RESEARCH ARTICLE

Exploration of susceptibility genes in type 2 diabetes mellitus among Chinese Hui and Han people

Jianhua Kang, Xingya Shen, Shaohua Feng, Delai Li, Haisheng Yuan, Na Li, Hexi Wei, Lishun Yang

Abstract

Objective: : To identify the differentially expressed genes among Chinese Hui and Han individuals to identify potential susceptibility genes for type 2 diabetes mellitus.

Method: The case-control study was conducted at Tianjin Beichen District Chinese Medicine Hospital, China, from January to September 2021, and comprised Hui patients with diabetes in group A1 and Han patients with diabetes in group B1. Hui healthy, non-diabetic patients were enrolled in group C1, and healthy, non-diabetic Han patients were enrolled in group D1. Gene expression in whole blood samples was analysed using microarray technology. Data was analysed to identify differentially expressed genes, followed by Gene Ontology terms and Kyoto Encyclopaedia of Genes and Genomes pathway-enrichment analyses. Additionally, a Protein-Protein Interaction network analysis was performed based on the identified differentially expressed genes. Finally, quantitative reverse transcription-polymerase chain reaction was employed to validate the selected susceptibility genes.

Results: Of the 51 subjects, 11(21.7%) were in group A1, 14(27.4%) in group B1, 14(27.4%) in group C1, and 12(23.5%) in group D1. On comparison of A1 vs. B1, A1 vs. C1, B1 vs. D1, and C1 vs. D1, 953, 289, 598 and 382 differentially expressed genes were identified, respectively. Additionally, 57, 52, 55 and 54 Gene Ontology terms, as well as 192, 103, 143 and 112 Kyoto Encyclopaedia of Genes and Genomes pathways were identified for the overlapping differentially expressed genes that were present in at least one pair of the 4 sets. The quantitative reverse transcription-polymerase chain reaction results for susceptibility genes were consistent with the findings of bioinformatics analysis.

Conclusion: Insulin-like Growth Factor 1 Receptor, chondrodysplasia punctata-diacylglycerol synthase 1, Laminin Subunit Alpha 4, Sialic Acid-Binding Immunoglobulin-like Lectin 14, Chromosome 18 Open Reading Frame 61 and Ribosomal Protein S26 were identified as susceptibility genes for type 2 diabetes mellitus in different study populations.

Keywords: Diabetes mellitus, Type 2, Differentially expressed genes, Ethnic groups, Microarray analysis, Disease susceptibility. (JPMA 75: 704; 2025) **DOI: https://doi.org/10.47391/JPMA.10365**

Introduction

Insulin resistance (IR) and dysfunction for pancreatic beta (β) cell may be the direct cause of type 2 diabetes mellitus (T2DM). It primarily affects adults, and up to 95% of diabetes patients with T2DM.[1-3] T2DM has a clearly established polygenic genetic basis.[4] Therefore, research on related marker genes and susceptibility genes holds significant importance in the detection and prediction of the disease, and has seen substantial development.

However, as of now, the relationship between gene expression and T2DM, as well as the mechanism of gene interaction, remains not fully understood.[5] In other words, the causes of T2DM are still blurry. Additionally, despite recent advancements in the molecular genetics of T2DM, there are different susceptibility gene profiles for T2DM among various ethnic groups residing in different regions. These differences are attributed to diverse genetic backgrounds, living environments, and behavioural patterns.[6] The association of genetic polymorphisms with different ethnic types of T2DM necessitates a more extensive statistical analysis, and many less-studied susceptibility genes in humans are yet to be identified. As the second most populous ethnic minority in China, some scholars have sequenced the whole genome of Hui nationality in Ningxia, and discovered the genetic distinction between the Hui and Han ethnicities (Fixation index [FST]=0.0015). These differences include highly differentiated functional variations in genes related to lipid metabolism between the two ethnic groups.[7]

The current study was planned to identify differentially expressed genes (DEGs) in T2DM patients from various populations and their respective normal controls using microarray technology.

Materials and Methods

The case-control study was conducted at Tianjin Beichen District Chinese Medicine Hospital, China, from January to

Tianjin Beichen District Chinese Medicine Hospital, China.
Correspondence: Lishun Yang. e-mail: yanglishun@163.com
ORCID ID: 0000-0002-7313-9308

Submission complete: 26-09-2023 **1st Revision received:** 27-02-2024
Acceptance: 29-01-2025 **Last Revision received:** 28-01-2025

September 2021. The sample size was calculated according to the World Health Organisation (WHO) calculator (https://cdn.who.int/media/docs/default-source/ncds/ncd-surveillance/steps/sample-size-calculator.xls) with 95% confidence interval (CI), T2DM population proportion 10% of Chinese and margin of error 0.1.[8] The formula used was based on an earlier case-control study:[9]

$$n = \frac{(z_{\alpha/2}\sqrt{2pq} + z_{\beta}\sqrt{p_0q_0 + p_1q_1})^2}{(p_1 - p_0)^2}$$

We set the p_0=0.1, p_0=1-p_0, OR=3.0, p_1=(p_0*OR)/ (1-p_0+p_0*OR), q_1=1-p_1, p=(p_0+p_1)/2, q=1-p, $z_{0.05/2}$=1.96, z_{β}=0.1, respectively.

The sample was raised using simple random sampling technique. Those included were Hui patients with T2DM in group A1 and Han patients with T2DM in group B1. Hui healthy, non-diabetic controls were enrolled in group C1, and healthy, non-diabetic Han controls were enrolled in group D1. Hui and Han patients with T2DM had fasting blood sugar (FBS) levels ≥7.0mmol/L (126mg/dl), or random blood sugar (RBS) levels ≥11.1mmol/L (200mg/dl) 2 hours after a glucose tolerance test (GTT). Individuals were excluded across the board if they had mental disorders, autoimmune diseases and malignant tumours.

After taking informed consent from the subjects, peripheral blood samples were collected. Total ribonucleic acid (RNA) was extracted from the samples using the BioTeck Rapid Extraction Kit (Sangon Biotech Co., China). The purity and concentration of RNA were detected using a spectrophotometer (Nanodrop ND-1000) (Thermo Fisher Scientific Inc, US). RNA integrity was assessed using 1% formaldehyde denaturing gel electrophoresis. The samples with RNA integrity ≥6 were extracted, and subjected to further analysis.

Total RNA and T7 Oligo (dT)(Sangon Biotech Co., China) primer were utilised to synthesise the first-strand complementary deoxyribonucleic acid (cDNA) using the First Strand Enzyme Mix(Sangon Biotech Co., China). The RNA strand in the DNA-RNA hybrid was then converted into second-strand cDNA using the Second Strand Enzyme Mix(Sangon Biotech Co., China), resulting in the production of double-stranded DNA (dsDNA). After dsDNA synthesis was complete, the products were purified using the polymerase chain reaction (PCR) NucleoSpin Extract II Kit(Sangon Biotech Co., China), and was subjected to reaction at 37°C for 14 hours. It was purified, reverse-transcripted, and labelled with cyanine-3 deoxycytidine triphosphate (Cy3-dCTP). Hybridisation was done overnight, and it was then washed out with 0.2% Sodium Dodecyl Sulfate (SDS) and Standard Saline Citrate (SCC) for 5 minutes.

Following the hybridisation, the chip was removed and subjected to a series of washes. Initially, it was washed in a solution of 0.2% SDS and 2× SSC (lotion I) at 42°C for 5 minutes, followed by a subsequent wash in room temperature 0.2× SSC (lotion II) for 5 minutes. It was then allowed to dry. Subsequently, the cleaned chip was scanned using an Agilent chip scanner (G2565CA) (Sangon Biotech Co., China) to produce a hybrid image.

Normalisation and quality control were ensured using GeneSpring software V13(https://genespring-support.com/genespring-13-new). Log2-transformed and median-centred genes were subjected to CLUSTER 3.0 software (https://www.encodeproject.org/software/cluster). Following this, the DEGs for the comparisons of group A1 vs. group B1, group A1 vs. group C1, group B1 vs. group D1, and group C1 vs. group D1 were identified using limma V3.32.2 (p<0.05 and |log2 (fold change)| ≥1) (https://www.bioconductor.org/packages/release/bioc/html/limma.html).

Gene Ontology (GO) terms and Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways enrichment analyses were performed using DAVID (version 6.7) (http://david.abcc.ncifcrf.gov/) and KEGG PATHWAY (http://www.genome.jp/kegg), with p<0.05 being the threshold value.

The protein-protein pairs of DEGs were screened out using STRING (Version 10.5) (https://string-db.org/), with the cut-off value being a confidence score >500. The Protein-Protein Interaction (PPI) network was further constructed and visualised using Cytoscape (Version 3.5.1) (http://www.cytoscape.org/download.php). The hub genes were identified using cytoHubba (Version 3.0) (https://apps.cytoscape.org/apps/cytohubba).

Another set of subjects were enrolled in A2, B2, C2 and D2 groups with similar characteristics and recruitment criteria as for groups A1, B1, C1 and d1. Peripheral whole blood samples were collected from these subjects, and total RNA was extracted. Quantitative reverse transcription-PCR (qRT-PCR) was performed to measure the relative expression level of the selected susceptibility genes in the following comparisons: group A2 vs. group B2, group A2 vs. group C2, group B2 vs. group D2, and group C2 vs. group D2. This analysis was conducted using SYBR® Premix Ex Taq™ Kit(Sangon Biotech Co., China) following the manufacturer's instructions. All the primers (Takara Biomedical Technology (Beijing) Co., China.) were noted (Table-1), and β-actin served as the inner control.

Table-1: The primer sequences.

Gene	Primer
LAMA4-F	5'-GGAAAATAAGCGAGGCACCG-3'
LAMA4-R	5'-AGCCACAGAGGCAGAACCGA-3'
IGF1R-F	5'-TCGACATCCGCAACGACTATC-3'
IGF1R-R	5'-CCAGGGCGTAGTTGTAGAAGAG-3'
RPS26-F	5'-CTGAAGCGAGCGTCTTCGAT-3'
RPS26-R	5'-TGAATTGCACAACTCACACAGTA-3'
CDS1-F	5'-GCTTCAGACTTACTCACTTCCAC-3'
CDS1-R	5'-GCAAAGGTTGACAGTGCAATG-3'
SIGLEC14-F	5'-GATCCTGAGCAATGGCATGTC-3'
SIGLEC14-R	5'-TCTGGGAAGGATTGAGGGCT-3'
C18orf61-F	5'-AGGCCTCCTTGTCTCAGTTCC-3'
C18orf61-R	5'-GATCACAGCATGCGCCAA-3'
β-actin-F	5'-CTGGACTTCGAGCAAGAGAT-3'
β-actin-R	5'-GATGTCCACGTCACACTTCA-3'

IGF1R: Insulin-like Growth Factor 1 Receptor, CDS1: Cytidine diphosphate diacylglycerol synthase 1, LAMA4: Laminin Subunit Alpha 4, SIGLEC14: Sialic Acid-Binding Immunoglobulin-like Lectin 14, C18orf61: Chromosome 18 Open Reading Frame 61, RPS26: Ribosomal Protein S26, β: Beta.

Results

Of the 51 subjects, 11(21.7%) were in group A1, 14(27.4%) in group B1, 14(27.4%) in group C1, and 12(23.5%) in group D1. The four sets of DEGs were labelled DEG-1, DEG-2, DEG-3 and DEG-4, respectively. On comparison of A1 vs. B1, A1 vs. C1, B1 vs. D1, and C1 vs. D1, 953, 289, 598 and 382 DEGs were identified, respectively. The top 20 DEGs were noted along with their value of significance (Table-2).

A total of 57, 52, 55, and 54 enriched GO terms and 192, 103, 143, and 112 KEGG pathways were identified for the four sets of DEGs, respectively (Figure-1A-D), The top 30 enriched KEGG pathways for the four sets of DEGs were noted with their value of significance (Figure-1E-H).

Table-2: The top 20 most significant differentially expressed genes (DEGs) in 4 sets of DEGs.

DEG-1		DEG-2		DEG-3		DEG-4	
Gene	***p*-value**	**Gene**	***p*-value**	**Gene**	***p*-value**	**Gene**	***p*-value**
CNTNAP3P2	3.149E-6	ATXN3	9.459E-6	FM01	3.494E-6	**SIGLEC14**	8.402E-7
FSHR	6.876E-6	lnc-LIPG-2	1.135E-5	**C18orf61**	6.128E-6	**RPS26**	7.143E-6
MRAP2	1.334E-5	LOC93432	6.037E-5	VPREB1	1.574E-5	lnc-NNT-1	5.209E-5
lnc-KIAA1462-2	1.614E-5	**LOC100129203**	1.005E-4	LM07	6.796E-5	C8orf89	1.553E-4
MAP7D2	2.606E-5	**lnc-RP11-1220K2.2.1-1**	1.566E-4	**DENND1A**	2.044E-4	SPAG6	1.553E-4
lnc-WFDC8-1	3.598E-5	A0X1	1.771E-4	EFCAB1	2.240E-4	lnc-UTRN-2	2.395E-4
lnc-SLC15A4-12	4.4829E-5	**HCG4**	2.313E-4	**CCDC144A**	2.308E-4	**ADARB2**	6.044E-4
PTPLA	6.952E-5	LOC101927894	2.542E-4	**ANKRD36**	2.909E-4	USP17L7	6.873E-4
lnc-FTSJD2-1	7.511E-5	lnc-DLEU1-1	2.720E-4	**RAMP1**	2.970E-4	LOC101928669	7.208E-4
PCBP3-0T1	9.786E-5	**LOC101927354**	3.300E-4	LEP	3.073E-4	DENND1A	1.094E-3
lnc-RP5-1047A19.4.1-1	1.217E-4	MEI0B	3.575E-4	**lnc-SPSB4-1**	3.339E-4	**CPED1**	1.198E-3
lnc-CTIF-2	1.273E-4	**LOC100129203**	5.715E-4	LOC100506351	3.583E-4	ENH0	1.518E-3
CES1	1.395E-4	**lnc-PDSS2-2**	7.522E-4	**ZFX**	3.740E-4	XLOC_I2_000706	1.675E-3
lnc-GRSF1-1	1.655E-4	**PIGC**	7.546E-4	DPY19L2	4.072E-4	lnc-GRHL2-2	1.960E-3
WAC-AS1	1.855E-4	ABI3BP	8.096E-4	XLOC_I2_012847	4.347E-4	OR5M9	1.995E-3
LCORL	1.944E-4	lnc-C11orf41-1	9.004E-4	**LEPR0T**	4.841E-4	lnc-MORF4L1-2	2.067E-3
ZFX	1.995E-4	FAR2P1	9.523E-4	**MAP3K4**	5.355E-4	MID1IP1-AS1	2.228E-3
C4orf36	2.013E-4	**HIST1H4K**	9.656E-4	**RAPGEF6**	5.483E-4	TTC39C-AS1	2.751E-3
TMEM176A	2.476E-4	**FAM19A1**	9.857E-4	KIF14	5.532E-4	EMP1	2.809E-3
A2MP1	2.695E-4	lnc-DCTN5-2	0.001	TMEM150B	5.998E-4	lnc-PRKACB-4	3.409E-3

Bold font represents overlapping that were found in at least two groups of DEGs. CNTNAP3P2: Contactin Associated Protein Like 3 Pseudogene 2; ATXN3: Ataxin 3; FSHR: Follicle-Stimulating Hormone Receptor; lnc-LIPG-2: Long Non-Coding RNA Associated with Lipase G, Endothelial Type, Transcript 2; E: exponent

Table-3: The top 5 nodes with the highest degree the in Protein-Protein Interaction (PPI) network.

Set	Gene	Degree
DEGs-1	IGF1R	4
	CDS1	2
	PLD1	2
	DGKE	2
	CSF1	1
DEGs-2	EGF	1
	FGFR2	1
DEGs-3	KITLG	1
	PLD1	1
	LEP	1
	F2R	1
	PLA2G4C	1
DEGs-4	LAMA4	2
	ITGB1	2
	VCAM1	1
	BMP6	1
	NRXN1	1

DEG: Differentially expressed genes. IGF1R: Insuline-like Growth Factor 1 Receptor; CDS1: Cytidine diphosphate diacylglycerol synthase 1; PLD1: Phospholipase D1; DGKE: Diacylglycerol Kinase E; CSF1: Colony Stimulating Factor 1 (Macrophage-CSF); EGF: Epidermal Growth Factor; FGFR2: Fibroblast Growth Factor Receptor 2; KITLG: KIT Ligand; LEP: Leptin; F2R: Coagulation Factor II Receptor (Thrombin Receptor); PLA2G4C: Phospholipase A2 Group IVA (Cytosolic, Calcium-Dependent); LAMA4: Laminin Subunit Alpha 4; ITGB1: Integrin Subunit Beta 1; VCAM1: Vascular Cell Adhesion Molecule 1; BMP6 – Bone Morphogenetic Protein 6; NRXN1: Neurexin 1.

A total of 9, 1, 4, and 4 protein -protein pairs were selected from the four sets of DEGs, and the corresponding PPI networks were noted (Figure-2). Also, the top 5 nodes with the highest degree in the PPI network were noted separately (Table-3), among which Insulin-like Growth Factor 1 Receptor (IGF1R), Cytidine diphosphate diacylglycerol synthase 1 (CDS1) and Laminin Subunit Alpha 4 (LAMA4) had higher degrees in the PPI network of DEGs-1 and DEGs-4. Chromosome 18 Open Reading Frame 61 (C18orf61), Sialic Acid-Binding Ig-like Lectin 14 (SIGLEC14) and Ribosomal Protein S26

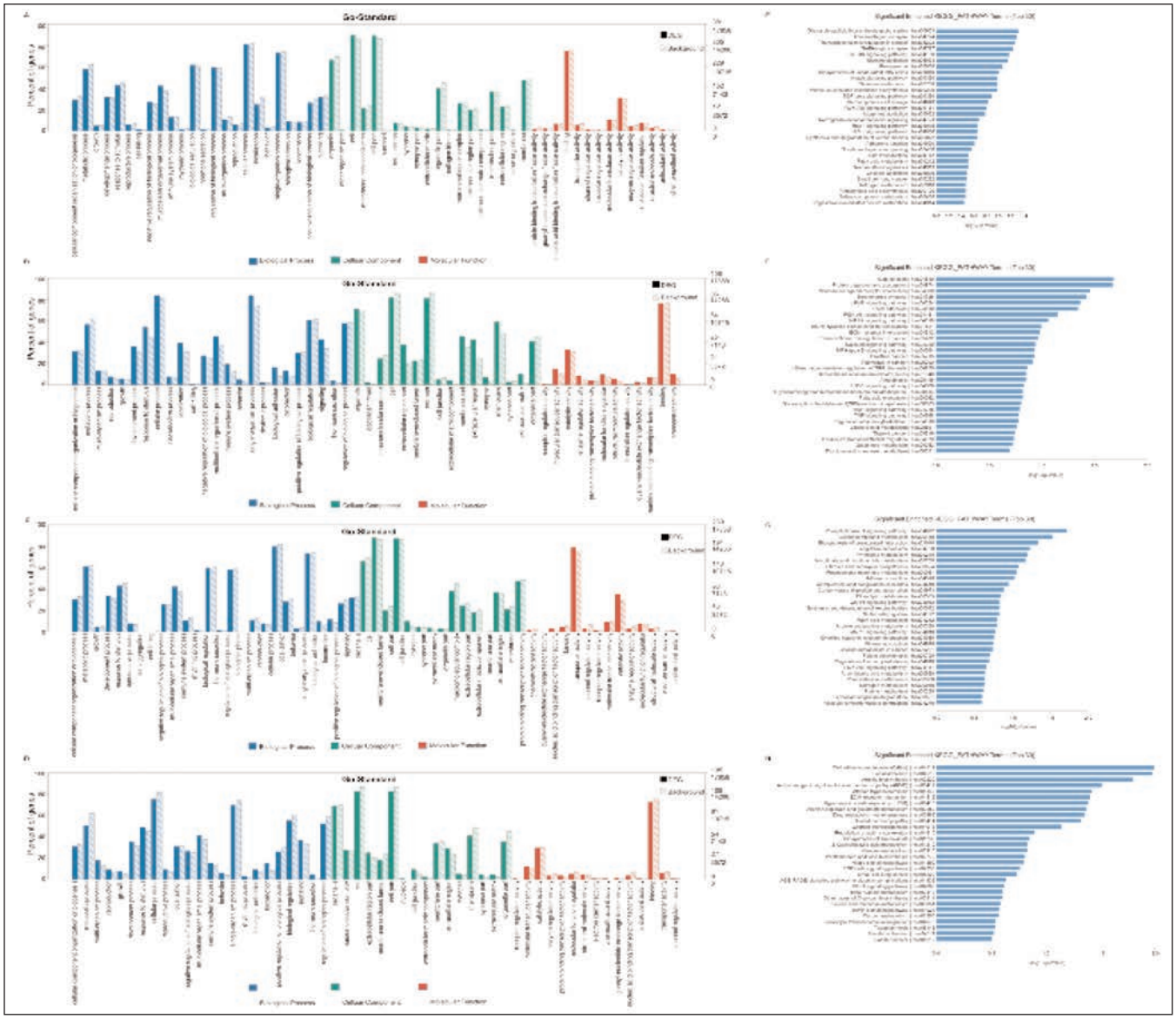

Figure-1: Gene Ontology (GO) terms for differentially expressed genes (DEGs) 1 (A), DEGs-2 (B), DEGs-3 (C) and DEGs-4 (D), and the top 30 enriched Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways for DEGs-1 (E), DEGs-2 (F), DEGs-3 (G) and DEGs-4 (H), according to respective *p*-values.

(RPS26) overlapped in more sets of DEGs with lower values of significance (Table 2), establishing IGF1R, CDS1, LAMA4, C18orf61, SIGLEC14 and RPS26 as susceptibility genes for T2DM in the context of comparisons between Chinese Hui and Han ethnicities.

The qRT-PCR results for susceptibility genes (Figure 3) were consistent with the findings of bioinformatics analysis.

Discussion

Over the last few decades, considerable efforts have been made worldwide to study the potential genetic determinants of T2DM. Several major genes associated with T2DM, including insulin, insulin receptor, glucokinase genes, mitochondrial DNA mutations, as well as the gene encoding hepatocyte nuclear factor (hnf1-α, hnf4-α, hnf1-β) and insulin promoter factor 1 (ipf1), have been identified in rare families. In recent years, with the development and improvement of whole-genome association studies and single nucleotide polymorphism (SNP) analysis techniques, hundreds of common gene mutations have been reported to be closely related to T2DM.[10]

Currently, there are many gene diagnosis techniques for mutations in T2DM. However, due to the complexity of the onset of T2DM and the diversity of influencing factors, the existing genetic diagnosis techniques are not accurate, and are narrowly applicable. Furthermore, the adverse reactions

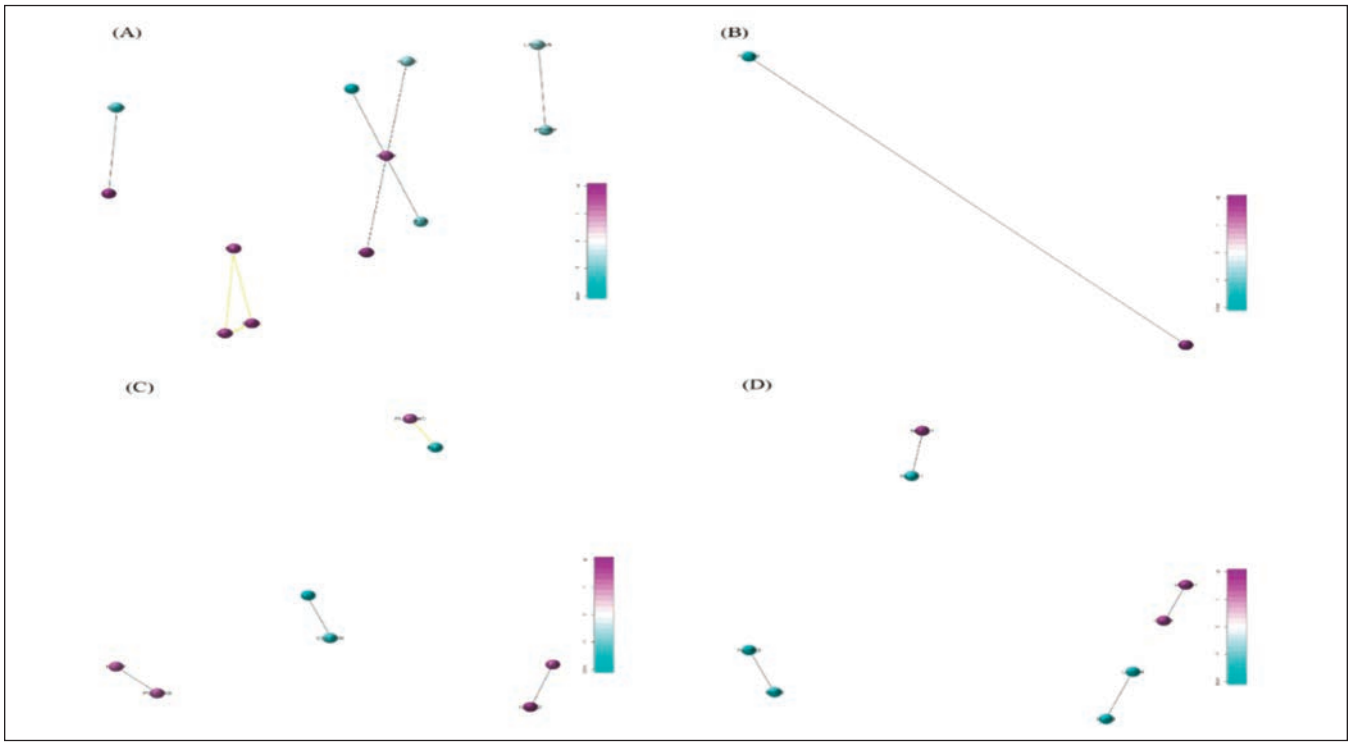

Figure-2: Protein-Protein Interaction (PPI) network for differentially expressed genes (DEGs) 1 (A), DEGs-2 (B), DEGs-3 (C) and DEGs-4 (D).

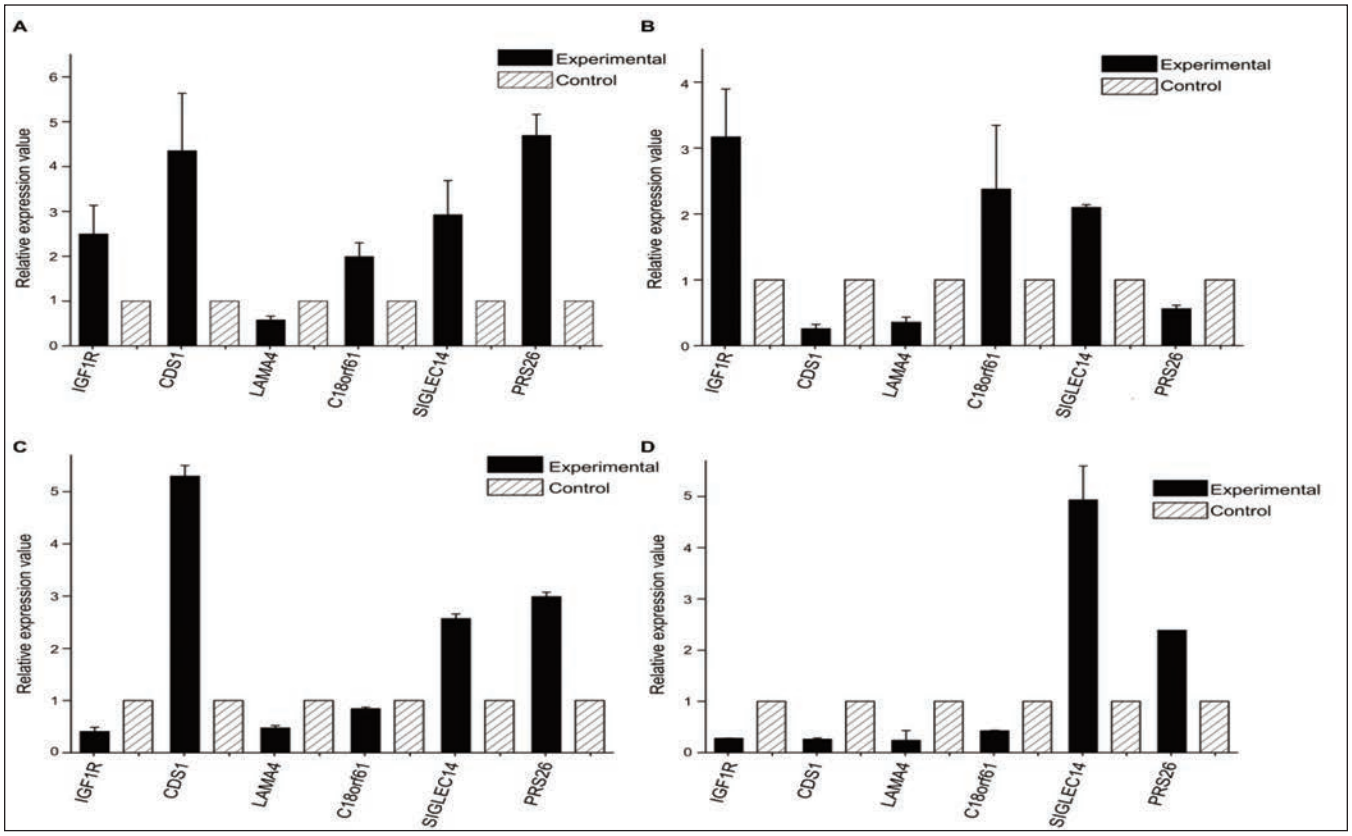

Figure-3: Relative expression values of Insulin-like Growth Factor 1 Receptor (IGF1R), Cytidine diphosphate diacylglycerol synthase 1 (CDS1), Laminin Subunit Alpha 4 (LAMA4), Chromosome 18 Open Reading Frame 61 (C18orf61), Sialic Acid-Binding Immunoglobulin-like Lectin 14 (SIGLEC14) and Ribosomal Protein S26 (RPS26) in group A2 vs. group B2 (A), group A2 vs. group C2 (B), group B2 vs. group D2 (C) and group C2 vs. group D2 (D).

of existing targetted drugs are significant, and their efficacy needs to be improved. Although Whole Genome Sequencing (WGS) is the most direct and comprehensive strategy for genome analysis, large-scale WGS research still requires significant funding in many laboratories and clinical environments.[11] DNA microarray, also known as gene chip, consists of microarrays containing tens of millions of nucleic acid molecules immobilised in a small area on a solid support as per a predetermined position. Under specific conditions, the nucleic acid molecules on the carrier can hybridise with complementary nucleic acid fragments from the sample sequence. As the DNA analysis technology developed in the post-genomic era, microarray analysis could investigate the expression of thousands of genes in various physiological and pathological states.[12] It enables large-scale, high-throughput, and parallel detection, facilitating the screening of specific expression genes, specific activation pathways, and the prediction of regulatory mechanisms at the level of the human genome.[12] This makes microarray analysis unique in the prediction of biological characteristics and molecular typing of diseases.

In the current study, 6 T2DM susceptibility genes were identified using microarray technology, namely IGF1R, CDS1, LAMA4, SIGLEC14, C18orf61 and RPS26. Besides, qRT-PCR was employed to validate the candidate susceptibility genes.

Differential expression analysis showed that 3 genes, namely SIGLEC14, RPS26 and C18orf61, were significantly differentially expressed between Chinese Hui and Han people. SIGLEC14 is a member of the sialic-acid binding lectin family that regulates immune responses to pathogens through the activating domain.[13] Yamanaka et al.[14] discovered that the expression of SIGLEC14 on a monocytic cell line could enhance lipopolysaccharide-elicited tumour necrosis factor-alpha (TNF-α) secretion. TNF-α, encoded by the TNF-α gene, is thought to regulate the transcriptional level of TNF and is associated with susceptibility to IR, chronic hepatitis B, autoimmune diseases, cancer and other diseases.[15-17] Therefore, SIGLEC14 may be linked to susceptibility to T2DM.

The RPS26 gene is a component of the ribosomal small subunit 40S and belongs to the ribosomal protein gene (RPG) family. Recent studies have shown that the abnormality of RPG is related to the occurrence and development of tumours.[18] Studies have indicated that the expression of RPS26 is significantly down-regulated in human nasal type natural kill cell and T cell lymphoma, while it is up-regulated in varying degrees in other non-Hodgkin lymphoma.[19] Vincent et al.[20] identified RPS26 as the most likely susceptibility gene for type 1 diabetes (T1D) in this genomic region. Additionally, a study found that the rs1131017 locus in the 5' untranslated region of RPS26 is highly correlated with susceptibility to T1D.[21] However, to our knowledge, no studies have yet been conducted on the susceptibility of RPS26 to T2DM.

C18orf61 is uncharacterised Locus 497259 (LOC497259) in Homo sapiens and is a type of long non-coding RNA. Its role in T2DM and other diseases has not been reported to date. Therefore, exploring its significance in susceptibility to T2DM will be one of the key points of future research.

The most enriched GO terms for the DEGs were related to cellular component organisation or biogenesis, organelle, and metabolic process, suggesting a potential connection between these terms and the mechanism. Cellular component organisation, or biogenesis, encompasses processes that lead to the creation of macromolecules, the assembly of constituent parts, and the arrangement or disassembly of cellular components.[22] Many of these processes are likely to play important roles in insulin production. Apart from supplying energy for cells, mitochondria also play roles in processes like cell differentiation, cell signalling, and apoptosis, and they can influence cell growth and cell cycle.[23] There is substantial evidence suggesting that mitochondrial dysfunction is responsible for causing T2DM.[24] It is clear that mitochondria play a vital role in adenosine triphosphate (ATP) production in cells, and are involved in glucose metabolism and insulin secretion.[25] Metabolic processes encompass chemical reactions and pathways, including anabolism and catabolism. Metabolic syndrome (MS) is a combination of metabolically related risk factors often associated with obesity, hyperglycaemia, dyslipidaemia and hypertension. These factors directly contribute to the development of atherosclerotic cardiovascular diseases.[26] Isomaa et al. found that the prevalence of MS for men and women was as high as 78% and 84% in the T2DM population, and it could cause a series of chronic complications.[27]

In the PPI network, the top 3 nodes were IGF1R, CDS1 and LAMA4. IGF1R is the receptor of IGF1, and the IGF1 pathway is associated with a variety of diseases, including diabetes, cancer and ageing.[28] Currently, the expression of IGF1R has been demonstrated to impact the proliferation of various cancers, such as lung cancer,[29] gastric cancer,[30] prostate cancer[31] and breast cancer.[32] CDS1, also recognised as checkpoint kinase 2 (CHK2), is linked to a heightened risk of autosomal dominant breast, colon, thyroid and prostate cancers.[33,34] CDS1 encodes a cell cycle regulator and putative tumour suppressor. When activated in response to DNA damage, it inhibits cell division cycle 25C (CDC25C) phosphatase, thereby preventing entry into mitosis.

Additionally, it stabilises the tumour suppressor protein p53 and interacts with phosphorylated breast cancer gene 1(BRCA1), enabling it to restore cell survival following DNA damage. CDS1 has been revealed as a multi-organ cancer susceptibility gene.[35] LAMA4 is a protein coding gene that produces alpha-laminin 4, a variant A chain of laminin. Laminin is a multidomain glycoprotein that is a primary component of basement membranes in the heart and blood vessels.[36] Diseases associated with LAMA4 include dilated cardiomyopathy and familial isolated dilated cardiomyopathy.[37] Its related pathways include beta-adrenergic signalling and the blood-brain barrier pathway.

In the current study, IGF1R, CDS1 and LAMA4 exhibited a higher level of connectivity in the PPI network, indicating their potential role in regulating the development of T2DM.

The current study has limitations, because, owing to financial constraints, it suffered from a lack of transgenic mouse and cell line experiments. Also, the sample size was small, and larger samples of T2DM patients are needed to validate the findings.

Conclusion

IGF1R, CDS1, LAMA4, SIGLEC14, C18orf61 and RPS26 were identified as potential susceptibility genes for T2DM, and their expression levels were validated using qRT-PCR. Additional research is necessary to validate the findings.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Besides, studies concerning human research participants have been approved by Ethics Committee of Tianjin Beichen District Chinese Medicine Hospital (No. 2016-SHGY-13).

Informed consent: Informed consent was obtained from all individuals included in this study.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: The Tianjin Beichen District Science and Technology Development Plan Project(No. 2016-SHGY-13); Tianjin Health Science and Technology Project (No. TJWJ2024qn107)

References

1. Richardson A, Park WG. Acute pancreatitis and diabetes mellitus: a review. Korean J Intern Med 2021;36:15-24. doi: 10.3904/kjim.2020.505.
2. Raman PG. Environmental Factors in Causation of Diabetes Mellitus. In: Larramendy ML, Soloneski S, eds. Environmental Health Risk - Hazardous Factors to Living Species. London, UK: IntechOpen Limited; 2016. Doi: 10.5772/62543.
3. Faselis C, Katsimardou A, Imprialos K, Deligkaris P, Kallistratos M, Dimitriadis K. Microvascular Complications of Type 2 Diabetes Mellitus. Curr Vasc Pharmacol 2020;18:117-24. doi: 10.2174/1570161117666190502103733.
4. Zulfiqar S, Hussain F, Jamil A, Ahmed N. Association of RAGE gene polymorphism with Type-2 diabetes mellitus in local population. Pak J Med Sci 2018;34:226-9. doi: 10.12669/pjms.341.14359.
5. Shi-huan X, Wei Y, Song J, Yuming C, Xiaoli L, Jun W. Progress in researches on mechanism of therapeutic effect of vitamin D on type 2 diabetes. Chin J Public Health 2016;32:1267-70. doi: 10.11847/zgggws2016-32-09-36.
6. Fang-lin Y, Ying Y, Yan-sheng Y. Progress in research of single nucleotide polymorphism of susceptibility genes of type 2 diabetes. Chin J Dis Control Prev 2016;20:1095-100. doi: 10.16462/j.cnki.zhjbkz.2016.11.005.
7. Ma X, Yang W, Gao Y, Pan Y, Lu Y, Chen H, et al. Genetic Origins and Sex-Biased Admixture of the Huis. Mol Biol Evol 2021;38:3804-19. doi: 10.1093/molbev/msab158.
8. Althubaiti A. Sample size determination: A practical guide for health researchers. J Gen Fam Med 2022;24:72-8. doi: 10.1002/jgf2.600.
9. Wang X, Ji X. Sample Size Estimation in Clinical Research: From Randomized Controlled Trials to Observational Studies. Chest 2020;158 (Suppl 1):s12-20. doi: 10.1016/j.chest.2020.03.010.
10. Cirillo E, Kutmon M, Gonzalez Hernandez M, Hooimeijer T, Adriaens ME, Eijssen LMT, et al. From SNPs to pathways: Biological interpretation of type 2 diabetes (T2DM) genome wide association study (GWAS) results. PLoS One 2018;13:e0193515. doi: 10.1371/journal.pone.0193515.
11. Bagger FO, Borgwardt L, Jespersen AS, Hansen AR, Bertelsen B, Kodama M, et al. Whole genome sequencing in clinical practice. BMC Med Genomics 2024;17:39. doi: 10.1186/s12920-024-01795-w.
12. Harrington CA, Rosenow C, Retief J. Monitoring gene expression using DNA microarrays. Curr Opin Microbiol 2000;3:285-91. doi: 10.1016/s1369-5274(00)00091-6.
13. Graustein AD, Horne DJ, Fong JJ, Schwarz F, Mefford HC, Peterson GJ, et al. The SIGLEC14 null allele is associated with Mycobacterium tuberculosis- and BCG-induced clinical and immunologic outcomes. Tuberculosis (Edinb) 2017;104:38-45. doi: 10.1016/j.tube.2017.02.005.
14. Yamanaka M, Kato Y, Angata T, Narimatsu H. Deletion polymorphism of SIGLEC14 and its functional implications. Glycobiology 2009;19:841-6. doi: 10.1093/glycob/cwp052.
15. Patel R, Palit SP, Rathwa N, Ramachandran AV, Begum R. Genetic variants of tumor necrosis factor-α and its levels: A correlation with dyslipidemia and type 2 diabetes susceptibility. Clin Nutr 2019;38:1414-22. doi: 10.1016/j.clnu.2018.06.962.
16. Zhang Q, Cui F, Fang L, Hong J, Zheng B, Zhang JZ. TNF-α impairs differentiation and function of TGF-β-induced Treg cells in autoimmune diseases through Akt and Smad3 signaling pathway. J Mol Cell Biol 2013;5:85-98. doi: 10.1093/jmcb/mjs063.
17. Yang L, Wang Y, Shi S, Xie L, Liu T, Wang Y, et al. The TNF-α-induced expression of miR-130b protects cervical cancer cells from the cytotoxicity of TNF-α. FEBS Open Bio 2018;8:614-27. doi: 10.1002/2211-5463.12395
18. Ji X, Liang X, Ma J. Correlation between ribosomal protein gene S26 and endocrine hormone levels in patients with polycystic ovarian syndrome. [Online] 2018 [Cited 2024 September 08]. Available from URL: https://kns.cnki.net/kcms2/article/abstract?v=sSXGFc3NEDJHgfhvK4J5LMclZkmqRl24zcMl2tk8i2UBL-LK9UP8cfhpOvBOKpdh09Exdpj_k3zJtk5Li5HDuEBYzwz_8IhP6qzErRBgOJ8laplB

wpmPUMdqvSUV9kbD16G8sUV18oU=&uniplatform=NZKPT&language=CHS.

19. Pongpruttipan T, Kummalue T, Bedavanija A, Khuhapinant A, Ohshima K, Arakawa F, et al. Aberrant antigenic expression in extranodal NK/T-cell lymphoma: a multi-parameter study from Thailand. Diagn Pathol 2011;6:79. doi: 10.1186/1746-1596-6-79.
20. Plagnol V, Smyth DJ, Todd JA, Clayton DG. Statistical independence of the colocalized association signals for type 1 diabetes and RPS26 gene expression on chromosome 12q13. Biostatistics 2009;10:327-34. doi: 10.1093/biostatistics/kxn039.
21. Kasela S, Kisand K, Tserel L, Kaleviste E, Remm A, Fischer K, et al. Pathogenic implications for autoimmune mechanisms derived by comparative eQTL analysis of CD4+ versus CD8+ T cells. PLoS Genet 2017;13:e1006643. doi: 10.1371/journal.pgen.1006643.
22. Neal S, Ohtake F, Cuervo AM, Hegde RS, Jakob U, Lazarou M, et al. Quality Control: Maintaining molecular order and preventing cellular chaos. Mol Cell 2022;82:1390-7. doi: 10.1016/j.molcel.2022.04.002
23. Maeda Y, Chida J. Control of cell differentiation by mitochondria, typically evidenced in dictyostelium development. Biomolecules 2013;3:943-66. doi: 10.3390/biom3040943.
24. Pinti MV, Fink GK, Hathaway QA, Durr AJ, Kunovac A, Hollander JM. Mitochondrial dysfunction in type 2 diabetes mellitus: an organ-based analysis. Am J Physiol Endocrinol Metab 2019;316:E268-85. doi: 10.1152/ajpendo.00314.2018.
25. Sangwung P, Petersen KF, Shulman GI, Knowles JW. Mitochondrial Dysfunction, Insulin Resistance, and Potential Genetic Implications. Endocrinology 2020;161:bqaa017. doi: 10.1210/endocr/bqaa017.
26. Lin H, Song Q, Zhang M, Xiao W, Zhao M, Cui Y. Metabolically healthy obesity increase risk for hypertension, T2DM and the metabolic syndrome: A community-based cohort study. Guangxi Yi Ke Da Xue Xue Bao 2018;35:794-9. doi: 10.16190/j.cnki.45-1211/r.2018.06.013.
27. Tian S, Xu Q, Jiang R, Han T, Sun C, Na L. Dietary Protein Consumption and the Risk of Type 2 Diabetes: A Systematic Review and Meta-Analysis of Cohort Studies. Nutrients 2017;9:982. doi: 10.3390/nu9090982.
28. Chen L, Chen Q, Rong P, Wang HY, Chen S. The energy sensing LKB1-AMPKα1 pathway regulates IGF1 secretion and consequent activation of the IGF1R-PKB pathway in primary hepatocytes. FEBS J 2017;284:2096-109. doi: 10.1111/febs.14106.
29. Zhou F, Nie L, Feng D, Guo S, Luo R. [Retracted] MicroRNA-379 acts as a tumor suppressor in non-small cell lung cancer by targeting the IGF-1R-mediated AKT and ERK pathways. Oncol Rep 2022;48:128. doi: 10.3892/or.2022.8339.
30. Xu L, Zhou R, Yuan L, Wang S, Li X, Ma H, et al. IGF1/IGF1R/STAT3 signaling-inducible IFITM2 promotes gastric cancer growth and metastasis. Cancer Lett 2017;393:76-85. doi: 10.1016/j.canlet.2017.02.014.
31. Xiong W, Huang C, Deng H, Jian C, Zen C, Ye K, et al. Oncogenic non-coding RNA NEAT1 promotes the prostate cancer cell growth through the SRC3/IGF1R/AKT pathway. Int J Biochem Cell Biol 2018;94:125-32. doi: 10.1016/j.biocel.2017.12.005.
32. Iravani O, Bay BH, Yip GW. Silencing HS6ST3 inhibits growth and progression of breast cancer cells through suppressing IGF1R and inducing XAF1. Exp Cell Res 2017;350:380-9. doi: 10.1016/j.yexcr.2016.12.019.
33. Xiang HP, Geng XP, Ge WW, Li H. Meta-analysis of CHEK2 1100delC variant and colorectal cancer susceptibility. Eur J Cancer 2011;47:2546-51. doi: 10.1016/j.ejca.2011.03.025.
34. Hale V, Weischer M, Park JY. CHEK2 (*) 1100delC Mutation and Risk of Prostate Cancer. Prostate Cancer 2014;2014:294575. doi: 10.1155/2014/294575.
35. Muranen TA, Greco D, Blomqvist C, Aittomäki K, Khan S, Hogervorst F, et al. Genetic modifiers of CHEK2*1100delC-associated breast cancer risk. Genet Med 2017;19:599-603. doi: 10.1038/gim.2016.147.
36. Aumailley M, Smyth N. The role of laminins in basement membrane function. J Anat 1998;193:1-21. doi: 10.1046/j.1469-7580.1998.19310001.x.
37. Chen J, Ma Y, Li H, Lin Z, Yang Z, Zhang Q, et al. Rare and potential pathogenic mutations of LMNA and LAMA4 associated with familial arrhythmogenic right ventricular cardiomyopathy/dysplasia with right ventricular heart failure, cerebral thromboembolism and hereditary electrocardiogram abnormality. Orphanet J Rare Dis 2022;17:183. doi: 10.1186/s13023-022-02348-z.

Author Contribution:
JK & XS: Data acquisition, drafting and revision.
SF, DL, HY & NL: Data analysis, interpretation, software and validation.
HW: Methodology.
LY: Concept, design, drafting and revision.