Integration of metabolomics and transcriptomics reveals major metabolic pathways and potential biomarkers involved in aging mice with type 2 diabetes mellitus

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To the Editor: Aging can cause changes in human metabolic capacity[1] and metabolic syndrome is one of the important causes of type 2 diabetes mellitus (T2DM). In recent years, T2DM has become one of the top ten killers of humans.[2] T2DM has been reported in ≥25% of the population aged over 65 years.[3] The liver is the most important metabolic center and its metabolic disorders are associated with T2DM.[4] However, studies on liver metabolic disorders in older adult patients with T2DM are limited. Therefore, it is necessary to analyze liver metabolism in these patients in combination with modern omics methods. In this study, we integrated metabonomics and transcriptomics analyses to analyze the specific metabolites and genes that change in the liver of aging mice with T2DM, and to provide a theoretical basis for the treatment of older adult patients with T2DM.

Ten 8-week-old and twenty 20-week-old male Kunming (KM) mice were acquired from the specific pathogen-free (SPF) Laboratory at Gansu University of Chinese Medicine (Gansu, China) (License Number: SCXK (Gan) 2017-0002). All the 8-week-old mice were assigned to the young group, and the twenty 20-week-old mice were randomly divided into a non-diabetic aging group ($n = 10$) and an aging-with-T2DM group ($n = 10$). The young group and the non-diabetic aging group were given a normal diet and the aging-with-T2DM group was given a high carbohydrate and fat diet for 28 consecutive days. From the 29th day, the mice in the aging-with-T2DM group were injected with 70 mg/kg streptozotocin (STZ) for 3 days[5] and the mice in the other groups were injected with an equal amount of saline. Blood samples were collected 7 days after the last STZ injection, and then the mice were euthanized using cervical dislocation. All experimental procedures and protocols were in accordance with the guidelines of the Institutional Animal Ethics Committee of Gansu University of Chinese Medicine (No. 2017-064).

The body weight, daily food intake, and water consumption of mice in each group were measured and recorded every 2 days. One week after the last STZ injection, the mice in each group were made to fast without water for 12 h; fasting blood glucose was measured using test paper and the result was obtained after 5 s of a chemical reaction. We found that aging mice with T2DM showed obvious symptoms of polydipsia, polyphagia, and slow weight gain, or even weight loss. After model establishment, the fasting blood glucose was >20 mmol/L, which is higher than the upper limit for clinical diagnosis of diabetes. The detailed results are provided in Supplementary Figure 1, http://links.lww.com/CM9/A590. All the data mentioned above indicate that the T2DM model was successfully established.

After the mice were euthanized, the liver was harvested, fixed with 4% paraformaldehyde, dehydrated, embedded with paraffin, and sliced (3 μm thickness). Hematoxylin and eosin (H&E) staining and Periodic acid-Schiff (PAS) staining were used. H&E staining showed that the liver cells of the young mice were intact, while in the non-diabetic aging mice, some of the liver cells were swollen. In the liver tissue of the aging mice with T2DM, the liver cells were obviously swollen and disordered, and even the central vein was deformed and collapsed [Supplementary Figure 2A–C, http://links.lww.com/CM9/A590]. PAS staining showed that the liver cells of the aging mice with T2DM had significant accumulation of glycogen, whereas the liver cells of the young and non-diabetic aging mice were normal [Supplementary Figure 2D–F, http://links.lww.com/CM9/A590].

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000001554

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Chinese Medical Journal 2022;135(2)
Received: 06-01-2021; **Online:** 01-07-2021 **Edited by:** Lishao Guo

The above general physiological parameters and histological results indicated that there were significant differences between the aging mice with T2DM and the other two groups, but there were no significant differences between the young and non-diabetic aging mice. To examine the difference in liver metabolism between aging mice with and without T2DM, we used only the non-diabetic aging mice and the aging mice with T2DM in the subsequent experiments.

To determine the changes in the metabolic profile of non-diabetic aging mice and aging mice with T2DM,

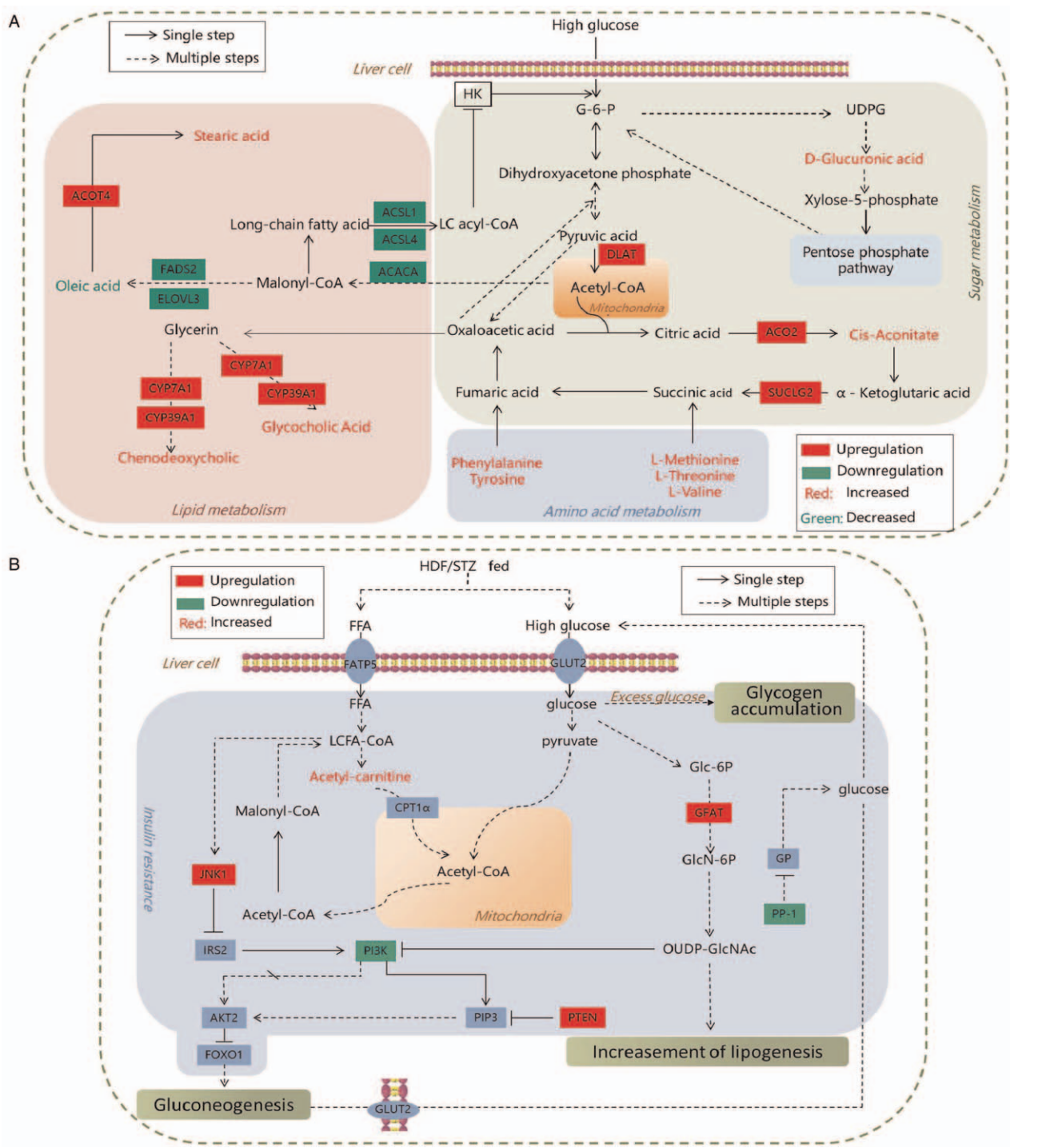

Figure 1: Pathways associated with aging with T2DM. (A) Carbohydrate, lipid, and amino acid metabolism. (B) Insulin resistance. ACACA: Acetyl-coenzyme A carboxylase alpha; ACSL1: Acyl-CoA synthetase-1; CYP7A1: Cytochrome P450 family 7 subfamily A member 1; DLAT: Dihydrolipoamide S-acetyltransferase; ELOVL3: Elongase of very long chain fatty acids-3; FOXO1: Forkhead box transcription factor O1; FADS2: Fatty acid desaturase 2; GFAT: Glutamine fructose 6-phosphate amidotransferase; GLUT2: Glucose transporter protein 2; IRS2: Insulin receptor substrates 2; JNK1: c-Jun N-terminal kinase 1; LCFA: Long-chain fatty acid; PI3K: Phosphoinositide 3-kinases; PIP3: Phosphatidylinositol-3,4,5-trisphosphate; PTEN: Phosphatase and tension homolog; T2DM: Type 2 diabetes mellitus.

high-performance liquid chromatography quadrupole time-of-flight mass spectrometry (MS) analysis was performed on frozen liver tissues of non-diabetic aging mice and aging mice with T2DM. The MS data were processed using Mass Profiler software (Agilent, Shanghai, China) and further subjected to partial least squares discriminant analysis (PLS-DA) and orthogonal projections to latent structures-discriminant analysis (OPLS) using SIMCA-P 14.1 for multivariate biochemical patterns recognition. The PLS-DA and OPLS results showed significant differences in metabolites between aging mice with and without T2DM. The Multi Experiment Viewer software (Version 4.5.1, http://www.tm4.org) was used for the hierarchical clustering analysis and significance analysis for microarrays. Sixty-four significantly altered metabolites were found between aging mice with and without T2DM (detailed information about these metabolites is provided in Supplementary Table 1, http://links.lww.com/CM9/A591). Using Metabolomics Pathway Analysis (MetPA) (https://www.metaboanalyst.ca/) for statistical, functional, and integrative analysis of metabolomics data, 39 altered pathways were identified and visualized [Supplementary Figure 3A–D, http://links.lww.com/CM9/A590; see detailed information about these pathways in Supplementary Table 2, http://links.lww.com/CM9/A592].

To elucidate the reasons for the changes in the metabolic profile, messenger RNA (mRNA) expression profiles were examined in the liver of non-diabetic aging mice and aging mice with T2DM. GeneSpring software V13.0 (Agilent) was used to calculate the gene expression differences. There were 2486 and 3131 mRNAs that were down and upregulated, respectively, in aging mice with T2DM compared with non-diabetic aging mice ($|FC| \geq 2$; $P \leq 0.05$). Cluster 3.0 was used for cluster analysis. KEGG Orthology Based Annotation System (KOBAS) (http://kobas.cbi.pku.edu.cn/kobas3) was used for Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment pathway analysis of differentially expressed genes, revealing 57 significantly enriched KEGG signaling pathways [Supplementary Figure 4A and 4B, http://links.lww.com/CM9/A590; see detailed information about these pathways in Supplementary Table 2, http://links.lww.com/CM9/A592].

To clarify the regulatory relationship between metabolites and differential genes, integrated molecular pathway analysis of transcriptomics and metabolomics data was performed using the IMPaLA (http://impala.molgen.mpg.de/) web tool. By integrating 64 remarkable metabolites and 5617 differentially expressed genes, 31 pathways were identified, 13 of which were metabolism-related pathways, and six were related to human disease (see detailed information about these pathways in Supplementary Table 2, http://links.lww.com/CM9/A592). Signaling pathways related to three nutrient metabolism disorders [Figure 1A] and insulin resistance [Figure 1B] were found in this study, and all were related to the aging-with-T2DM group.

In conclusion, our combined metabolomics and transcriptomics analyses indicated that the mechanism behind aging with T2DM may involve disorders of glucose, fat and amino acid metabolism, and insulin resistance. This study explored the liver metabolic characteristics of aging mice with T2DM at the gene and metabolic levels; however, further verification of our findings is required.

Acknowledgements

The authors thank Mr. Jun-Jun Liu for his help with conducting animal experiments, and Dr. Yong Wang for assisting us in histopathological analysis.

Funding

This work was supported by grants from the National Natural Science Foundation of China (Nos. 81760835, 82060829), Postgraduate Innovation Fund of Gansu University of Chinese Medicine (No. CX2020-45) and Natural Science Foundation of Gansu Province (No. 1606RJZA192).

Conflicts of interest

None.

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How to cite this article: Meng J, Liu J, Chen D, Li D, Wang J. Integration of metabolomics and transcriptomics reveals major metabolic pathways and potential biomarkers involved in aging mice with type 2 diabetes mellitus. Chin Med J 2022;135:247–249. doi: 10.1097/CM9.0000000000001554