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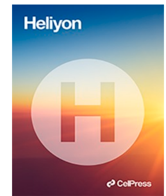

Bioinformatics analysis of the diversity of gut microbiota and different microbiota on insulin resistance in diabetes mellitus patients

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A R T I C L E I N F O

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A B S T R A C T

Introduction: It aimed to explore the diversity of gut microbiota (GM) and the effect of different microbiota on insulin resistance in diabetes mellitus (DM) patients through bioinformatics analysis. Material and Method: Microarray data were obtained from GEO database. GM samples from DM patients and healthy controls were collected, and 16S rRNA gene sequencing was carried out adopting high-throughput sequencing technology. The differential expression genes were screened using the Qlucore Omics Explorer 3.0 software. Subsequently, online tools such as STRING and DAVID were utilized for bioinformatics analysis of the differential expression genes. The differences in bacterial diversity between DM patients and healthy controls were evaluated by analyzing the diversity indicators of the microbiota, such as Shannon and Chao1 indexes. Differential abundance and functional prediction analysis were adopted to explore the different microbiota and its possible metabolic pathways between DM patients and controls. And differences in insulin resistance in specific bacterial taxa were analyzed. Result: GM diversity between DM patients and controls had significant differences. GM diversity was lower in DM patients compared with controls, as indicated by a decrease in Shannon and Chao1 indexes. The differential abundance analysis showed that there were multiple different bacterial communities between DM patients and controls, including some bacterial communities at the genus-level. Functional prediction analysis also revealed potential metabolic pathways related to GM and insulin resistance in DM patients. HEXB, ZC3H12A, CCR, CXCR3, GBR10, CDK9, TXN, IGFBP3, PDHA1, and NDUFB3 genes may be potential targets for treatment. Conclusion: There are differences in GM diversity between DM patients and healthy controls, and the different microbiota may be related to the occurrence and development of insulin resistance.

1. Introduction

Diabetes mellitus (DM) is a global widespread chronic metabolic disease characterized by hyperglycemia [1]. According to the World Health Organization (WHO), there are about 460 million people with DM in the world, which is expected to increase to 640 million by 2030. Patients with DM are usually accompanied by the occurrence and development of insulin resistance, which is one of the core links in the pathogenesis of DM [2]. Insulin resistance leads to the decrease of the body's response to insulin, and then causes blood glucose regulation disorder [3]. DM is a metabolic disease, and gut microbiota (GM) is closely related to the occurrence and

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development of DM. GM diversity studies mainly reveal the role of GM in the disease by comparing GM between DM patients and non-DM population, and by observing GM changes in DM patients after treatment or dietary intervention [4,5]. GM is a collection of various microorganisms living in the human gut, including bacteria, fungi, viruses, etc. Its composition and function play an important role in the host's metabolism and immune system. The GM of DM patients is different from that of normal people, and its diversity is affected. These differences may be closely related to the occurrence of insulin resistance and glucose metabolism disorders. Dysbacteriosis increases the risk of DM. Studies have found that GM diversity in DM patients is relatively low, and some specific species are lost or increased [6]. Compared with non-DM population, the abundance and diversity of some bacterial flora are different in DM patients. Inflammatory bowel disease-associated bacterial flora was increased while beneficial bacterial flora was decreased in DM patients.

Bioinformatics, the study of biological data using methods such as computer science and statistics, has many advantages. Biological research generates a large amount of data, such as genome sequencing data, proteomics data, and transcriptomics data. Bioinformatics analysis can use computers and related tools to efficiently process and analyze these large-scale data and mine the information hidden in the data [7]. With the development of high-throughput technology, large-scale and high-dimensional biological data can be obtained, such as gene expression data, DNA methylation data, and protein interaction network data. Bioinformatics analysis can integrate biological data from different sources, including genome, transcriptome, proteome, and metabolome data, to comprehensively analyze different levels of biological information and reveal complex biological processes and interrelationships. It can also predict biological problems such as gene function, protein structure, and gene regulatory network, which can provide guidance for experimental design and biological research. It can also perform simulation and modeling of biological processes to help to understand the operation mechanism of biological systems. It can also identify candidate biomarkers and drug targets, accelerate the drug discovery process, and reduce research and development costs [8].

Bioinformatics analysis has the advantages of efficient processing of big data, comprehensive analysis and integration of multi-level data, problem prediction and simulation, marker and target discovery, data sharing and cooperation, which provides powerful tools and methods for biological research. As of 2021, research has shown a relationship between the gut microbiota and diabetes as well as insulin resistance. The gut microbiota constitutes a microbial ecosystem within the human body, comprising bacteria, fungi, viruses, and other microorganisms. They can influence the host's metabolism and immune system, thereby impacting the development of diabetes and insulin resistance. The identification of microbiota species/metabolites related to diabetes and insulin resistance relies on techniques such as 16S rRNA sequencing, whole-genome sequencing, metabolomics, and functional genomics. Transgenic diversity typically pertains to crop production and food safety, with no direct association with diabetes and its pathogenesis. Diabetes is a complex metabolic disorder involving multiple factors, including genetics, environment, and lifestyle. Some studies have identified certain microbiota species and metabolites associated with diabetes and insulin resistance [9]. For instance, the relative abundance of specific bacterial species may be related to diabetes risk, and certain microbial metabolites might directly or indirectly influence host insulin sensitivity. However, these studies are still evolving and exploring, and absolute conclusions cannot be drawn yet. The relationship between microbiota and diabetes is a complex field, and further research is required to gain a better understanding of the specific mechanisms by which these microorganisms influence diabetes and insulin resistance. Studies on the diversity of gut microbiota in diabetes patients still face challenges and controversies, such as individual variations, environmental factors, and the impact of different types of diseases on research outcomes. Based on the current research on metabolomics analysis of diabetes patients, this study explores the correlation between diabetes occurrence and gut microbiota diversity, and conducts pathway analysis of differentially expressed metabolites, providing a theoretical basis for diagnosis and treatment.

2. Materials and methods

2.1. Sources of microarray data

NCBI web site: https://www.ncbi.nlm.nih.gov/into Gene Expression Omnibus (GEO) database page: https://www.ncbi.nlm.nih.gov/geo/, keywords, such as "diabetes gut microbiota" or "diabetic microbiome". The dataset GSE176230 platform GPL24676 had 34 samples. The data set GSE162622 platform GPL570 had 50 samples. 10 cases of controls and 10 cases of T2D group were selected in the data set.

Inclusion criteria: (1) participants aged above 18 years; (2) all patients diagnosed with T2D; (3) the study included a control group, i.e., healthy individuals without T2D; (4) the study design was a randomized controlled trial; (5) detailed statistics of differential metabolites between diabetes patients and the control group were available. Exclusion criteria: (1) gastrointestinal disorders; (2) acute infections; (3) secondary type 1 diabetes; (4) malignant tumors, autoimmune diabetes; (5) coexisting moderate to severe liver or kidney impairment.

2.2. Screening of differentially expressed genes (DEGs)

The Qlucore Omics Explorer 3.0 (http://www.qlucore.com/) was used for the analysis of gene chips, Mi-RNA chips, and protein chips, and the software was relatively easy to operate. In this work, the Qlucore Omics Explorer 3.0 software was utilized for statistical analysis of chip data. The data were imported into the software and then subjected to standardization (mean centered to 0, and scaled to a standard deviation of 1). The statistical method used for data analysis was the two-group comparison (*t*-test) to effectively filter and identify differentially expressed genes. The criteria for selecting differentially expressed genes were: $P < 0.05$, fold change ≥ 2, and $Q < 0.01$.

DEGs in the data set were analyzed using zero code analysis tools GEO2R (https://www.ncbi.nlm.nih.gov/geo). GEO2R is an application in the GEO2 online database that can identify DEGs in 2 or more samples and rank gene importance. GEO2R compared the microbiota among DM patients and the DEGs with GM abnormalities. When $P < 0.05$ and $|\log FC| \geq 1.5$, there were differences in gene expression between the two groups.

2.3. Analysis of DEGs

After screening out the DEGs, it further performed Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis on the DEGs. GO analysis mainly included three parts: biological process (BP), cellular component (CC), and molecular function (MF). DEGs were selected to upload to DAVID 6.8 bioinformatics resource database (https://david.ncifcrf.gov/) to perform GO enrichment and KEGG pathway analysis for DEGs, P value was obtained by using the Fisher's exact probability method, The screening conditions were all $P < 0.05$, and the P values were ranked from small to large.

2.4. Sequencing analysis

The high-throughput sequencing of the two groups of subjects was retrospectively analyzed. Through the quality initial screening sequences, according to the primer and Barcode information, the sequences were identified and assigned to the corresponding samples, and the doubts such as chimeras were removed. Sequences were obtained based on operational taxonomic unit (OTU) division with 97 % sequence similarity. The taxonomic status of the representative sequences of each OTU was identified. According to the richness distribution of OTU in the samples, the diversity of the sample flora (ACE, Chao1, Simpson, and Shannon) was evaluated. Partial least squares-discriminant analysis (PLS-DA) model was constructed based on the abundance matrix of the flora using R software, and OTU rarefaction curve was drawn using QIIME software to analyze the differences in the phylum level of the flora.

2.5. Protein-protein interaction (PPI) network analysis

The search tool for the retrieval of interacting genes (STRING) (https://string-db.org/) database was used to predict PPI. Through the PPI score results, the possibility of PPI can be evaluated. The proteins encoded by the DEGs were input into the STRING library, and the screening threshold was set as: binding score >0.4. Cytoscape v3.6.1 was adopted to construct the PPI network, and the top 30 key genes were screened, and the color was adopted to indicate the score predicted by Degree algorithm.

3. Results

3.1. Analysis of two datasets

The GSE162622 dataset contained 50 samples, mainly for the analysis of systemic inflammatory response of probiotics in DM patients. The results of data sample visualization are illustrated in Fig. 1. Fig. 1A showed GSE162622: expression density, and Fig. 1B

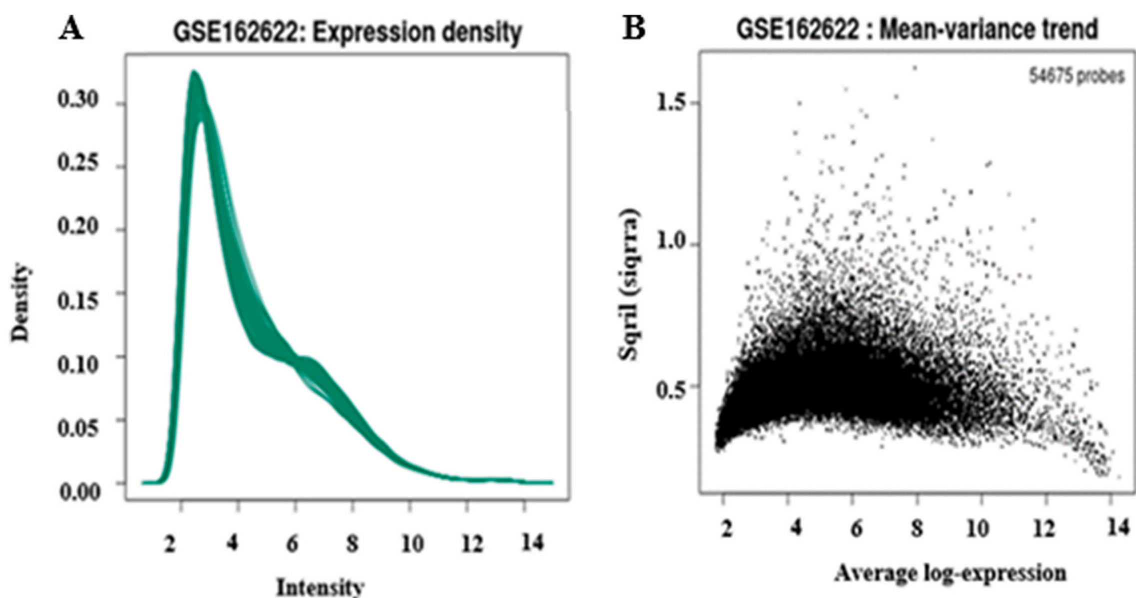

Fig. 1. Sample visualization of GSE162622 dataset. (Note: A: GSE162622: expression density; B: GSE162622: mean-varlance trend).

displayed GSE162622: mean-varlance trend. The distribution characteristics of the entire data set can be observed, and it was found that most of the data were relatively clustered, while only a few were discrete.

Fig. 2A was the GSE176230: UMAP plot, while Fig. 2B displayed the GSE176230 data distribution. Samples from the GSE176230 dataset were subjected to Next gene sequencing of the patient's whole blood transcriptome using the Illumina NovaSeq platform to obtain sequence depth of 50 million reads/sample. Patient metabolite-based dietary supplementation was associated with microbiota and immune modulation (Fig. 2).

3.2. PLS-DA

PLS-DA was performed based on partial least squares regression model, which had sample distribution information and discriminant community structure data. The difference between the normal and the DM group was higher ($P < 0.05$) (Fig. 3).

3.3. Sample diversity

The microbial Alpha diversity indices of the two groups are given in Table 1, and the results of Simpson, Shannon, ACE, and Chao1 were calculated for each sample at the same sequencing depth.

In Fig. 4, the mean values of Simpson, Shannon, ACE, and Chao1 were taken to analyze the Alpha diversity index. There was similar in the diversity index parameters (Simpson, Shannon, ACE, and Chao1) between the two groups of samples ($P > 0.05$). In Fig. 4, the mean values of Simpson, Shannon, ACE and Chao1 of the two groups of research objects were taken to analyze the Alpha diversity index. In Fig. 4A, there was no significant difference between ACE and Chao1 in the diversity index parameters between the two groups of samples ($P > 0.05$). In Fig. 4B, there was no significant difference in the diversity index parameters of Simpson and Shannon between the two groups ($P > 0.05$).

3.4. Sample diversity

In bioinformatics analysis, each sequence sequenced comes from a bacterium. In order to understand the number of species and genus in the sequencing results of the sample, it is necessary to classify the sequence. All sequences were subjected to OTU for statistical analysis of biological information. Fig. 5 had 965 OTU in the intersection, with 562 OTU unique to the controls, and 298 OTU unique to the T2D group.

Rank abundance curves were used to account for species abundance and species evenness. In the horizontal direction, the abundance of species is reflected by the width of the curve, the higher the abundance of species, the larger the range of the curve on the horizontal axis. The smoothness of the curve reflects the evenness of the species, the flatter the curve, the more uniform the species distribution. The main species included in the two groups of samples in Fig. 6 are Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, Verrucomicrobia, Fusobacteria, Tenericutes, Synergistetes, Cyanobacteria, Patescibacteria. Among them, Tenericutes was the most evenly distributed. Bacteria accounted for most of this microorganism. Proteobacteria, Bacteroidetes, Actinobacteria, and Firmicutes accounted for most of the bacterial flora.

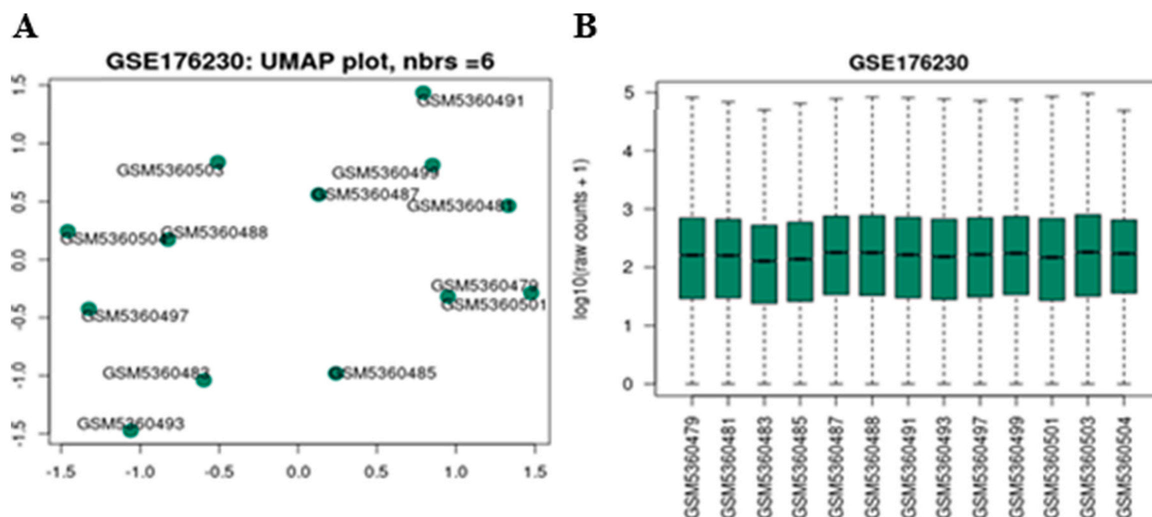

Fig. 2. Sample of GSE176230 dataset. (Note: A: GSE176230: UMAP plot; B: GSE176230 Data distribution).

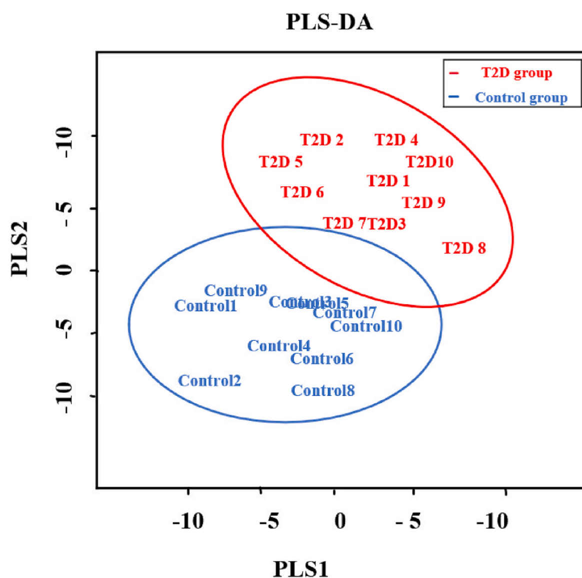

Fig. 3. PLS-DA.

Table 1
Specific values of microbial Alpha diversity index of the two groups of bacteria.

Sample	Simpson	Shannon	ACE	Chao1
T2D 1	0.897064	5.64	1243.6	114.65
T2D 2	0.876578	6.23	897.65	876.55
T2D 3	0.954367	7.32	897.45	902.4
T2D 4	0.942368	6.89	745.98	785.43
T2D 5	0.917654	7.54	1087.4	1134.9
T2D 6	0.925436	7.65	1124.3	1254.61
T2D 7	0.956324	6.32	987.65	1056.86
T2D 8	0.965437	5.38	1134.67	1065.45
T2D 9	0.944653	5.93	869.4	978.56
T2D 10	0.854376	6.34	965.3	986.54
Control 1	0.934562	6.34	879.45	965.32
Control 2	0.921753	6.85	1231.4	1135.6
Control 3	0.935426	6.45	986.54	997.64
Control 4	0.942306	6.84	543.87	974.56
Control 5	0.960435	6.34	678.34	692.33
Control 6	0.876540	6.97	1145.34	108.76
Control 7	0.963423	7.32	932.54	967.81
Control 8	0.953425	7.14	945.78	967.82
Control 9	0.917543	7.34	968.43	978.65
Control 10	0.970543	7.23	532.68	567.84

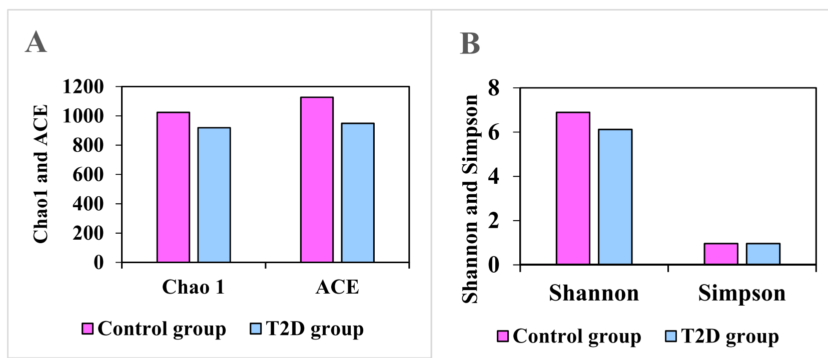

Fig. 4. Comparison of Alpha diversity index. (Note: A: ACE, Chao1; B: Simpson, Shannon).

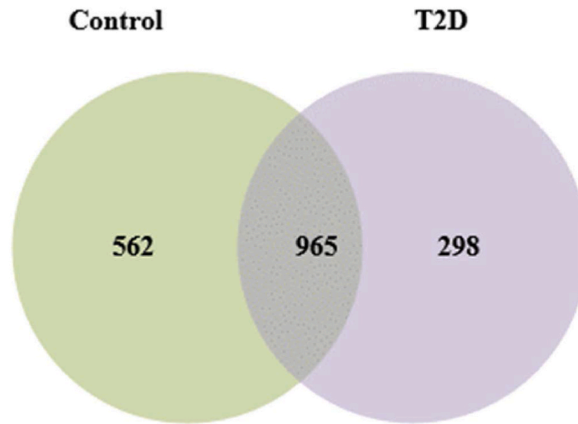

Fig. 5. Venn diagram of OTU intersection.

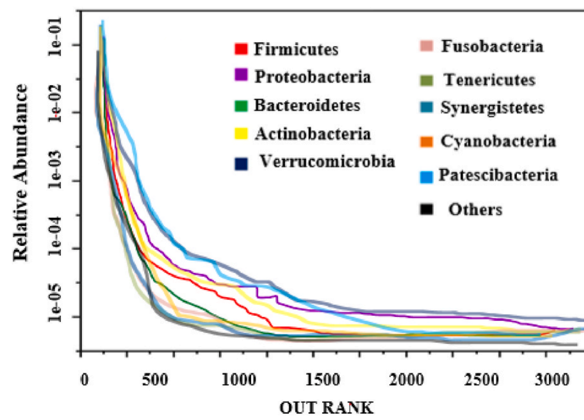

Fig. 6. Rank abundance curves of the bacterial flora.

3.5. OTU rarefaction curve

The length of the curve represents the level of sample sequencing, and the smoothness of the curve reflects the effect of depth on the diversity of observed samples. The rarefied curves of the two groups gradually flattened, indicating that the current sequencing depth could reflect the diversity contained in the samples, and no new OTU could be detected if the sequencing depth continued to increase, which also reflected the quality control pass of 16sRNA sequencing (Fig. 7).

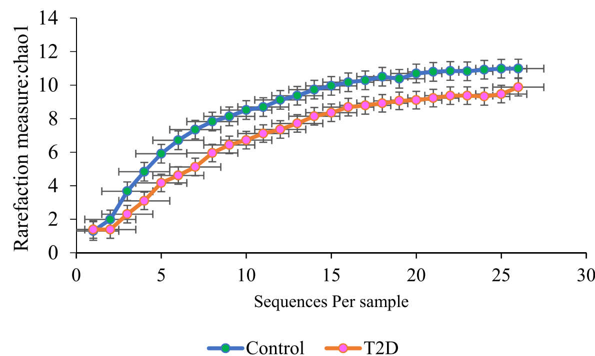

Fig. 7. OTU rarefaction curve.

3.6. Composition and abundance distribution of samples at Actinobateria taxonomic level

KEGG enrichment pathways of DEGs included adipocytokine signaling pathway, oxidative phosphorylation, citrate cycle, Parkinson's disease, Huntington's disease, Alzheimer's disease. Differential genes were also involved in the following processes: transcription initiation from RNA polymerase, generation of precursor metabolites and energy, muscle system proces, phosphorylation, phosphorus metabolic process, oxidation reduction, cytokine mediated signaling pathway, energy derivation by oxidation of organic compounds (Fig. 8).

3.7. PPT network analysis

The main coding PPI network of differential genes in mild AD was 46 nodes, 41 edges. The average node degree was 1.78, the average local clustering coefficient was 0.445, the expected number of edges was 22, and the PPT enrichment *P* value was 0.000292. The top 10 key genes were HEXB, ZC3H12A, CCR, CXCR3, GBR10, CDK9, TXN, IGFBP3, PDHA1, and NDUFB3 (Fig. 9).

4. Discussion

The dysregulation of GM is closely related to the development and progression of insulin resistance. Some studies have found that the abundance and composition of some bacterial flora in DM patients are related to insulin resistance. Some studies have also shown that adjusting GM through dietary intervention, probiotics, and prebiotic supplementation can improve the condition of DM patients. These therapeutic interventions may improve insulin sensitivity, glycemic control, and metabolic markers [10]. Huang et al. (2022) [11] observed the changes of GM in T2DM patients on a low-fat diet during a 6-month follow-up, and the composition of bacterial flora in T2DM patients had no obvious differences as against healthy persons. According to Wu et al. (2023) [12], FMT improved the metabolic status of T2D patients. The transplantation of donor-derived microbiota, with or without metformin, significantly improved insulin resistance, BMI, and gut microbiota composition in T2D patients. Yang et al. (2021) [13] proposed that GM was clearly abnormal in DM patients relative to healthy people. In the DM group, the abundance of Enterobacter faecalis, Prevotella, and Roseburia was higher, while the abundance of Shigella and Bifidobacterium was lower. GM is crucial for the occurrence and development of DM. The study conducted by Zhang et al. (2021) [14] suggests that early intervention in prediabetes (PreDM) patients may have an impact on the transition of gut microbiota towards T2D. This article found that the number of Lactobacillus and Bifidobacterium in the samples of T2DM patients was less as against controls, and there was GM imbalance in T2DM patients. The specific dysbiosis pattern observed in diabetic patients is a complex field of study, and related research is continuously evolving, with no consistent dysbiosis pattern identified yet. Some studies [15] have found certain dysbiosis trends in diabetic patients, such as alterations in the gut microbiota compared to non-diabetic individuals. Particularly, the abundance of beneficial bacteria (such as Bifidobacterium and Lactobacillus) may decrease, while potential harmful bacteria (such as Clostridium) may increase in diabetic patients. Furthermore, the abundance of inflammatory bacteria may also increase in diabetic patients, potentially related to the development of chronic inflammatory states. Compared to healthy individuals, diabetic patients may exhibit lower gut microbiota diversity, which may be associated with disease progression and metabolic disturbances. Other research [16] has indicated that the gut microbiota in diabetic patients may produce more metabolites related to glucose metabolism, potentially affecting host glucose metabolism. It has been observed [17] that the relative abundance ratio of Firmicutes to Bacteroidetes in the gut may change in insulin-resistant populations. This study also suggests that the gut microbiota may play a significant role in the development of insulin resistance. Certain gut microbes may produce harmful metabolites, such as endotoxins, triggering host inflammatory responses. Prolonged inflammatory reactions may interfere with insulin signaling pathways, leading to insulin resistance. Dysbiosis of the microbiota may also result in the disruption of the intestinal mucosal barrier, allowing harmful substances to enter the circulation system and provoke insulin resistance. Dysbiosis in diabetic patients may cause a variety of effects, further promoting the development and progression of insulin resistance.

There is a certain correlation between GM diversity and insulin resistance in DM patients. As a marker of the development of PreDM, it can be adjusted, combined with quantitative aerobic exercise, to achieve adjustment of insulin sensitivity and improve the development of DM in PreDM people [18]. The research has revealed that the reduced abundance of Akkermansia muciniphila in T2D patients may be associated with blood glucose fluctuations. Das et al. (2023) [19] has suggested the identification of novel biomarkers for early detection of diabetic kidney disease (DKD), as well as potential therapies to modulate the gut microbiota and improve host immune response. Ye et al. (2023) [20] has identified the inadequacy of circulating dopamine, imbalanced short-chain fatty acid (SCFA) production, and excessive metabolic inflammation as multiple parallel impacts driven by the gut microbiota in the development of gestational diabetes mellitus (GDM). The gut microbiota may hold promise as a target for interventions in gestational diabetes. This article also observed that lower microbiota diversity may be associated with the development of insulin resistance and an increased risk of DM. This may be related to impaired stability and function of the microbiota. Differential microbiota characteristics associated with insulin resistance were identified. These features may have specific expression or abundance changes in DM patients, and may play an important role in insulin signaling or metabolic pathways. Phenylalanine and tryptophan metabolic pathways have some links with the development of DM. HEXB, ZC3H12A, CCR, CXCR3, GBR10, CDK9, TXN, IGFBP3, PDHA1, and NDUFB3 are closely related to the pathogenesis of T2D and have potential targets. Studies have shown that the interaction between GM and host metabolism has an important impact on the regulation of insulin resistance [21]. The different microbiota may affect insulin sensitivity by regulating intestinal barrier function, inflammatory response, energy metabolism, and other pathways. Cani et al. (2008) [22] used drugs to reduce intestinal endotoxin absorption and successfully prevented the development of PreDM. Changes in intestinal microbiota control metabolic endotoxemia, inflammation, and related diseases by increasing intestinal permeability. This shows that GM

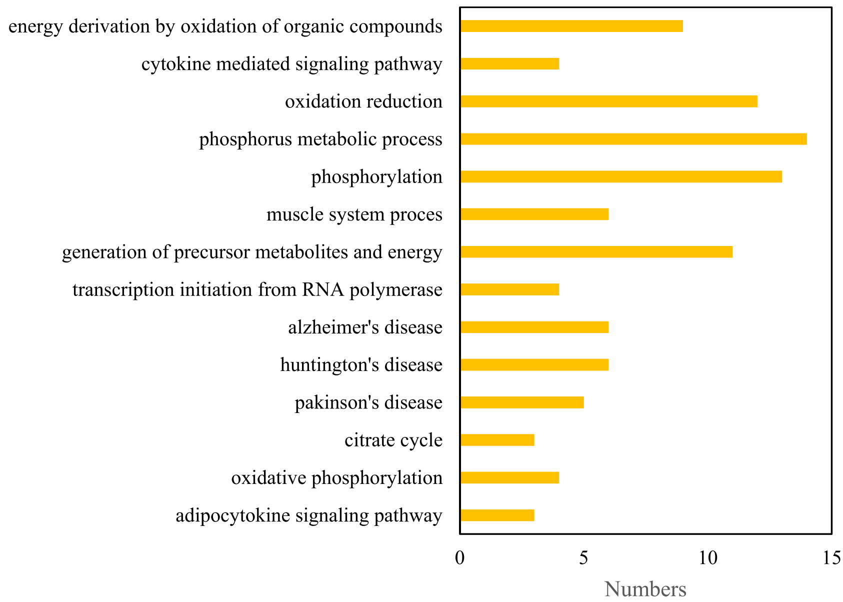

Fig. 8. Results of enrichment pathway analysis.

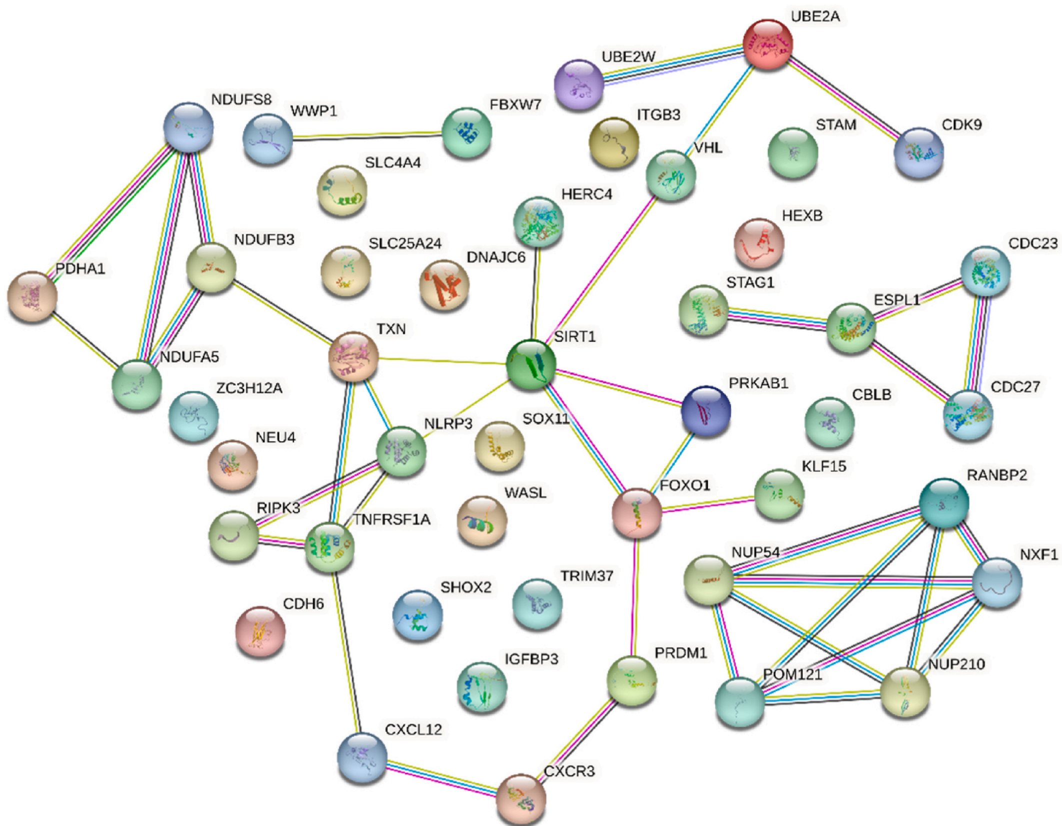

Fig. 9. PPT diagram between related proteins.

can reduce inflammation and improve insulin sensitivity. Cani et al. (2009) [23] showed that selective gut microbiota changes control and increase endogenous glucagon-like peptide-2 (GLP-2) production, therefore improving intestinal barrier function through a GLP-2-dependent mechanism, contributing to the improvement of intestinal barrier function during DM. GM, as a biological marker,

can predict the occurrence of DM and the risk of cardiovascular disease [24]. Many studies have shown that GM can regulate host energy metabolism, regulate the secretion of gut hormones and gut peptides, and participate in the occurrence of DM. Bai et al. (2022) [25] indicated that the changes in the gut microbiota of diabetic retinopathy patients are associated with the depletion of Firmicutes, Bacteroidetes, Synergistetes, and Desulfovibrionaceae. The alterations in the composition and function of the gut microbiota in diabetic patients suggest that it can serve as a non-invasive biomarker to improve clinical diagnostic methods and identify potential therapeutic targets for diabetic retinopathy (DR). Zhang et al. (2022) [26] observed that gut microbiota dysbiosis in type 2 diabetes is characterized by decreased diversity, increased abundance of Fusobacteria, and decreased abundance of Firmicutes. Lv et al. (2023) [27] emphasized that understanding the characteristic bacterial taxa of the gut microbiota in T2DM patients is beneficial for the prevention, diagnosis, and treatment of endocrine disorders. There is a certain correlation between GM and insulin resistance in DM patients. These consistent findings help to deepen the understanding of the pathogenesis of DM and provide new targets and strategies for future clinical intervention and treatment. In the future, long-term follow-up studies can be conducted to observe the dynamic changes of GM in DM patients and its relationship with insulin resistance, to help better understand the stability and plasticity of flora, as well as the causal relationship between GM and insulin resistance. This study explored the relationship between gut microbiota and insulin resistance in diabetic patients. However, there are limitations in this study. The gut microbiota is highly complex, composed of various types of microorganisms. The study may have focused on only a small subset of microbial species, neglecting other potential microorganisms that could influence insulin resistance. Additionally, there may be other confounding factors such as lifestyle, dietary habits, and medication use that could impact the study results, and not fully controlling for these factors could introduce bias. Considering the individual variations in gut microbiota composition and function, personalized treatment strategies may be more effective in addressing the needs of each individual and improving treatment outcomes. Personalized therapeutic approaches can modulate the gut microbiota composition to influence the functionality of the immune system, which holds potential implications for the treatment of autoimmune diseases and enhancing immune function.

5. Conclusion

This article analyzed the potential mechanism of GM and insulin resistance based on bioinformatics. There is dysbiosis of bacterial flora in DM patients. The gut microbiota diversity in diabetic patients was observed to be lower compared to the healthy control group, as indicated by a decrease in the Shannon and Chao1 indices. Differential abundance analysis revealed the presence of various distinct bacterial communities at the genus level between diabetic patients and healthy controls, including some taxonomic groups. Considering the variability in GM composition among individuals, future studies can explore individualized treatment strategies. Through understanding the individual microbiota, as well as its relationship with insulin resistance, it is possible to provide individualized GM regulation programs for patients with DM, improving the therapeutic effect and preventing the development of DM. For example, GM regulation can be combined with drug therapy and dietary intervention to achieve better therapeutic outcome and DM management.

CRediT authorship contribution statement

Qian Guo: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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