Pharmacogenomics and Personalized Medicine

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Association of MiRNA Polymorphisms Involved in the PI3K/ATK/GSK3β Pathway with T2DM in a Chinese Population

Xing Zhou[1–3,*], Man Yang[1,*], Ying Yang[1], Fan Xu[1], Feiying Wang[1], Ming Jiao[2,4], Wenyu Tao[1], Yiping Li[1]

[1]Department of Endocrinology, The Affiliated Hospital of Yunnan University & The Second People's Hospital of Yunnan Province, Kunming, Yunnan, People's Republic of China; [2]Kunming Medical University, Kunming, Yunnan, People's Republic of China; [3]Department of Endocrinology, Dongguan Tungwah Hospital, Dongguan, Guangdong, People's Republic of China; [4]Yunnan Emergency Center, Kunming, Yunnan, People's Republic of China

*These authors contributed equally to this work

Correspondence: Wenyu Tao; Yiping Li, Department of Endocrinology, The Affiliated Hospital of Yunnan University & The Second People's Hospital of Yunnan Province, Kunming, Yunnan, People's Republic of China, Email twy623@126.com; yyflyp@aliyun.com

Background: Single nucleotide polymorphisms (SNPs) in miRNA genes can influence the expression of miRNAs that modulate the PI3K/AKT/GSK3β pathway and play crucial roles in type 2 diabetes mellitus (T2DM) susceptibility. The purpose of this study was to investigate the association of SNPs in miRNA genes targeting the PI3K/AKT/GSK3β pathway with T2DM.

Methods: This case-control study included 1,416 subjects with T2DM and 1,694 non-diabetics. Eleven SNPs in miRNA genes (rs895819 in miR-27a, rs11888095 in miR-128a, rs2292832 in miR-149, rs6502892 in miR-22, rs13283671 in miR-31, rs1076063 and rs1076064 in miR-378a, rs10061133 in miR-449b, rs3746444 in miR-499a and rs678956 and rs476364 in miR-326) involved in PI3K/AKT/GSK3β pathway were genotyped by TaqMan Genotyping Assay, and the associations of these SNPs with T2DM were analyzed using online SHesis and SNPstats.

Results: The results showed that miR-378a rs1076064 G allele could be a protective factor against T2DM ($p<0.001$, OR=0.828; 95% CI:0.749–0.916), whereas the miR-31 rs13283671 C allele could increase the risk of developing T2DM ($p=0.003$, OR=1.193; 95% CI:1.060–1.342). In addition, the miR-378a rs1076063A-rs1076064G haplotype could be a protective against T2DM ($p<0.001$, OR=0.731; 95% CI:0.649–0.824). According to inheritance mode analysis, compared with the AA-AG genotype, the GG genotype of rs1076064 showed a protective effect in T2DM in the recessive mode ($p<0.01$, OR=0.71; 95% CI: 0.59–0.84). For rs13283671, compared with the TT genotype, the CT-CC genotype showed a risk effect in T2DM in the dominant inheritance model ($p<0.01$, OR=1.29; 95% CI: 1.12–1.49). Genotype-Tissue Expression (GTEx) Portal database analysis showed that miR-31 rs13283671 CT and CC genotypes had lower AKT expression than TT genotypes.

Conclusion: In conclusion, rs13283671 in miR-31 and rs1076064 in miR-378a involved in the PI3K/AKT/GSK3β pathway were associated with T2DM susceptibility in a Chinese population.

Keywords: T2DM, GSK3β, microRNA, polymorphisms, Chinese population

Introduction

Type 2 diabetes mellitus (T2DM) is a common chronic metabolic disease caused by a combination of genetic and environmental factors. According to the International Diabetes Federation (IDF), the number of people with diabetes worldwide already reached 537 million in 2021 and was expected to increase to 643 million by 2030, and China has the highest number of cases of diabetes in the world.[1] T2DM accounts for more than 95% of all diabetes cases and the age of onset tends to be younger.[2]

The main characteristics of T2DM are insulin resistance and reduced insulin secretion. The phosphoinositide 3-kinase (PI3K)/protein kinase B(AKT)/glycogen synthase kinase-3β(GSK3β) signaling pathway is a key component in the

Received: 30 August 2024
Accepted: 3 February 2025
Published: 14 February 2025

regulation of glucose homeostasis, and has been linked to a multitude of biological activities, such as cellular metabolism, migration, apoptosis, proliferation and inflammation.[3] Insulin binds to the cognate insulin receptor to activate insulin receptor substrate 1(IRS1), which induces the phosphorylation of molecules in the downstream cascade, such as PI3K and AKT.[4] Activated AKT further phosphorylates the Ser9 of GSK3β resulting in transient inactivation of GSK3β,[5] which was found to be beneficial for cellular metabolism and mitochondrial biogenesis, such as glucose transport, glycogen synthesis and ATP production.[6,7] In 2001, Nikoulina et al reported that the PI3K/AKT/GSK3β pathway was blunted by persistent hyperinsulinism in the skeletal muscle of individuals with T2DM, resulting in diminished GSK3β inhibition.[8] Prolonged overactivation of GSK3β inhibits glucose transport and glycogen synthase activity and causes an increase in blood glucose.[8] In addition, Jere et al reported that the dysregulation of the PI3K/AKT/GSK3β pathway has a significant impact on wound healing in diabetic ulcers.[9] Thus, the PI3K/AKT/GSK3β pathway has been implicated in the development and adverse prognosis of T2DM.

MicroRNAs (miRNAs) are a class of short, single-stranded, noncoding RNAs that regulate approximately 60% of protein-coding genes.[10] As a negative regulator after gene transcription, miRNAs bind to the 3′ untranslated region (3′ UTR) of the target mRNAs, which reduces and silences the expression of target genes by directly cleaving the mRNA or inhibiting mRNA translation or accelerating mRNA decay.[11] A number of studies have shown that miRNA target genes are involved in different stages of the insulin signaling pathway.[12–14] Aberrant miRNA expression leads to dysregulation of proteins in the cascade response, affecting PI3K/AKT/GSK3β signaling.[14,15] SNPs in miRNA genes have an effect on the miRNA processing, maturation and expression.[16] Therefore, SNPs in miRNAs may affect their interaction with target genes of signaling pathways, and ultimately be associated with T2DM susceptibility.

In the current study, our aims were to investigate the association of SNPs in miRNA genes involved in the PI3K/AKT/GSK3β pathway with T2DM. Firstly, we mined and selected potential miRNAs which were related to T2DM. With respect to candidate miRNAs, we predicted the target genes of the miRNAs enriched in the PI3K/AKT/GSK3β pathway. Secondly, eleven SNPs related to nine miRNA genes of PI3K/AKT/GSK3β pathway (rs895819 in miR-27a, rs11888095 in miR-128a, rs2292832 in miR-149, rs6502892 in miR-22, rs13283671 in miR-31, rs1076063 and rs1076064 in miR-378a, rs10061133 in miR-449b, rs3746444 in miR-499a and rs678956 and rs476364 in miR-326) were selected, and the association of these SNPs with T2DM was evaluated in a Chinese population.

Materials and Methods

Subjects

A total of 3110 Chinese Han people were recruited as study subjects. From July 2022 to January 2024, 1416 individuals with T2DM who were admitted to the ward or attended to the outpatient clinic of the Department of Endocrinology at the Affiliated Hospital of Yunnan University were selected as the T2DM group. During the same period, 1694 individuals without a history and family history of diabetes in health checkups at the same hospital were recruited as the control group. Inclusion and exclusion criteria for T2DM and control subjects were described in our previous study.[17] In brief, the diagnosis of T2DM was in accordance with the World Health Organization criteria published in 1999 and American Diabetes Association (ADA) guidelines in 2021.[18] The criteria for inclusion of T2DM group was the T2DM subjects with fasting plasma glucose ≥7.0mmol/L or 2-hours postprandial plasma glucose during oral glucose tolerance test≥11.1mmol/L or a random plasma glucose≥11.1mmol/L. The diagnosis for diabetes was required to have the above two abnormal test results. The criteria for exclusion of T2DM group was: ①Specific types of diabetes were excluded based on the history illness, such as the diseases of the exocrine pancreas and glucocorticoid use. ②Gestational women were not included; ③The islet β-cell function was evaluated by measurement of insulin and C peptides level of fasting and glucose load to exclude subjects with type 1 diabetes; ④The islet cell autoantibodies and glutamic acid decarboxylase autoantibodies were detected to exclude subjects with latent autoimmune diabetes in adults.[19] For control, subjects with prediabetes were excluded from the control group (subjects with fasting plasma glucose (FPG) greater than 6.1 mmol/L and/or glycosylated haemoglobin (HbA1C) greater than 5.7%). The protocol involving human participants was reviewed and approved by the Institutional Review Board of the Second People's Hospital of Yunnan Province (2020093). All participants provided written informed consent to participate in this study.

Data Collection and Laboratory Measurements

General clinical information, such as age, race, and sex, was collected via questionnaire. Venous blood was drawn early in the morning after 8 hours of fasting. All metabolic parameters were measured on a Hitachi 7600–020 autoanalyzer. Total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured enzymatically. FPG was measured by the glucose oxidase method. HbA1C was measured by immunoturbidimetric method.

MiRNA Selection, Target Gene Prediction and Signaling Pathway Enrichment

MiRWalk 3.0 (http://mirwalk.umm.uni-heidelberg.de/) was used to mine miRNAs correlated with T2DM. Three overlapping miRNAs (TargetScan, miRDB and miRTarBase) with a score greater than 0.95 were selected. The miRPathv4 database (https://diana-lab.e-ce.uth.gr/app/miRPathv4) was used to predict target genes of these candidate miRNAs, and pathway enrichment was then performed for these predicted target genes.

SNP Selection and Genotyping

First, miRNAs involved in PI3K/AKT/GSK3β pathway regulation were selected through target prediction and enrichment. Candidate SNPs in the primary sequences, precursor sequences, mature sequences, or transcriptional regulatory regions of these miRNAs were subsequently selected. In addition, the minor allele frequency (MAF) of the SNPs was used as a selection criterion for the selection of SNPs with a MAF greater than 0.05 in Asian population (https://asia.ensembl.org/). As a result, eleven SNPs (rs895819 in the noncoding transcript exon of miR-27a, rs11888095 in the upstream transcript of miR-128a, rs2292832 in the noncoding transcript exon of miR-149, rs6502892 in the upstream transcript of miR-22, rs13283671 in the 500 bp downstream region of miR-31, rs1076063 and rs1076064 are both located in upstream transcript of miR-378a, rs10061133 in the mature sequence of miR-449b, rs3746444 in the mature sequence of miR-499a; and rs678956 in the upstream transcript and rs476364 in the 500 bp downstream of miR-326) were screened. Details of miRNA SNPs selected in this study are shown in Table 1.

All sample genomic DNA was obtained by a QIAamp Blood Mini Kit (Qiagen, Hilden, Germany) and extracted from EDTA anticoagulated whole blood samples. The purity of DNA was measured on a Multiskan GO Protein Nucleic Acid Microtester. The A260/A280 ratio value was 1.8–2.0. Eleven SNPs were subsequently genotyped using the TaqMan Assay. The PCR primers used were designed by Thermo Scientific. The conditions and procedures for PCR were

Table 1 The Information of the Eleven SNPs Selected in the Current Study

Genes	SNPs	Location	Function Consequence	Alleles	MAF in Asia
MIR27A	rs895819	Chr 19:13836478	Non coding transcript	T>C	0.280
MIR128A	rs11888095	Chr 2:135665214	Upstream transcript	C>T	0.176
MIR149	rs2292832	Chr 2:240456086	Non coding transcript	C>T	0.363
MIR22	rs6502892	Chr 17:1714314	Upstream transcript	C>T	0.107
MIR31	rs13283671	Chr 9:21511741	500bp downstream	T>C	0.251
MIR378A	rs1076063	Chr 5:149732632	Upstream transcript	T>A	0.247
	rs1076064	Chr 5:149732603	Upstream transcript	G>A	0.491
MIR449B	rs10061133	Chr 5:55170716	Mature miRNA	A>G	0.265
MIR499A	rs3746444	Chr 20:34990448	Mature miRNA	A>G	0.145
MIR326	rs678956	Chr 11:75335478	Upstream transcript	A>G	0.417
	rs476364	Chr 11:75334856	500bp downstream	C>G	0.429

Abbreviations: SNP, single nucleotide polymorphisms; MAF, minor allele frequency.

https://doi.org/10.2147/PGPM.S487873

performed as described in previous studies.[18] Raw genotyping data were obtained using QuantStudio Real-Time PCR Software (Agena, Inc, San Diego, CA, USA). The samples were subsequently subjected sequencing to verify the genotyping results of the SNPs.

Statistical Analyses

SPSS 26.0 software (SPSS, Chicago, IL, USA) and web-based analysis tools were used for statistical analysis of the current study. Continuous variables including (age, TC, TG, HDL, LDL, FPG and HbA1C) are presented as the mean ±SD and were compared by Student's *t* test. The chi-square test was used to compare sex differences between the T2DM group and the control group. The Hardy-Weinberg equilibrium (HWE) for every SNP in the two groups was evaluated with a threshold set at P>0.05. Differences in allele and genotype distributions, odds ratios (ORs) and 95% confidence intervals (95% CIs) for allele-specific risk of each SNP in T2DM and Control groups were analyzed using SHEsis software,[20] with Bonferroni-corrected significance threshold set at P<0.0045 (0.05/n, n=11). The statistical power was calculated using PS Software.[21,22] Linkage disequilibrium (LD) among these SNPs was estimated using SHEsis software, and the LD coefficient D′ was calculated. The haplotypes constructed and differences in the haplotypes between the T2DM and control groups were determined with the SHEsis software.[20] The SNPstats software was used to determine the best fit model for each SNP.[23] In addition, genotype-phenotype correlations (https://gtexportal.org/home/) were analysed using SNP-related data from the public database Genotype-Tissue Expression (GTEx) portal, and a P value below 0.05 was considered statistically significant.

Results

Demographic Characteristics and Metabolic Indicators of the Study Subjects

A total of 3110 subjects participated in this study. The demographic characteristics and metabolic indices of the subjects are shown in Table 2. The mean ages of the T2DM and control subjects were 53.710±0.321 years and 53.887±0.246 years, respectively. There were 872 males and 544 females with T2DM. In the control group, there were 1033 males and 661 females. There were no differences in age or sex between the two groups (p>0.05). Significant differences were observed between the T2DM and control groups in TC, TG, HDL-C, LDL-C, FPG, and HbA1C (p<0.05). Specifically, the TC and LDL-C levels were greater in the control group than in the T2DM group, as anti-hyperlipidemic drugs were prescribed at the diagnosis of T2DM and hyperlipidemia.

Table 2 Demographic and Clinical Characteristics of the T2DM and Control Group

Variables	T2DM (1416)	Control (1694)	T/χ^2	p
Age (Year)	53.710±0.321	53.887±0.246	0.438	0.662
Sex (male/female)	872/544	1033/661	0.140	0.708
Glycated hemoglobin (%)	9.340±0.075	5.128±0.007	55.689	<0.001
Fasting plasma glucose (mmol/L)	8.150±0.074	5.153±0.012	39.785	<0.001
Total cholesterol (mmol/L)	4.556±0.04	4.987±0.026	−8.998	<0.001
Triglycerides (mmol/L)	2.683±0.061	1.728±0.029	14.055	<0.001
High-density lipoprotein-cholesterol (mmol/L)	1.082±0.009	1.360±0.009	−21.530	<0.001
Low-density lipoprotein-cholesterol (mmol/L)	2.778±0.026	3.138±0.022	−10.700	<0.001

Abbreviation: T2DM, type 2 diabetes.

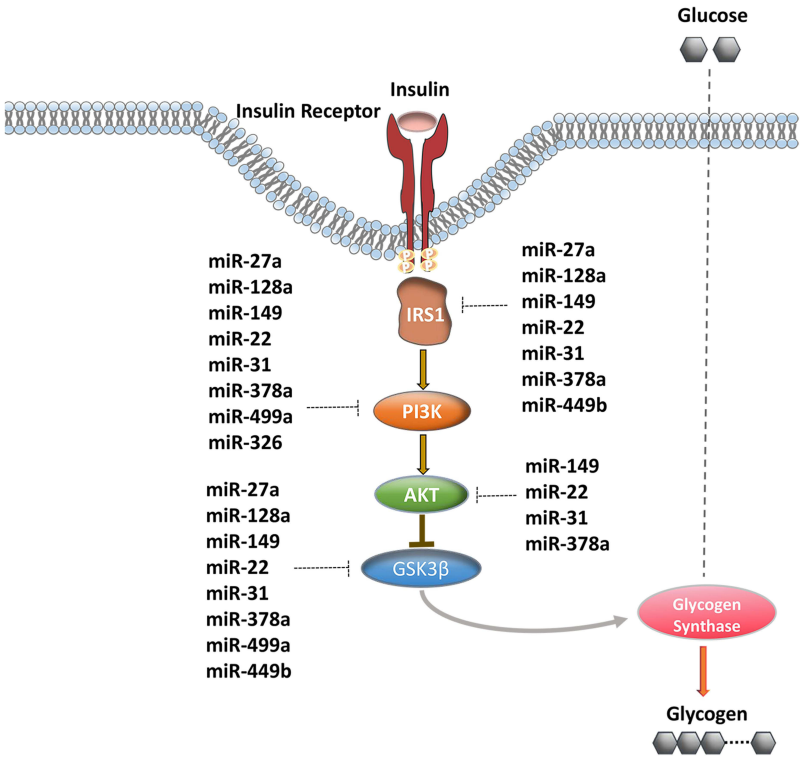

Figure 1 Nine miRNAs involving in PI3K/AKT/GSK3β Signaling Pathway.

Signaling Pathway Enrichment of miRNAs

The target genes of the miRNAs were predicted using TargetScan 8.0 of miRPathv4.0. The potential target genes were subsequently subjected to enrichment analysis. The enrichment results showed that nine miRNAs were involved in the PI3K/AKT/GSK3β pathway (p<0.01) (Figure 1).

Association of the Eleven SNPs with T2DM

The genotype frequencies for eleven SNPs were all in HWE in the T2DM and control groups (p>0.05) (Table 3). The results showed that the allele frequencies of rs1076064 in the miR-378a and rs13283671 in miR-31 were significantly different between the T2DM and control groups. The G allele of rs1076064 in miR-378a could be a protective factor for T2DM (p<0.001, OR=0.828; 95% CI: 0.749–0.916). The C allele of rs13283671 in miR-31 could increase the risk of developing T2DM (p=0.003, OR=1.193; 95% CI:1.060–1.342). No significant difference in the allelic or genotypic distribution of the other SNPs were observed between the two groups (p>0.0045).

Table 3 Comparison of Genotype and Allele Distribution of 11 SNPs Between T2DM and Control Group

SNPs	Alleles/Genotypes	T2DM n (%)	Control n (%)	χ 2	*P* value	T2DM vs Control Odds Ratio (95% CI)
rs895819	C	773(27.3)	909(26.8)	0.169	0.680	1.023 (0.915–1.145)
	T	2059(72.7)	2479(73.2)			
	T/C	577(40.8)	695(41.0)			
	C/C	98(6.9)	107(6.3)	0.457	0.795	
	T/T	741(52.3)	892(52.7)			

(*Continued*)

https://doi.org/10.2147/PGPM.S487873

Table 3 (Continued).

SNPs	Alleles/Genotypes	T2DM n (%)	Control n (%)	χ 2	*P* value	T2DM vs Control Odds Ratio (95% CI)
rs11888095	C	2340(82.6)	2826(834)	0.675	0.411	1.057 (0.925–1.207)
	T	492(17.4)	562(166)			
	C/C	971(68.6)	1179(69.6)			
	C/T	398(28.1)	468(27.6)	0.938	0.626	
	T/T	47(3.3)	47(2.8)			
rs2292832	C	965(34.1)	1198(35.4)	1.123	0.289	0.944 (0.850–1.049)
	T	1867(65.9)	2190(64.6)			
	T/T	618(43.6)	725(42.8)			
	T/C	631(44.6)	740(43.7)	2.064	0.356	
	C/C	167(11.8)	229(13.5)			
rs6502892	C	2543(89.8)	3042(89.8)	0.000	0.992	0.999 (0.847–1.178)
	T	289(10.2)	346(10.2)			
	C/C	1145(80.9)	1371(81.0)			
	T/C	253(17.9)	300(17.7)	0.055	0.972	
	T/T	18(1.3)	23(1.3)			
rs13283671	C	710(25.1)	742(21.9)	8.661	0.003	1.193 (1.060–1.342)
	T	2122(74.9)	2646 (78.1)			
	T/T	778(54.9)	1035(61.0)			
	T/C	566(40.0)	576(34.0)	12.549	0.001	
	C/C	72(5.1)	83(5.0)			
rs1076063	A	706(24.9)	847(25.8)	0.612	0.433	0.955 (0.851–1.071)
	T	2126(75.1)	2514(74.2)			
	T/T	789(55.7)	943(55.7)			
	T/A	548(38.7)	628(37.0)	3.900	0.142	
	A/A	79(5.6)	123(7.3)			
rs1076064	G	1281(45.2)	1691(49.9)	13.531	0.000	0.828 (0.749–0.916)
	A	1551(54.8)	1697(50.1)			
	G/G	272(19.2)	427(25.2)			
	A/A	407(28.8)	430(25.4)	16.639	0.000	
	A/G	737(52.0)	837(49.4)			
rs10061133	A	2034(71.8)	2450(72.3)	0.185	0.666	1.024 (0.916–1.145)
	G	798(28.2)	938(27.7)			
	A/G	536(37.9)	668(39.4)			

(*Continued*)

Table 3 (Continued).

SNPs	Alleles/Genotypes	T2DM n (%)	Control n (%)	χ 2	*P* value	T2DM vs Control Odds Ratio (95% CI)
	A/A	749(52.9)	891(52.6)	1.992	0.369	
	G/G	131(9.2)	135(8.0)			
rs3746444	A	2419(85.4)	2929(86.5)	1.372	0.241	1.089 (0.943–1.257)
	G	413(14.6)	459(13.5)			
	A/A	1029(72.7)	1263(74.6)			
	G/G	26(1.8)	28(1.7)	1.434	0.488	
	A/G	361(25.5)	403(23.7)			
rs678956	A	1140(40.3)	1355(40.0)	0.043	0.834	1.010 (0.913–1.119)
	G	1692(59.7)	2033(60.0)			
	G/G	499(35.2)	607(35.8)			
	A/A	223(15.7)	268(15.8)	0.148	0.928	
	A/G	694(49.0)	819(48.4)			
rs476364	C	1644(58.1)	1934(57.1)	0.590	0.442	0.961 (0.868–1.063)
	G	1188(41.9)	1454(42.9)			
	C/G	710(50.1)	848(50.1)			
	C/C	467(33.0)	543(32.1)	0.654	0.721	
	G/G	239(16.9)	303(17.8)			

Abbreviations: SNP, single nucleotide polymorphisms; T2DM, type 2 diabetes.

Linkage Disequilibrium (LD) Analysis Between SNPs in miRNA Genes

Based on the location of the SNPs (rs1076063 and rs1076064 in miR-378a on chromosome 5; rs678956 and rs476364 in miR-326 on chromosome 11), LD among SNPs located on the same chromosome was estimated. D′ values above 0.8 indicate the existence of LD between the sites. For the SNPs in chromosome 5 (rs1076063 and rs1076064), the LD test results showed that rs1076063 and rs1076064 were in linkage disequilibrium (D′=0.83). For rs678956 and rs476364 on chromosome 11, the LD tests result showed that two SNPs were in linkage disequilibrium (D′=0.98).

Correlations Between SNP Haplotypes in miRNA Genes and T2DM

According to the results of LD analysis, the rs1076063-rs1076064 haplotype on chromosome 5 and the rs678956-rs476364 haplotype on chromosome 11 were constructed. The haplotype analysis results showed that the rs1076063A-rs1076064G haplotype of miR-378a was a protective factor against T2DM (p<0.001, OR=0.731; 95% CI: 0.649–0.824) (Table 4). However, the results showed that the frequency of the rs678956-rs476364 haplotypes was no significant differences between T2DM and control subjects (data not shown).

Inheritance Model Analysis of the Eleven SNPs with T2DM

The association of the genotypes of the eleven SNPs with T2DM was evaluated using inheritance model analysis (Table 5 and Supplement Table S1). Five models of inheritance (codominant, dominant, recessive, super-dominant, and log-additive) were analyzed for each SNP. Based on the Akaike information criterion (AIC) and Bayesian information criterion (BIC) values, the lowest AIC and BIC values for each SNP were selected to determine the best-fitting model.[23] The results showed that the genotype distribution of rs13283671 in miR-31 and rs1076064 in miR-378a were significantly different between the T2DM and

Table 4 Haplotype - Analysis of rs1076063-rs1076064 in the MiRNA-378 Between T2DM and Control

rs1076063-rs1076064	T2DM (N/%)	Control (N/%)	χ 2	*p* value	Odds Ratio (95%)
TG	708(25.0)	819(24.1)	0.568	0.450	1.045 (0.931–1.174)
TA	1418(50.0)	1695(50.0)	0.001	0.974	1.001 (0.906–1.106)
AG	573(20.2)	872(25.7)	26.210	0.000	0.731 (0.649–0.824)

Note: Haplotypes with frequency <0.03 are ignored.

Table 5 Different Inheritance Models of rs1076064 and rs13283671 Between T2DM and Control Group (n=3110, Adjusted by Sex+age)

SNPs	Models	Genotypes	T2DM (N/%)	Control (N/%)	Odds Ratio (95CI)	*P* value	AIC	BIC
rs13283671	Codominant	T/T	778 (54.9)	1035 (61.1)	1.00	0.00	4283.70	4314.00
		C/T	566 (40.0)	576 (34.0)	1.31 (1.13–1.52)			
		C/C	72 (5.1)	83 (4.9)	1.15(0.83–1.60)			
	Dominant	T/T	778 (54.9)	1035 (61.1)	1.00	0.00	4282.30	4306.40
		C/T-C/C	638 (45.1)	659 (38.9)	**1.29 (1.12–1.49)**			
	Recessive	T/T-C/T	1344 (94.9)	1611 (95.1)	1.00	0.82	4294.20	4318.30
		C/C	72 (5.1)	83 (4.9)	1.04 (0.75–1.44)			
	Overdominant	T/T-C/C	850 (60.0)	1118 (66.0)	1.00	0.00	4282.40	4306.60
		C/T	566 (40.0)	576 (34.0)	1.29 (1.12–1.50)			
	Log-additive	-	-	-	1.20 (1.06–1.35)	0.00	4285.40	4309.60
rs1076064	Codominant	A/A	407 (28.7)	430 (25.4)	1.00	0.00	4279.50	4309.70
		A/G	737 (52.0)	837 (49.4)	0.93(0.79–1.10)			
		G/G	272 (19.2)	427 (25.2)	0.67 (0.55–0.83)			
	Dominant	A/A	407 (28.7)	430 (25.4)	1.00	0.03	4289.70	4313.90
		A/G-G/G	1009 (71.3)	1264 (74.6)	0.84 (0.72–0.99)			
	Recessive	A/A-A/G	1144 (80.8)	1267 (74.8)	1.00	0.00	4278.20	4302.40
		G/G	272 (19.2)	427 (25.2)	**0.71 (0.59–0.84)**			
	Overdominant	A/A-G/G	679 (48.0)	857 (50.6)	1.00	0.15	4292.10	4316.30
		A/G	737 (52.0)	837 (49.4)	1.11 (0.96–1.28)			
	Log-additive	-	-	-	0.80(0.70–0.91)	0.00	4280.40	4304.60

Abbreviations: SNP, single nucleotide polymorphisms; T2DM, type 2 diabetes; AIC, akaike information criterion; BIC, bayesian information criterion.

control groups. The best-fit inheritance model for rs1076064 was recessive. In this model, the GG genotype of rs1076064 was a protective factor for T2DM compared to the (AA-AG) genotype after adjusted by sex and age (p<0.01, OR=0.71, 95% CI: 0.59–0.84). The best-fit inheritance model for rs13283671 was dominated. According to this model, the (CT-CC) genotype of rs1328367 was a risk factor for T2DM compared to the TT genotype after adjusted by sex and age (p<0.01, OR=1.29, 95% CI: 1.12–1.49).

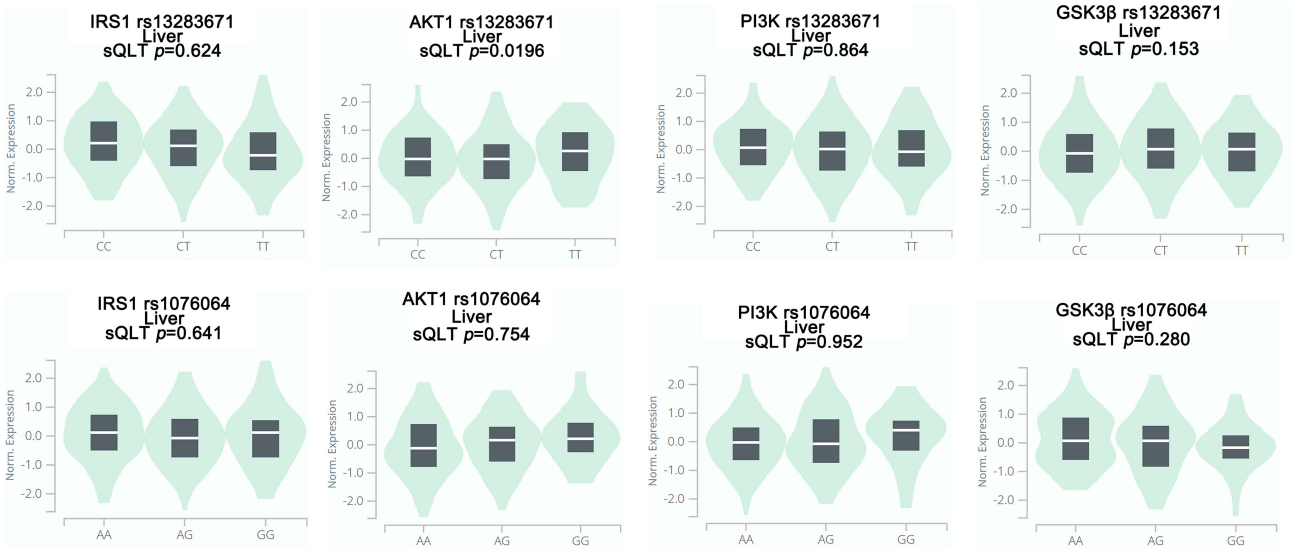

Figure 2 SQTL analysis of miR-31 rs13283671 in liver tissues based on data from the GTEx portal was performed to assess genotype correlation with gene expression in PI3K/AKT/GSK3β signaling pathways. p-values were calculated using a linear regression model.

The Correlations of miR-378a rs1076064 and miR-31 rs13283671 Genotypes with Gene Expression Levels

Since miR-378a rs1076064 and miR-31 rs13283671 were associated with T2DM susceptibility, we further functionally analyzed using Genotype-Tissue Expression (GTEx) portal to compare the expression levels of target genes among genotypes (Figure 2). The results showed that in the liver, miR-31 rs13283671 CC and CT genotypes had lower AKT expression than TT genotypes (p = 0.0196). Similarly, miR-378a rs1076064 GG genotypes had higher expression of PI3K and AKT compared to AG and AA genotypes, whereas GSK3β expression was lower in GG genotypes than in AG and AA genotypes, although not reaching statistical significance (p>0.05).

Discussion

MiRNAs are potential biomarkers for T2DM,[24] cancer,[25] and autoimmune diseases.[26] Aberrant expressions of miRNAs associated with insulin signaling pathway played a crucial role in the pathogenesis of T2DM. We investigated the association of SNPs in miRNA genes involved in the PI3K/AKT/GSK3β pathway with T2DM. Our results showed that rs13283671 in miR-31 and rs1076064 in miR-378a were associated with T2DM susceptibility in a Han Chinese population, and the statistical power reached 0.540 and 0.805 respectively.

According to the results of our research, miR-378a rs1076064 G allele could be a protective factor against T2DM (p<0.001, OR=0.828; 95% CI: 0.749–0.916). The rs1076063A-rs1076064G haplotype could be protective against T2DM (*p*<0.001, OR=0.731; 95% CI: 0.649–0.824), and the haplotype result was consistent with the single SNP results. The association between miR-378a rs1076064 and cancer survival has been reported in previous studies.[27,28] In fact, the role of miR-378a in glucose homeostasis and lipid metabolism cannot be ignored. It was shown that miR-378a expression was positively correlated with insulin resistance.[29] MiR-378 was reported to be upregulated in the plasma of obese and insulin resistant individuals.[29] Similarly, compared with those in non-diabetic individuals, the levels of miR-378a in the mitochondria of T2DM subjects and mouse models of T2DM were found to be elevated.[30] Indeed, in 2014, Liu et al reported that miR-378a targeted the p110α subunit of PI3K in liver to directly downregulate PI3K, causing disturbances in glucose and lipid metabolism.[31] In 2021, Wang et al identified that miR-378a plays a crucial role in the pathogenesis of hepatic insulin resistance by downregulating IRS1, AKT and PPARα.[32] Notably, in 2012, Carrer et al demonstrated that miR-378a was linked to mitochondrial dysfunction.[33] MiR-378a knockout mice fed with high-fat diet have lower blood glucose and serum triglyceride levels than wild-type mice.[33] These results suggested that miR-378a is a mediator of a wide range of biological processes involved in T2DM.

https://doi.org/10.2147/PGPM.S487873

The association between miR-378a rs1076064 and T2DM could be attributed to its effect on the expression of miR-378a. Rs1076064 is located at the 222 bp upstream of the coding region of the stem-loop precursor of miR-378a. Since this genetic variant is located within the predicted regulatory region of miR-378a, it may alter the expression of mature miR-378 by affecting the transcription rate of pri-miR-378. In 2014, Jiang et al demonstrated the region around the rs1076064 locus (−476 bp to 128 bp) as the major promoter region of miR-378a.[34] An et al verified in an in vitro study that rs1076064 affects the transcriptional activity of miR-378, and the promoter activity of the rs1076064 G allele was higher than that of the A alleles in hepatocellular carcinoma cells.[28] In addition, Feng et al found that this variant is located in the C-Myc binding region of transcription factors, which could affect the affinity of transcription factors.[35] Taken together, these studies suggested that rs1076064 is a functional genetic polymorphism site that may affect miR-378a expression by altering promoter activity or transcription factor affinity. GTEx Portal database analysis revealed that miR-378a rs1076064 GG genotypes had higher expression of PI3K and AKT compared to AG and AA genotypes, whereas GSK3β expression was lower in GG genotypes than in AG and AA genotypes. Therefore, rs1076064 led to the dysregulation of miR-378a target genes involved in the PI3K/AKT/GSK3β pathway and then was correlated with the pathogenesis of T2DM.

We also found that the miR-31 rs13283671 C allele could be a risk factor for T2DM (p=0.003, OR=1.193; 95% CI: 1.060–1.342). To date, the relationship between miR-31 rs13283671 and T2DM has not been investigated. In 2011, Hu et al found that the C allele of rs13283671 was associated with survival in subjects with non-small cell lung cancer.[27] Upregulation of miR-31 was observed in serum and adipose tissue in the mice with T2DM.[36] In 2018, Gottman et al reported the detrimental effects of miR-31 on insulin signaling in individuals withT2DM and obesity.[36] In detail, the overexpression of miR-31 impaired the adipocyte differentiation and insulin induced glucose uptake by directly acting on peroxisome proliferator-activated receptor-γ and IRS1.[36] Moreover, in 2018, Yu et al reported that miR-31 could downregulate Integrin Alpha5 which can participate in the activation of the PI3K/Akt pathway.[37] Besides, its effects on the insulin signaling pathway, this molecule has also been linked to pancreatic β-cell development. In vitro cellular experiment showed miR-31 was downregulated during islet stem cell differentiation.[38] The miR-31 rs13283671 could be linked to T2DM by impacting the expression of miR-31. In 2009, Sun et al detected an RNA polymerase II binding site downstream of the miR-31 gene by chromatin immunoprecipitation assay, which indicated a promoter in the downstream region.[39] In 2014, Yao et al demonstrated that the proximal region of the miRNA promoter was located within 2 kb upstream and downstream of the start transcription site, and these regional SNPs affected the transcriptional activity of the gene.[40] Due to the fact that rs13283671 is located 500bp downstream of the stem-loop structure of the precursor miR-31. Rs13283671 may alter the transcriptional activity of miR-31, thereby affecting miR-31 expression. Moreover, this SNP might be related to Drosha's recognition and cleavage of pri-miRNA-31.[41] Our results also revealed that miR-31 rs13283671 CC and CT genotypes had lower AKT expression than TT genotypes. Therefore, the rs13283671 CC and CT genotypes affect the interaction of miR-31 with molecules in the PI3K/AKT/GSK3β signaling pathway, which increases the risk of developing T2DM. As PI3K/AKT/GSK3βpathway has been implicated in the development and adverse prognosis of T2DM, Teli et al reported that the GSK3β has been considered as a potential target for diabetes in 2023.[42] Thus, the T2DM subjects with the risk genotypes would prefer to be prescribed by GSK3β inhibitors to improve glycogen synthesis in the future.

MiR-449b, miR-326, miR-149 and miR-22 mediate metabolic dysregulation and promote T2DM development.[43–46] MiR-449b,[43] miR-326,[47] and miR-22[48] were observed to be upregulated in T2DM serum, whereas miR-149 was downregulated in T2DM serum.[38] In 2023, Meng et al reported that miR-449b inhibited the expression of genes involved in oxidative metabolism, such as nuclear respiratory factor-1 and membrane uncoupling protein 3,[49] which induced insulin resistance and T2DM.[50] MiR-149, miR-326 and miR-22 directly or indirectly modulate the expression of the metabolic factor SIRT1.[44–46] SIRT1 expression is positively correlated with insulin signaling activation and glucose uptake, and negatively correlated with the development of T2DM.[51] We predicted that these miRNAs could target genes in the PI3K/AKT/GSK3β pathway by bioinformatics analysis. However, our results suggested that the miR-449b, miR-326, miR-149 and miR-22 SNPs were no relationship with T2DM susceptibility in a Chinese population. Neither miR-449b rs10061133 nor miR-149 rs2292832 was found to be associated with T2DM susceptibility in studies from other populations,[52,53] which was consistent with our current findings.

Recent studies have shown that miR-27a, miR-499a and miR-128a played an important role in development of T2DM and diabetic neuropathy (DNP).[54–56] It has been shown that the miR-27a expression increases not only in the serum of T2DM subjects,[56] but also in the tissue of T2DM subjects, such as liver and adipose tissue.[57] Interestingly, miR-499a was reported to be downregulated in the serum and nerve tissue of subjects with T2DM.[14] For miR-128a, it was reported that miR-128a was significantly upregulated in skeletal muscle cells.[58] MiR-27a, miR-499a and miR-128a play a role by targeting genes in the insulin signaling pathway.[14,58–60] It has been suggested that miR-27a accounts for the early development of hepatic insulin resistance by suppressing peroxisome proliferator-activated receptor gamma (PPARγ), INSR and IRS1.[59,60] With regard to miR-499a, it has been reported that low expression of miR-499a in the mouse liver increases the expression of PTEN, which impaired the PI3K/AKT/GSK3β pathway in the liver, leading to inhibition of hepatic glycogen synthesis.[14] Furthermore, Motohashi et al revealed that miR-128a downregulates the expression of INSR, INRS1, and PI3K in the muscle cells, resulting in reduced glucose uptake in skeletal muscle cells of mice.[58] In this study, we did not find a significant correlation between the SNPs miR-27a, miR-499a and miR-128a and T2DM. A study on T2DM showed that the miRNA-27a rs895819 C allele was a risk factor for T2DM in an overweight Han Chinese population in northern China.[61] Nevertheless, in a cohort study of Italians[62] and Iranians,[63] the miR-27a rs895819 C allele was shown to be a protective factor against T2DM. A study conducted in Rome showed that the miR-128a rs11888095 T allele increased the risk of DNP.[54] Furthermore, in 2023, Burada et al identified the miR-499 rs3746444 G allele as a risk factor for T2DM and DNP in a Romanian population.[64] One of the reasons for the discrepancy between different studies is the varying genetic backgrounds and ethnicities of the subjects. The other reason is that sample size may also be an important factor affecting the reliability of association studies. For example, in 2023, Zeng et al showed that the miR-27a rs895819 was associated with gestational diabetes mellitus, but further meta-analysis revealed no association between rs895819 and GDM.[12] The third reason could be that these miRNAs are involved in different pathways in T2DM and DNP.[54,55]

There are several limitations in the current study. Firstly, the body weight and BMI were useful to evaluate the enrolled subjects, however, we had not collected the data about the body weight and BMI, which was one of limitation in the current study. Besides, the expression levels of these miRNAs and predicted target genes in the plasma were not been validated in our subjects. Secondly, environmental factors are also important for influencing genetic susceptibility. We did not perform further analyses of the interaction effects of SNPs with environmental factors on T2DM, as we did not collect data on environmental factors in T2DM subjects. At last, further functional and molecular experiments are needed to test the effects of miRNA polymorphisms on T2DM.

Conclusion

SNP located in miRNAs affect miRNA expression and their interaction with target genes, thus further affecting the PI3K/AKT/GSK3β pathway in T2DM. Our results suggest that rs1076064 in the miR-378a gene and rs13283671 in the miR-31 gene are associated with T2DM susceptibility in the Chinese population.

Data Sharing Statement

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

Ethical Approval

The protocol complied with the Declaration of Helsinki in 1964 and its amendments, and was approved by the Institutional Review Board of the Second People's Hospital of Yunnan Province (2020093).

Acknowledgments

We appreciate all participants in the Second People's Hospital of Yunnan Province for their kind donation and cooperation.

Funding

This work was supported by grants from the Association Foundation Program of Yunnan Provincial Science and Technology Department and Kunming Medical University (202101AY070001-281), Yunnan Province "Xingdian Talent Support Program" famous doctor special (XDYC-MY-2022-0019), Yunnan Province clinical key specialty (Endocrinology).

Disclosure

All authors have no conflict of interest to declare.

References

1. Kumar A, Gangwar R, Zargar AA, Kumar R, Sharma A. Prevalence of Diabetes in India: a Review of IDF Diabetes Atlas 10th Edition. *Curr Diabetes Rev*. 2024;20(1):e130423215752. doi:10.2174/1573399819666230413094200
2. GBD 2021 Diabetes Collaborators. Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: a systematic analysis for the Global Burden of Disease Study 2021. *Lancet*. 2023;402(10397):203–234. doi:10.1016/S0140-6736(23)01301-6
3. Guo H, Wu H, Li Z. The Pathogenesis of Diabetes. *Int J mol Sci*. 2023;24(8):6978. doi:10.3390/ijms24086978
4. White MF. IRS proteins and the common path to diabetes. *Am J Physiol Endocrinol Metab*. 2002;283(3):E413–E422. doi:10.1152/ajpendo.00514.2001
5. Dajani R, Fraser E, Roe SM, et al. Crystal structure of glycogen synthase kinase 3 beta: structural basis for phosphate-primed substrate specificity and autoinhibition. *Cell*. 2001;105(6):721–732. doi:10.1016/S0092-8674(01)00374-9
6. Yang K, Chen Z, Gao J, et al. The key roles of GSK-3β in regulating mitochondrial activity. *Cell Physiol Biochem*. 2017;44(4):1445–1459. doi:10.1159/000485580
7. Beurel E, Grieco SF, Jope RS. Glycogen synthase kinase-3 (GSK3): regulation, actions, and diseases. *Pharmacol Ther*. 2015;148:114–131. doi:10.1016/j.pharmthera.2014.11.016
8. Nikoulina SE, Ciaraldi TP, Carter L, Mudaliar S, Park KS, Henry RR. Impaired muscle glycogen synthase in type 2 diabetes is associated with diminished phosphatidylinositol 3-kinase activation. *J Clin Endocrinol Metab*. 2001;86(9):4307–4314. doi:10.1210/jcem.86.9.7872
9. Jere SW, Houreld NN, Abrahamse H. Role of the PI3K/AKT (mTOR and GSK3β) signalling pathway and photobiomodulation in diabetic wound healing. *Cytokine Growth Factor Rev*. 2019;50:52–59. doi:10.1016/j.cytogfr.2019.03.001
10. Jiang Q, Wang Y, Hao Y, et al. miR2Disease: a manually curated database for microRNA deregulation in human disease. *Nucleic Acids Res*. 2009;37(Database issue):D98–104. doi:10.1093/nar/gkn714
11. Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet*. 2008;9(2):102–114. doi:10.1038/nrg2290
12. Zeng Q, Zou D, Liu N, et al. Association of miR-196a2 and miR-27a polymorphisms with gestational diabetes mellitus susceptibility in a Chinese population. *Front Endocrinol*. 2023;14:1127336. doi:10.3389/fendo.2023.1127336
13. Li Y, Li C, Yang M, et al. Association of single nucleotide polymorphisms of miRNAs involved in the GLUT4 pathway in T2DM in a Chinese population. *mol Genet Genomic Med*. 2019;7(9):e907. doi:10.1002/mgg3.907
14. Wang L, Zhang N, Pan H-P, Wang Z, Cao Z-Y. MiR-499-5p contributes to Hepatic insulin resistance by suppressing PTEN. *Cell Physiol Biochem*. 2015;36(6):2357–2365. doi:10.1159/000430198
15. Latouche C, Natoli A, Reddy-Luthmoodoo M, Heywood SE, Armitage JA, Kingwell BA. MicroRNA-194 modulates glucose metabolism and its skeletal muscle expression is reduced in diabetes. *PLoS One*. 2016;11(5):e0155108. doi:10.1371/journal.pone.0155108
16. Hrovatin K, Kunej T. Classification of miRNA-related sequence variations. *Epigenomics*. 2018;10(4):463–481. doi:10.2217/epi-2017-0126
17. Li Y, Yang J, Tao W, et al. The single nucleotide polymorphisms (rs1292037 and rs13137) in miR-21 were associated with T2DM in a Chinese population. *Diabetes Metab Syndr Obes*. 2022;15:189–198. doi:10.2147/DMSO.S345758
18. American Diabetes A. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes-2021. *Diabetes Care*. 2021;44(Suppl 1):S15–S33.
19. Buzzetti R, Tuomi T, Mauricio D, et al. Management of latent autoimmune diabetes in adults: a consensus statement from an international expert panel. *Diabetes*. 2020;69(10):2037–2047. doi:10.2337/dbi20-0017
20. Shi YY, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res*. 2005;15(2):97–98. doi:10.1038/sj.cr.7290272
21. Dupont WD, Plummer WD. Power and sample size calculations. A review and computer program. *Control Clin Trials*. 1990;11(2):116–128. doi:10.1016/0197-2456(90)90005-M
22. Dupont WD, Plummer WD. Power and sample size calculations for studies involving linear regression. *Controlled Clin Trials*. 1998;19(6):589–601. doi:10.1016/S0197-2456(98)00037-3
23. Solé X, Guinó E, Valls J, Iniesta R, Moreno V. SNPStats: a web tool for the analysis of association studies. *Bioinformatics*. 2006;22(15):1928–1929. doi:10.1093/bioinformatics/btl268
24. Shantikumar S, Caporali A, Emanueli C. Role of microRNAs in diabetes and its cardiovascular complications. *Cardiovasc Res*. 2012;93(4):583–593. doi:10.1093/cvr/cvr300
25. Fabris L, Ceder Y, Chinnaiyan AM, et al. The potential of microRNAs as prostate cancer biomarkers. *Eur Urol*. 2016;70(2):312–322. doi:10.1016/j.eururo.2015.12.054
26. Alevizos I, Illei GG. MicroRNAs as biomarkers in rheumatic diseases. *Nat Rev Rheumatol*. 2010;6(7):391–398. doi:10.1038/nrrheum.2010.81
27. Hu Z, Shu Y, Chen Y, et al. Genetic polymorphisms in the precursor microRNA flanking region and non-small cell lung cancer survival. *Am J Respir Crit Care Med*. 2011;183(5):641–648. doi:10.1164/rccm.201005-0717OC

28. An J, Liu J, Liu L, et al. A genetic variant in primary miR-378 is associated with risk and prognosis of hepatocellular carcinoma in a Chinese population. *PLoS One*. 2014;9(4):e93707. doi:10.1371/journal.pone.0093707
29. Jones A, Danielson KM, Benton MC, et al. miRNA signatures of insulin resistance in obesity. *Obesity*. 2017;25(10):1734–1744. doi:10.1002/oby.21950
30. Shepherd DL, Hathaway QA, Pinti MV, et al. Exploring the mitochondrial microRNA import pathway through Polynucleotide Phosphorylase (PNPase). *J mol Cell Cardiol*. 2017;110:15–25. doi:10.1016/j.yjmcc.2017.06.012
31. Liu W, Cao H, Ye C, et al. Hepatic miR-378 targets p110α and controls glucose and lipid homeostasis by modulating hepatic insulin signalling. *Nat Commun*. 2014;5:5684. doi:10.1038/ncomms6684
32. Wang H, Song Y, Wu Y, Kumar V, Mahato RI, Su Q. Activation of dsRNA-dependent protein Kinase R by miR-378 sustains metabolic inflammation in Hepatic insulin resistance. *Diabetes*. 2021;70(3):710–719. doi:10.2337/db20-0181
33. Carrer M, Liu N, Grueter CE, et al. Control of mitochondrial metabolism and systemic energy homeostasis by microRNAs 378 and 378*. *Proc Natl Acad Sci U S A*. 2012;109(38):15330–15335. doi:10.1073/pnas.1207605109
34. Jiang X, Xue M, Fu Z, et al. Insight into the effects of adipose tissue inflammation factors on miR-378 expression and the underlying mechanism. *Cell Physiol Biochem*. 2014;33(6):1778–1788. doi:10.1159/000362957
35. Feng M, Li Z, Aau M, Wong CH, Yang X, Yu Q. Myc/miR-378/TOB2/cyclin D1 functional module regulates oncogenic transformation. *Oncogene*. 2011;30(19):2242–2251. doi:10.1038/onc.2010.602
36. Gottmann P, Ouni M, Saussenthaler S, et al. A computational biology approach of a genome-wide screen connected miRNAs to obesity and type 2 diabetes. *mol Metab*. 2018;11:145–159. doi:10.1016/j.molmet.2018.03.005
37. Yu T, Ma P, Wu D, Shu Y, Gao W. Functions and mechanisms of microRNA-31 in human cancers. *Biomed Pharmacother*. 2018;108:1162–1169. doi:10.1016/j.biopha.2018.09.132
38. Sebastiani G, Valentini M, Grieco GE, et al. MicroRNA expression profiles of human iPSCs differentiation into insulin-producing cells. *Acta Diabetol*. 2017;54(3):265–281. doi:10.1007/s00592-016-0955-9
39. Sun F, Wang J, Pan Q, et al. Characterization of function and regulation of miR-24-1 and miR-31. *Biochem Biophys Res Commun*. 2009;380(3):660–665. doi:10.1016/j.bbrc.2009.01.161
40. Yao L, Tak YG, Berman BP, Farnham PJ. Functional annotation of colon cancer risk SNPs. *Nat Commun*. 2014;5:5114. doi:10.1038/ncomms6114
41. Han J, Lee Y, Yeom K-H, et al. Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex. *Cell*. 2006;125(5):887–901. doi:10.1016/j.cell.2006.03.043
42. Teli DM, Gajjar AK. Glycogen synthase kinase-3: a potential target for diabetes. *Bioorg Med Chem*. 2023;92:117406. doi:10.1016/j.bmc.2023.117406
43. Sankrityayan H, Kulkarni YA, Gaikwad AB. Diabetic nephropathy: the regulatory interplay between epigenetics and microRNAs. *Pharmacol Res*. 2019;141:574–585. doi:10.1016/j.phrs.2019.01.043
44. Zhuang L, Wang Z, Hu X, Yang Q, Pei X, Jin G. CircHIPK3 alleviates high glucose toxicity to human renal tubular epithelial HK-2 cells through regulation of miR-326/miR-487a-3p/SIRT1. *Diabetes Metab Syndr Obes*. 2021;14:729–740. doi:10.2147/DMSO.S289624
45. Tang Q, Len Q, Liu Z, Wang W. Overexpression of miR-22 attenuates oxidative stress injury in diabetic cardiomyopathy via Sirt 1. *Cardiovasc Ther*. 2018;36(2). doi:10.1111/1755-5922.12318
46. Mohamed JS, Hajira A, Pardo PS, Boriek AM. MicroRNA-149 inhibits PARP-2 and promotes mitochondrial biogenesis via SIRT-1/PGC-1α network in skeletal muscle. *Diabetes*. 2014;63(5):1546–1559. doi:10.2337/db13-1364
47. Santovito D, De Nardis V, Marcantonio P, et al. Plasma exosome microRNA profiling unravels a new potential modulator of adiponectin pathway in diabetes: effect of glycemic control. *J Clin Endocrinol Metab*. 2014;99(9):E1681–E1685. doi:10.1210/jc.2013-3843
48. Parker DC, Wan M, Lohman K, et al. Monocyte miRNAs are associated with type 2 diabetes. *Diabetes*. 2022;71(4):853–861. doi:10.2337/db21-0704
49. Meng Z, Liang B, Wu Y, et al. Hypoadiponectinemia-induced upregulation of microRNA449b downregulating Nrf-1 aggravates cardiac ischemia-reperfusion injury in diabetic mice. *J mol Cell Cardiol*. 2023;182.
50. Patti ME, Butte AJ, Crunkhorn S, et al. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: potential role of PGC1 and NRF1. *Proc Natl Acad Sci U S A*. 2003;100(14):8466–8471. doi:10.1073/pnas.1032913100
51. Zhou Q, Sun -W-W, Chen J-C, et al. Phenylalanine impairs insulin signaling and inhibits glucose uptake through modification of IRβ. *Nat Commun*. 2022;13(1):4291. doi:10.1038/s41467-022-32000-0
52. Choi Y, Hong S-H. Genetic association between miR-27a and miR-449b polymorphisms and susceptibility to diabetes mellitus. *Biomed Rep*. 2022;16(5):37. doi:10.3892/br.2022.1520
53. Alipoor B, Meshkani R, Ghaedi H, Sharifi Z, Panahi G, Golmohammadi T. Association of miR-146a rs2910164 and miR-149 rs2292832 Variants with Susceptibility to Type 2 Diabetes. *Clin Lab*. 2016;62(8):1553–1561. doi:10.7754/Clin.Lab.2016.160124
54. Ciccacci C, Morganti R, Di Fusco D, et al. Common polymorphisms in MIR146a, MIR128a and MIR27a genes contribute to neuropathy susceptibility in type 2 diabetes. *Acta Diabetol*. 2014;51(4):663–671. doi:10.1007/s00592-014-0582-2
55. Latini A, Borgiani P, De Benedittis G, et al. Mitochondrial DNA copy number in peripheral blood is reduced in type 2 diabetes patients with polyneuropathy and associated with a MIR499A gene polymorphism. *DNA Cell Biol*. 2020;39(8):1467–1472. doi:10.1089/dna.2019.5326
56. Karolina DS, Tavintharan S, Armugam A, et al. Circulating miRNA profiles in patients with metabolic syndrome. *J Clin Endocrinol Metab*. 2012;97(12):E2271–E2276. doi:10.1210/jc.2012-1996
57. Srivastava A, Shankar K, Beg M, et al. Chronic hyperinsulinemia induced miR-27b is linked to adipocyte insulin resistance by targeting insulin receptor. *J Mol Med*. 2018;96(3–4):315–331. doi:10.1007/s00109-018-1623-z
58. Motohashi N, Alexander MS, Shimizu-Motohashi Y, Myers JA, Kawahara G, Kunkel LM. Regulation of IRS1/Akt insulin signaling by microRNA-128a during myogenesis. *J Cell Sci*. 2013;126(Pt 12):2678–2691. doi:10.1242/jcs.119966
59. Benito-Vicente A, Uribe KB, Rotllan N, et al. miR-27b modulates insulin signaling in Hepatocytes by regulating insulin receptor expression. *Int J mol Sci*. 2020;21(22):8675. doi:10.3390/ijms21228675
60. Wu H, Pula T, Tews D, et al. microRNA-27a-3p but not −5p is a crucial mediator of human adipogenesis. *Cells*. 2021;10(11):3205. doi:10.3390/cells10113205

61. Wang -T-T, Chen Y-J, Sun -L-L, Zhang S-J, Zhou Z-Y, Qiao H. Affection of single-nucleotide polymorphisms in miR-27a, miR-124a, and miR-146a on susceptibility to type 2 diabetes mellitus in Chinese Han people. *Chin Med J*. 2015;128(4):533–539. doi:10.4103/0366-6999.151112
62. Ciccacci C, Di Fusco D, Cacciotti L, et al. MicroRNA genetic variations: association with type 2 diabetes. *Acta Diabetol*. 2013;50(6):867–872. doi:10.1007/s00592-013-0469-7
63. Ghaedi H, Tabasinezhad M, Alipoor B, et al. The pre-mir-27a variant rs895819 may contribute to type 2 diabetes mellitus susceptibility in an Iranian cohort. *J Endocrinol Invest*. 2016;39(10):1187–1193. doi:10.1007/s40618-016-0499-4
64. Burada E, Roşu -M-M, Sandu RE, et al. miR-499a rs3746444 A>G polymorphism is correlated with type 2 diabetes mellitus and diabetic polyneuropathy in a Romanian cohort: a preliminary study. *Genes*. 2023;14(8):1543. doi:10.3390/genes14081543