The relation between CircRNA0056618 and type 2 diabetes mellitus and insulin resistance through miRNA-206/PTPn-1 pathway

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Background
Diabetes mellitus (DM) is a well-known metabolic syndrome characterized by hyperglycemia produced by a defect in insulin synthesis, insulin action, or a combination of the two.
Objective
The aim of this work was to find out how circular RNA 0056618 (circRNA 0056618) interacts with miRNA-206 to control blood sugar levels in type-2 diabetes mellitus (T2DM), as well as to test the role of PTPN-1 gene expression and protein phosphatase-2 (PP2A), insulin receptor substrate (IRS) protein level in diabetes. Also, whether circRNA 0056618, miRNA-206, PTPN-1, IRS, and PP2A protein could be used as biomarkers for T2DM diagnosis and prognosis.
Patients and methods
This cross-sectional analytic study was carried out on 110 patients. Participants were divided into two equal groups: patients' group (T2DM) and control group (normal participants). All participants were subjected to quantitative real-time PCR for assessed RNAs (circRNA 0056618, miRNA-206, and PTPn-1 gene expression), enzyme-linked immunosorbent assay technique for IRS, and PP2A protein levels.
Statistical analysis used
The Statistical Package for the Social Sciences (SPSS), version 28 was used to code and enter the data. All data will be presented as means and SDs. Correlations between quantitative variables will be done using Pearson correlation coefficient. Receiver operating characteristic (ROC) curve was constructed with area under the curve (AUC) analysis performed to detect the best cutoff value of significant parameters for detection of cases. *P* value will be considered significant less than 0.05.
Results and conclusion
There was a significant increase in circRNA 0056618 ($P<0.001$), PTPN-1 gene expression ($P=0.002$), and PP2A protein levels ($P<0.001$) and a significant decrease in miRNA-206 gene expression and IRS protein levels in diabetic cases ($P<0.001$) when compared with normal controls. There was a negative correlation between circRNA 0056618 and miRNA-206 and a positive correlation between circRNA 0056618 and PTPN-1. IRS at 0.840 showed 94.5% sensitivity and 90.9% specificity and AUC 0.905. PP2A at 0.868, sensitivity 96.4% and specificity 81.8%, and AUC 0.919. ROC curve for circRNA 0056618, at 0.882, sensitivity 89.1% and specificity 87.3%, and AUC 0.932, miRNA-206 at 0.785, sensitivity 85.5% and specificity 85.5%, and AUC 0.869. ROC curve for PTPN-1 at 0.556, sensitivity 67.3% and specificity 67.3%, and AUC 0.669.
We concluded that circRNA 0056618, PTPN-1, PP2A, miRNA-206 and IRS are considered diagnostic, predictive biomarkers in T2DM. Future RNA-based therapy approaches may benefit from an understanding of such new pathways.

Keywords:
circRNA0056618, diabetes mellitus type 2, insulin resistance, insulin receptor substrate, miRNA-206/PTPn-1 pathway, protein phosphatase-2

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Introduction

Diabetes is commonly associated with a slew of complications, including blindness, kidney failure, cardiovascular disease, stroke, and lower-limb amputation, all of which not only cause serious long-term damage but also result in a significant financial burden on the global healthcare system [1].

Many metabolic processes are mediated by micro-ribonucleic acid (microRNA), a noncoding RNA

that is substantially conserved in helminths, animals, and humans. Its ability to attach to the 30 nontranscribed sequences of the targeted mRNA for silencing matching target genes depends on its binding capacity [2].

With their high stability and conserved expression, several miRNAs have been identified as biomarkers in clinical medicine. They also play an important role in glucose homeostasis and type 2 diabetes mellitus (T2DM) regulation [3]. Because of their regulatory functions in the initiation and progression of the disease, miRNAs have attracted a lot of attention in the search for efficient biomarkers for early, prompt diagnosis and novel treatment target modalities for T2DM [4–7]. In the liver, cholesterol-induced miRNA-206 has been discovered as a powerful repressor of glucose and fat synthesis. MiRNA-206 targets protein tyrosine phosphatase, nonreceptor type 1 (PTPN-1), which can dephosphorylate insulin receptors (INSR), insulin receptor substrate (IRS), and protein phosphatase-2 (PP2A), impairing insulin signaling but enhancing lipogenesis through PTPN-1 pathways [8].

Circular ribonucleic acids (circRNAs) could act as microRNA (miRNA) sponges, regulating cellular activity after transcription [9]. CircRNAs also have the ability to control host gene expression via cis-acting regulation of gene expression [10]. Biologists used bioinformatics analysis to investigate the underlying mechanism and interrelationships of molecules. CircRNAs can sponge miRNA to regulate the targeted gene, as we all know. Using bioinformatics, we predicted miRNA sponged by circRNA0056618. MiRNA-206 was sponged with circRNA0056618, according to the analysis and results.

The aim of this work is to determine the role of circRNA0056618 and its sponging with miRNA-206 in glycemic control in T2DM, as well as to explain the role of PTPN-1 gene expression in the prediction of diabetes and its consequences. Furthermore, our study aims to demonstrate the potential of circRNA0056618, miRNA-206, PTPN-1, IRS protein, and PP2A protein as biomarkers for the diagnosis and prognosis of T2DM.

Materials and methods

The sample size was estimated as 51 patients for each group using the G*Power v3 software (A RED VENTURES COMPANY: USA, South Carolina) with a significance level of 0.05, an effect size of 0.5, and a power of 0.8. Based on this assumption, a total of 110 participants in the two studied groups were considered adequate.

This study was carried out on 110 participants aged from 20 to 65 years old, both sexes, with T2DM, as defined by the ADA, FBG level of 126 mg/dl or higher, 2-h PPG serum glucose level of 200 mg/dl, and HbA1c level of 6.5% or higher from March 2020 to April 2021.

The study was done after approval from the Ethical Committee Medical Biochemistry Department, Faculty of Medicine, Cairo University (Approval number: MD-83-2020). An informed written consent was obtained from the patient.

Exclusion criteria were advanced kidney disease, advanced liver disease, malignancies, pregnant and lactating women, untreated hypertension, and patients with evidence of autoimmune disorders.

Participants were divided into two groups:

Control group (55 patients): with normal FBG and 2-h PPG serum glucose level and normal KFTs.

Patients' group (55 patients): with T2DM have been included in the study.

All participants were subjected to.

Clinical evaluation

Biochemical and molecular investigations [biochemical analysis (complete blood count (CBC), liver function test (LFT), kidney function test (KFT), C-reactive protein (CRP), uric acid, fasting blood glucose (FBG), 2-h postprandial blood glucose (2-h PPG), fasting blood lipids; total cholesterol (TC), triglycerides (TAG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and HbA1c were done to all patients and controls)].

Quantitative real-time PCR for assessing RNAs (circRNA 0056618, miRNA-206, and PTPN-1 gene expression).

ELISA technique is used for IRS and PP2A protein-level measurement.

Sample collection

Trained laboratory staff collected 6 ml of peripheral venous blood from each participant after they had

fasted for 8 h and after eating for 2 h wkm n345n6 in EDTA vacutainer tubes that were used and centrifuged for 10 min at 4000×*g*. Plasma was separated and frozen at −80°C until RNA extraction. Another portion of blood was kept in plain tubes and left to clot for 15 min before centrifugation at 4000×*g* to collect serum for chemical and protein-level analysis.

Molecular analysis [11,12]

RNA extraction

Zymo Research Corp., USA's Direct-zol RNA Miniprep Plus for total RNA, including short RNA extraction (Catalog number# R2072), was used to extract total RNA. Using the NanoDrop (ND)-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, USA), the purity of RNA samples was examined. The total RNA yield was determined at 260 and 280 nm.

Circular RNA and ordinary mRNA gene expression

The prepared reaction mix samples were applied in real-time PCR (StepOne Applied Biosystem, Foster City, USA). Thermo Fisher Scientific's SuperScript IV One-Step RT-PCR kit was compatible with three-step cycling.

MiRNA gene expression

TransScript Green miRNA Two-Step qRT-PCR SuperMix kit (Catalog Number#AQ202-01) was used for reverse transcription of targeted miRNAs and quantitative RT-PCR. All primer sequences (Table 1).

The data were expressed in cycle threshold after the RT-PCR experiment (Ct). The Ct values of the examined gene (circ0056618, miRNA-206, and PTPN1) versus the housekeeping genes.

ELISA technique for human protein phosphatase 2A, PP2A levels: BT LAB bioassay technology laboratory provided the kit (Catalog Number# E6870Hu) for quantitative measurement of PP2A protein levels.

Table 1 Primer sequences of all studied genes

Gene symbol	Primer sequence from 5′ to 3′ F: forward primer, R: reverse primer
Circ-0056618	F: GAACCCACCCCACCTCTAC R: CTTCCCCGGGATAAACAAACC
miRNA-206	F: CGGGCTTGTGGAATGGTAAGC R: GGGCATACATCGGCTAATACA
PTPN-1	F: TTCTGAGCTGGGCTTGTTGT R: TGCAGCTAAAATGCAAACCCAT
GAPDH	F: 5′-ACCCACTCCTCCACCTTTGA R: CTGTTGCTGTAGCCAAATTCGT
U6	F: CGCTTCACGAATTTGCGTGTC R: TAAAACGCAGCTCAGTAACAGTC

ELISA technique for human IRS protein level LSBio provided the kit that was used. This ELISA kit (Catalog Number# LS-F9692) uses the Sandwich-ELISA technique to detect human IRS protein in blood, plasma, cell culture supernatants, tissue homogenates, and other biological fluids.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS), Version 28 was used to code and enter the data (IBM Corp., Armonk, New York, USA). In quantitative data, mean, SD, median, minimum, and maximum were used, while categorical data were summarized using frequency (count) and relative frequency (%). For normally distributed quantitative variables, the unpaired *t* test was employed, whereas for non-normally distributed quantitative variables, the nonparametric Mann–Whitney test was utilized [13]. The χ^2 test was used to compare categorical data. When the anticipated frequency is less than 5, the exact test was utilized instead [14]. The Spearman correlation coefficient was used to calculate correlations between quantitative variables [15]. Receiver operating characteristic (ROC) curve was constructed with area under the curve (AUC) analysis performed to detect the best cutoff value of significant parameters for detection of cases. Statistical significance was defined as a *P* value of less than 0.05.

Results and discussion

There was a statistically significant difference regarding occupation and eating habits and there was no statistically significant difference regarding age, sex, BMI, HCV, and sun exposure per day (Table 2).

Diabetes cases have a substantial increase in fasting blood glucose, HbA1c, microalbumin, triglycerides, and LDL (P=0.001), and have a significant increase in creatinine level (P=0.011), also, there was a substantial rise in HDL, vitamin D, and platelet count (P=0.001) when compared with normal controls (Table 3).

There was a significant increase in circRNA 0056618 (P<0.001), PTPN-1 (P=0.002) gene expression, and in PP2A protein levels (P<0.001) in diabetic cases when compared with normal controls. There was a significant decrease in IRS protein levels (P<0.001) and in miRNA-206 gene expression (P<0.001) in diabetic cases when compared with normal controls (Table 4).

There was a negative correlation between circRNA 0056618 and miRNA-206, IRS protein level also

Table 2 Demographic data for the studied groups

	Cases (*N*=55)	Controls (*N*=55)	*P* value
Age (years)	52.91±6.49	51.31±4.95	0.149
Sex			
Male	15 (27.3)	24 (43.6)	0.073
Female	40 (72.7)	31 (56.4)	
Occupation			
Worker	0	2 (3.6)	<0.001
Shopkeeper	1 (1.8)	2 (3.6)	
Retired	8 (14.5)	3 (5.5)	
Housewife	39 (70.9)	23 (41.8)	
Employer	7 (12.7)	22 (40.0)	
Driver	0	3 (5.5)	
BMI			
Normal	5 (9.1)	3 (5.5)	0.538
Overweight	20 (36.4)	16 (29.1)	
Obese	30 (54.5)	36 (65.5)	
Eating habit			
Normal	35 (63.6)	52 (94.5)	<0.001
Diet	20 (36.4)	3 (5.5)	
Sun exposure per day			
Rare	15 (27.3)	3 (5.5)	0.001
Short time	40 (72.7)	49 (89.1)	
Long time	0	3 (5.5)	
Current treatment			
Tablets	35 (63.6)	0	–
Insulin	20 (36.4)	0	
HCV (rapid test)			
Positive	3 (5.5)	3 (5.5)	1
Negative	52 (94.5)	52 (94.5)	

Data expressed as mean±SD and *n* (%). *P* value less than 0.05 and 0.001 was significant.

between miRNA-206 and PTPN-1, PP2A protein level also between PTPN-1 and IRS level, and between IRS level and PP2A. There was a positive correlation between circRNA 0056618 and PTPN-1, PP2A protein levels also between miRNA-206 and IRS, and triglycerides among cases also between PTPN-1 and PP2A level (Table 5).

The ROC curve for IRS showed that the best-chosen cutoff level was (<3.65), at which the sensitivity was 94.5% and the specificity was 90.9%. The AUC was 0.90, for PP2A plasma levels was more than 0.62, at which the sensitivity was 96.4% and the specificity was 81.8%. The AUC was 0.919. The ROC curve for circRNA0056618 gene expression: the best-chosen cutoff level was more than 2.95, at which the sensitivity was 89.1% and the specificity was 87.3%. The AUC was 0.93, for miRNA-206 gene expression was less than 0.95, at which the sensitivity was 85.5% and the specificity was 85.5%. The AUC was 0.869. The ROC curve for PTPN-1 gene expression showed that the best-chosen cutoff level was more than 1.18, at which the sensitivity was 67.3% and the specificity was 67.3%. The AUC was 0.669 (Fig. 1a, b).

CircRNAs are noncoding RNA molecules with covalently closed ends but no 5′ or 3′ ends. CircRNAs are widely expressed in humans, according to new computational approaches [16]. CircRNAs can range in length from a few hundred to several thousand nucleotides [17]. Furthermore, circRNAs are numerous, conserved, cell-specific RNA molecules, implying that they are not unintentional results of splicing errors. CircRNAs are key regulators of different diseases and cellular physiologies via influencing gene expression,

Table 3 Comparison between laboratory parameters among the studied groups

	Cases (*N*=55) (mean±SD)	Controls (*N*=55) (mean±SD)	*P* value
Fasting blood glucose (mg/dl)	264.65±71.73	90.24±10.87	<0.001
HbA1c (%)	6.96±0.39	4.79±0.22	<0.001
SGPT (ulU/ml)	18.76±5.04	19.05±5.49	0.767
Creatinine (mg/dl)	0.87±0.14	0.80±0.15	0.011
Albumin (g/dl)	3.88±0.25	3.97±0.33	0.085
Microalbumin (mg/dl)	47.09±51.69	9.18±5.01	<0.001
Calcium (mg/dl)	8.96±0.55	8.88±0.34	0.334
Phosphorus (mg/dl)	3.53±0.47	3.49±0.47	0.671
INR	1.00±0.01	1.00±0.02	0.757
Triglycerides (mg/dl)	211.25±48.10	147.25±24.89	<0.001
Cholesterol (mg/dl)	177.65±38.61	169.51±31.61	0.229
HDL (mg/dl)	49.47±12.91	60.40±13.99	<0.001
LDL (mg/dl)	130.20±38.91	94.74±22.90	<0.001
Vitamin D (ng/ml)	13.29±4.02	35.93±4.64	<0.001
Hb (g/dl)	12.22±0.53	12.22±0.66	0.987
RBCs (million/mm^3)	4.47±0.73	4.37±0.53	0.420
WBCs (K/μl)	6.61±2.13	6.68±1.84	0.848
Platelets (mcl)	271.69±57.59	318.71±30.74	<0.001

Data expressed as mean±SD. *P* value less than 0.05 was significant.

Table 4 CircRNA-0056618, PTPN-1 gene expression, IRS, PP2A protein levels, and miRNA-206 gene expression among the studied groups

	Cases	Controls	*P* value
circRNA-0056618	3.67	1.45	<0.001
PTPN-1	1.83	1.12	0.002
IRS	1.86	4.33	<0.001
PP2A	1.74	0.64	<0.001
miRNA-206	0.54	1.04	<0.001

Data expressed as median (range). *P* value less than 0.05 was significant.

Table 5 Correlation between circRNA-0056618 and miRNA-206, PTPN-1, IRS, and PP2A protein level, between miRNA-206 and PTPN-1, IRS, and PP2A, between PTPN-1 and IRS, PP2A, and platelet level, between PP2A and IRS level, age, fasting blood glucose and hemoglobin, and also correlation between miRNA-206 and triglycerides among cases

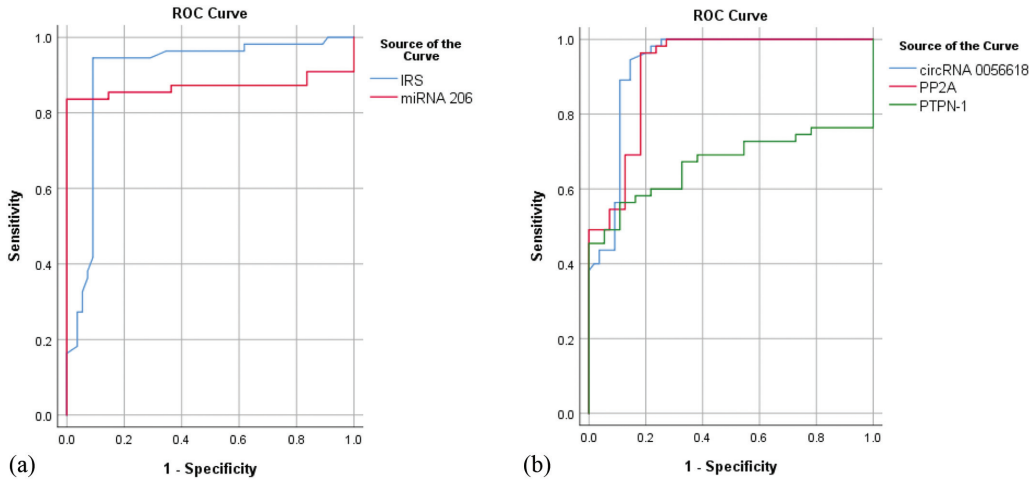
	r	*P* value
circRNA-0056618		
miRNA-206	−0.6657	<0.0001
PTPN-1	0.6846	<0.0001
IRS	−0.647	<0.0001
PP2A	0.5478	<0.0001
miRNA-206		
PTPN-1	−0.5718	<0.0001
IRS	0.3972	0.0003
PP2A	−0.5411	<0.0001
PTPN-1		
IRS	−0.4924	<0.0001
PP2A	0.5154	<0.0001
Platelets	−0.268	0.048
PP2A		
IRS	−0.4958	<0.0001
Age	0.372	0.005
Fasting blood glucose	0.705	<0.001
Hemoglobin	−0.269	0.047
MicroRNA-206		
Triglycerides	0.289	0.033

r, correlation coefficient.

according to recent research. They also operate as microRNA sponges, regulating posttranscriptional gene expression. CircRNA expression changes in various disorders led to the conclusion that circRNAs could be used as diagnostic and prognostic biomarkers. CircRNAs could possibly be translated into proteins, according to the researchers [18].

miRNAs are small noncoding RNAs with a length of about 22 nucleotides that target messenger RNA breakdown to regulate gene expression. Evidence suggests that dysregulated miRNA expression may have a role in the development of a variety of diseases, including T2DM. In the early stages of the disease, miRNA may be a useful and unique diagnostic biomarker [19]. PTPN-1 is a nonreceptor tyrosine phosphatase family (EC3.1.3.48). PTNPs are known to play a role in the etiology of IR and T2DM due to their relevance in the regulation of insulin signaling. Their expression was found to be higher in insulin-sensitive tissues, and it was thought to inhibit insulin signaling. Furthermore, PTPN-1 is reported to be expressed on tissues impacted by diabetic complications [20]. The IRS, an intracellular cytoplasmic adaptor protein, is phosphorylated by the IR. The binding and phosphorylation of IRS increase glucose absorption from the circulation into muscle cells and adipose tissue by boosting the expression of GLUT4 molecules on the outer membranes of these tissues. IR and DM are caused by defects in their expression and function in the insulin signaling pathway. IRS gene polymorphisms

Figure 1



ROC curve for (a) miRNA 206 gene expression and IRS ELISA levels, (b) for circRNAs 0056618 and PTPN-1 gene expressions and PP2A ELISA levels. IRS, insulin receptor substrate; ROC, receiver operating characteristic.

have been shown to play a pathogenic role in the development of T2DM [21]. PP2A is a serine/threonine phosphatase that preserves cell homoeostasis by inhibiting most kinase-driven signaling pathways in cells [22].

In our study, we found that there was a significant increase in HbA1c and micro-albumin ($P<0.001$) and creatinine level in diabetic cases when compared with normal controls ($P=0.011$).

This finding was discussed early by Lian *et al.* [23]. They observed a highly significant ($P=0.001$) positive link between HbA1C percent and diabetic kidney disease. In the current study, there was a significant increase in vitamin D and HDL in normal controls when compared with diabetic cases ($P<0.001$). This finding was also suggested by Ismail *et al.* [24], who implied that there is a link between this finding and the development of problems in diabetic patients. In our study, we found that there was a significant increase in platelet count in healthy controls compared with diabetic patients ($P<0.001$). Inversely, in a study conducted by Rodriguez and Johnson [25], they detected no link between platelet function, quantity, or aggregation with T2DM in both men and women. Our study revealed that there was a significant increase in circRNA 0056618 gene expression in diabetic cases when compared with normal controls ($P<0.001$). In a cross-sectional study conducted by Yan *et al.* [26], they revealed that there are numerous forms of circRNAs and their sponging with miRNA that could contribute to the development of DM, particularly T2DM. In gestational DM patients, according to Jiang *et al.* [27], microarray analysis was used to determine the expression of plasma exosomal circRNAs. They discovered that 1-mm FFA produced lipid accumulation. Stoll *et al.* [28], using a microarray technique, discovered that ciRS-7, a circRNA that has been linked to insulin secretion regulation, may have a role in the development of diabetes. They also discovered that circRNAs from intron of insulin gene (ci-INS) and ci-Ins2 are critical for normal insulin secretion.

In a study conducted by Zheng *et al.* [29], they discovered that in cases compared with controls, circRNA is upregulated in blood samples ($P=0.001$). In addition, circRNA expression is also linked to age ($r=0.259$, $P=0.001$), HDL levels ($r=0.196$, $P=0.009$), and glucose levels ($r=0.204$, $P=0.006$). These findings suggest that circRNA is involved in the development of diabetes and could be used as a predictor and diagnostic marker. In a study conducted by El-Hefnway *et al.* [30], they found that circRNA expression was significantly higher than in normal controls ($P=0.05$). Furthermore, they discovered that circRNAs, in combination with BMI, were an excellent predictor of prediabetes, implying that circRNAs could be an effective predictive and diagnostic biomarker for T2DM. In our current study, there was a significant decrease in miRNA-206 gene expression in diabetic cases when compared with normal controls ($P<0.001$). T2DM induces a decrease in miRNA-206 and AMPK, and T2DM is connected to muscle atrophy, according to this study. In a study performed by Lu *et al.* [31], they discovered that the level of serum miRNA-206 expression might be used to diagnose osteoporosis. In a study performed by Li *et al.* [3], they stated that dysregulation of miRNA was linked to the recurrence of cancer stem cells, and that quantitative PCR research indicated that an increase in seven miRNAs, including miRNA-206, significantly suppressed the proliferation of human malignant fibrous histiocytoma, suggesting that miRNA could be used as a marker and target for cancer diagnosis and treatment in the future. In our study, we revealed that there was a negative correlation between microRNA-206 and circ-0056618 ($r=-0.6657$, $P\leq0.0001$). Using bioinformatics analysis, Zheng *et al.* [32] predicted that miRNA-206 was the target of circ0056618 in a trial to understand the mechanism by which miRNA-206 controlled angiogenesis, migration, and proliferation in colorectal cancer cells. Their findings suggested that miR-206 may be a target for circ0056618, which has been linked to the development of malignancies. They investigated for miR-206 expression in colorectal cancer tissue samples and matching nontumor tissues. They revealed that miR-206 was downregulated in colorectal cancer tissues, particularly in patients with metastasis ($P=0.001$) compared with patients without metastasis ($P=0.001$). Additionally, they observed that the levels of miR-206 elevated following transfection of cells with circ0056618 ($P=0.001$). These findings showed that miR-206, which may be circ0056618's putative target, and circ0056618 had a negative correlation.

Chen *et al.* [33] discovered that the miRNA-206/KLB pathway can be used to diagnose and treat hepatoblastoma. They also performed another study that tested Adropin's preventive action as a secretory peptide against glucose and lipid metabolic problems is mediated by its effects on PP2A. In another study done by Wang [34], they speculated that miRNA-206 could play a role in both DM and CRC. In our study, we

found that there was a significant increase in PTPN-1 gene expression in diabetic cases when compared wiiith normal controls (P=0.002). In a study conducted by Yamakage *et al.* [35], they revealed that individuals with the T allele in the PTPN-1 SNP rs3787348 had significantly smaller BMI, bodyweight, and waist circumference decreases with weight loss medication (P=0.001). This research revealed that PTPN-1 polymorphisms are connected to weight loss therapy failure in obese Japanese people as determined by BMI and waist circumference. In addition, according to Villamar-Cruz *et al.* [36], the stimulation of several anti-apoptotic signaling pathways by PTPN-1 is hypothesized to play a role in the development of many cancers, as well as T2DM through the modulation of insulin and leptin signaling. Trials to suppress PTPN-1 activity or mutations in this gene have been discovered to have a direct effect on malignant transformations and the development of IR, which leads to diabetes. Several PTPN-1 effectors, including the INRS, IRS-1, and JAKs/STATs, have been linked to T2DM, obesity, and immune response modulation. The function and signaling pathways regulated by PTPN-1 appear to be cell-specific, according to research. Finally, PTPN-1's numerous activities and regulatory mechanisms may be crucial in the development of more targeted therapy options for diabetes and cancer. Byeon *et al.* [37] discovered that silencing PTPN-1 reduces the phosphorylation of various proteins in cells, including Akt, p38, and JNK and ER-stress response proteins. As a result, PTPN-1 could be a promising therapeutic target for DM diagnostics and treatment as an anti-inflammatory and antioxidant. Moreover, Krishnan *et al.* [38] concluded that small compounds could increase insulin and leptin signaling by stabilizing PTPN-1 in an inactive, oxidized form. This study demonstrated a novel paradigm for suppressing aberrant PTPN-1 signaling, which could pave the way for a new therapeutic function in diabetes and obesity.

In our study, we showed that there was a negative correlation between miRNA-206 and PTPN-1 (r=–0.5718, P≤0.0001). In study conducted by Wu *et al.* [39], they revealed that there was a negative correlation between microRNA-206 and PTPN-1 as they found that microRNA-206 improved insulin signaling by encouraging INSR (insulin receptor) phosphorylation and hampered hepatic lipogenesis by suppressing Srebp1c transcription and PTPN-1 expression. MicroRNA-206 decreased the production of lipid (P=0.006) and glucose (P=0.018) in human hepatocytes and the livers of fed obese mice (P=0.001 and 0.01, respectively). This was accomplished by simultaneously influencing lipogenesis and insulin signaling. PTPN-1 was reintroduced into livers, which counteracted microRNA-206's inhibitory effects. This suggests that PTPN-1 mediates microRNA-206's inhibitory effects on hepatosteatosis and hyperglycemia. Additionally, Sohail *et al.* [40] revealed that there was a negative correlation between PTPN-1 and IRS (r=–0.4924, P≤0.0001) in consistent with another study that revealed that PTPN-1 inhibited the phosphorylation of IR and IRS. Therefore, inhibition of PTPN-1 resulted in increased expression of IR, IRS1, PI3K, and GLUT4 in adipose tissues and IR, IRS, and PI3K in the pancreas in the diabetic-treated group as compared with diabetic group (P≤0.05). In our current study, we found that there was a significant decrease in IRS protein levels in diabetic cases when compared with normal controls (P<0.001). In one in-vivo study performed by Atmodjo *et al.* [41], they revealed that combining a high-fat meal with streptozotocin increased fasting blood glucose while lowering IRS and Langerhans' β-islets in diabetics (P=0.232). The scientists determined that a decrease in IRS was negatively associated with damage to the Langerhans' β-islets, hinting that there is a stage in-between both. According to Bedair *et al.* [42], the IRS polymorphism has been discovered to play a function in the development of T2DM. The IRS mutant allele (A) implies that this SNP may be a risk factor for T2DM in otherwise healthy people. Increased BMI may be a risk factor in and of itself for the development of T2DM. In our study, we revealed that there was a negative correlation between circRNA0056618 and IRS (r=–0.647, P≤0.0001). In another study, Rashad *et al.* [43] conducted to reveal the association between insulin receptor signaling and insulin resistance development, the authors also revealed a negative correlation between circRNA and IRS (r=–0.466, P≤0.001), indicating that circRNAs may play a role in inhibiting insulin signaling in patients with T2DM . In addition, our study revealed that there was a positive correlation between miRNA-206 and IRS (r=0.3972, P=0.0003), there were several miRNAs identified that modulate IR expression through binding to the 3-UTR of IR mRNA, downregulating the mRNA and protein levels of IR. Additionally, many miRNAs were found to control the amounts of IR mRNA, which affects insulin signaling. As regards Liu and Liu [44], miRNAs are shown to modify insulin signaling via modulating IR downstream elements in addition to directly controlling IR expression. Finally, in our study, we

found that there was a significant increase in PP2A protein levels in diabetic cases when compared with normal controls ($P<0.001$). In this study, PP2A activity and the expression of PP2Ac were upregulated in IR. Adropin treatment decreased PP2Ac expression and its binding with AMPKα leads to gluconeogenesis inhibition in IR hepatocytes. Overall, they identified inhibition of PP2A as the upstream molecular event in adropin-mediated AMPK activation in IR as PP2A was normally elevated in diabetic cases. Adropin could be a viable target for diabetes prevention and treatment. In our study, we found that there is a positive correlation between circRNA0056618 and PP2A ($r=0.5478$, $P\leq0.0001$). In a recent study conducted by Liu *et al.* [45], the authors tested the relation between circRNA and PP2A in inhibition of gastric cancer progression by targeting PP2A, they revealed that there was a protein named CM-248aa encoded by circRNA and its overexpression in cancer cells leads to failure of inhibition of PP2A and when the protein downregulated, the PP2A inhibited its activity, leading to activation of downstream pathways and progression of cancer.

Limitations

The sample size was relatively small. The study was in a single center. A future study aims to investigate the mechanism that reveals how circRNA 0056618 and miRNA regulate PTPN-1 gene expression and its link to T2DM. More research is needed into the canonical pathway of the PTPN-1 gene, circRNA 0056618, and miRNA-206 and to determine the involvement of IRS and PP2A in T2DM screening and early diagnosis. Prospective analytical and interventional investigations may be useful in determining the involvement of PP2A and IRS in the pathogenesis of T2DM, as well as modulating new treatment methods.

Conclusion

Expressions of circRNA 0056618, miRNA-206, PTPN-1, PP2A, and IRS protein levels could be used as diagnostic and prognostic tools and potential therapeutic targets in the future.

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Conflicts of interest

There are no conflicts of interest.

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