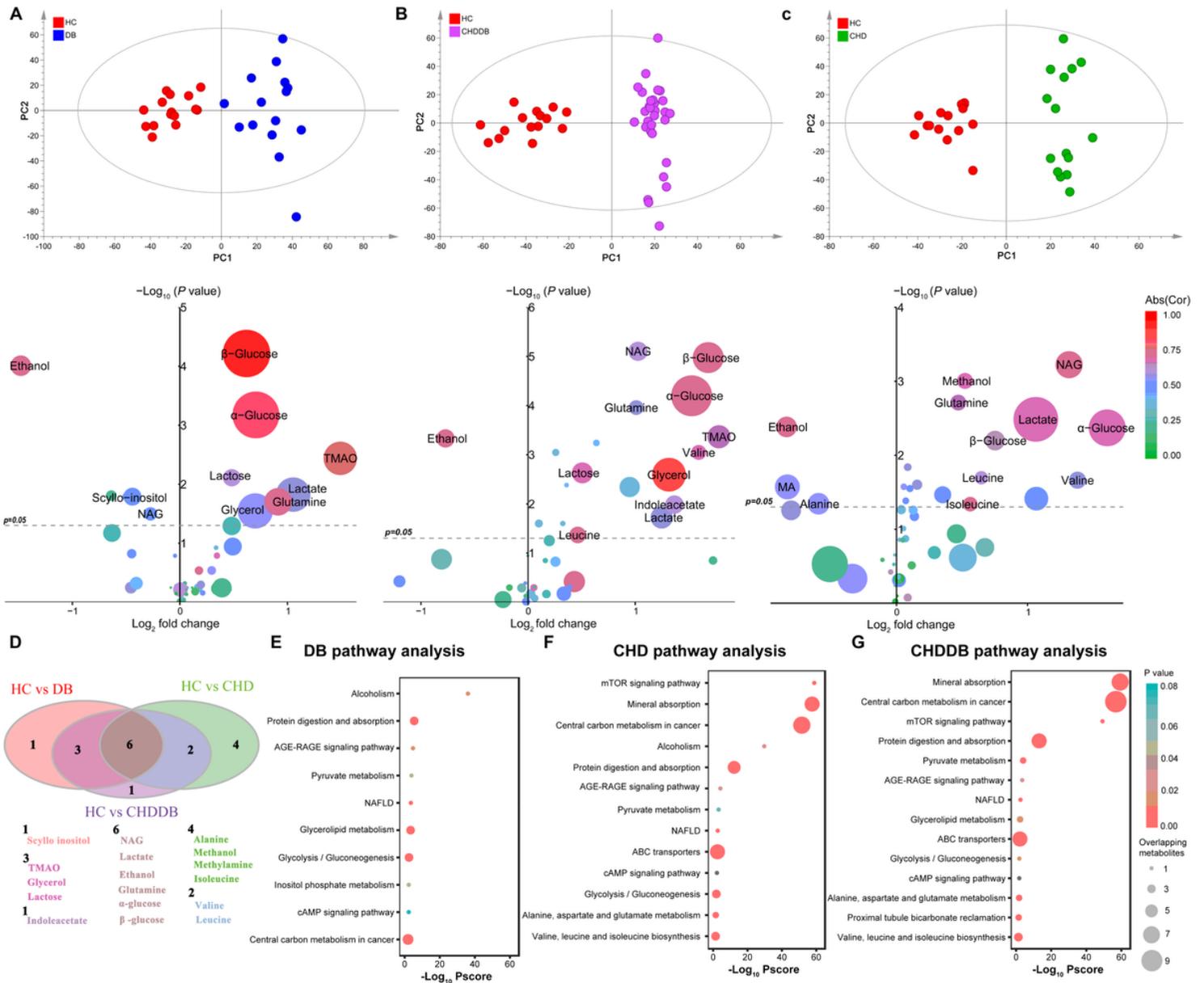


Figure 1

Plasma metabolomic differences between the patients and the healthy controls. OPLS-DA scores plots (top panels) and the corresponding enhanced volcano plots of all metabolites (middle panels) based on the NMR data of (A) HC vs. DB groups; (B) HC vs. CHDDB groups; and (C) HC vs. CHD groups. (D) The distribution of the differential metabolites in the DB, CHD and CHDDB patients. The disturbed metabolic pathways induced by DB (E), CHDDB (F), and CHD (G). The p-value of showed pathways is less than 0.05, which is filtered by ORA. HC, healthy controls; DB, diabetes mellitus; CHD, coronary heart disease; CHDDB, coronary heart disease with diabetes mellitus.

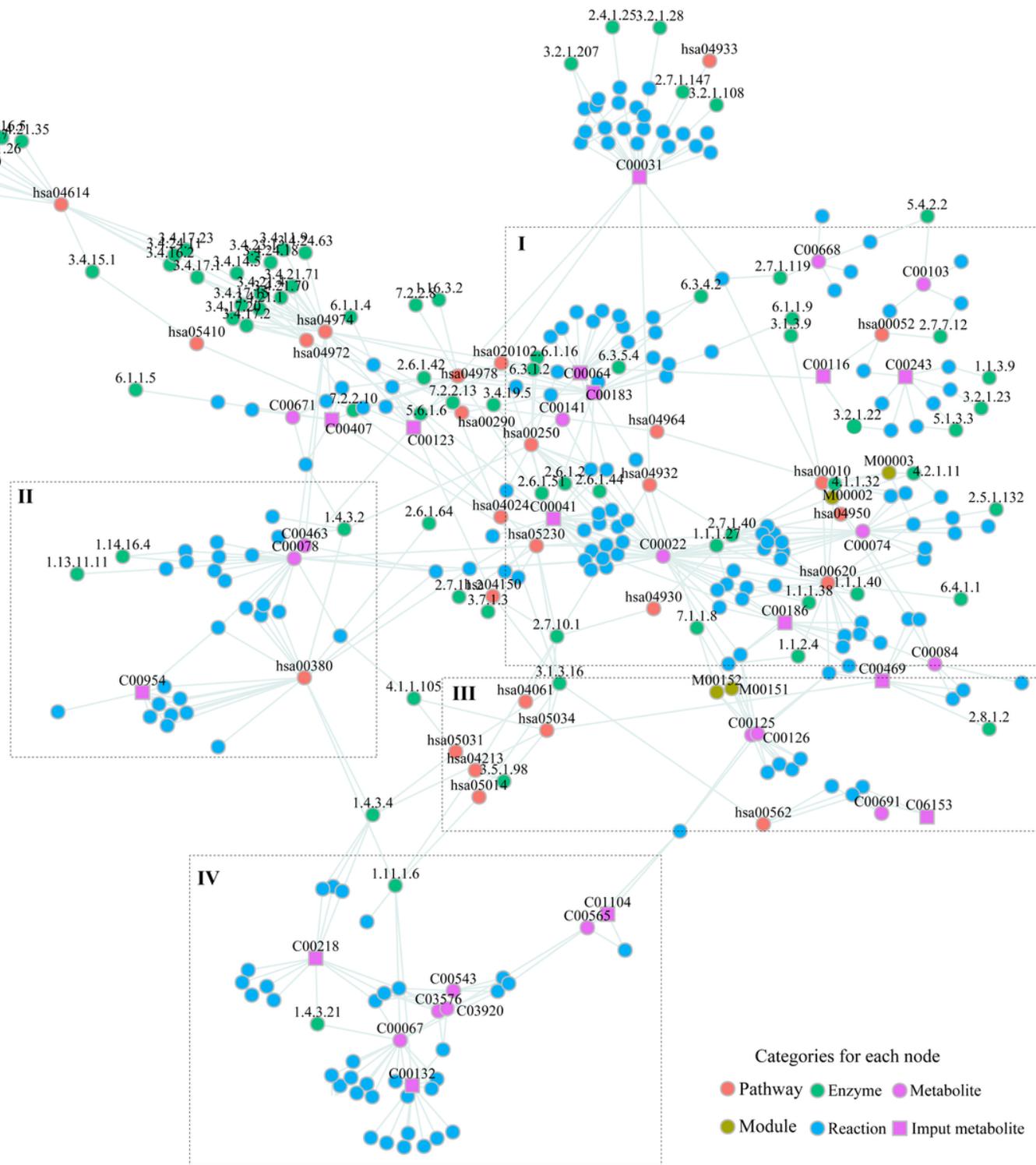


Figure 2

The network of pathway-module-enzyme-reaction-metabolites. The pathways, modules, enzymes and reactions are from KEGG database and the metabolites are from the characteristic metabolites of each disease. Four main subgraphs were identified by hierarchical agglomeration analysis (I, energy metabolism and amino acid synthesis; II, tryptophan metabolism; III, inositol phosphate metabolism module; IV, related to microbial metabolism module). The details of each subgraph are shown in Figure S3.

Supplementary Files

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