Research Article

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Diagnostic value and bioinformatics-based mechanistic exploration of serum miR-7977 in diabetic peripheral neuropathy

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Abstract

Objectives: Peripheral neuropathy, as a complication in patients with type 2 diabetes mellitus (T2DM), is easily overlooked in the early stage. The aim of this study was to investigate the significance of microRNA-7977 (miR-7977) for early warning of T2DM combined with diabetic peripheral neuropathy (DPN).
Methods: The study included T2DM patients two groups: those without DPN (n=63) and those with DPN (n=62). Quantitative Real-Time Polymerase Chain Reaction was used to detect serum levels of miR-7977 and mouse double minute 2 homolog (MDM2). Receiver Operating Characteristic Curve was used to assess their diagnose potential for DPN. Serum levels of malondialdehyde (MDA) and antioxidant markers (glutathione peroxidase and superoxide dismutase) were measured. Bioinformatics and dual luciferase reporter assays were used to validate MDM2 as a target of miR-7977.
Results: Serum miR-7977 levels were elevated in T2DM patients compared to healthy controls and further increased in DPN patients. Elevated miR-7977 was an independent risk factor for DPN and was positively correlated with MDA and negatively correlated with antioxidant markers in patients. MDM2, as a target gene of miR-7977, showed an opposite trend of expression to that in patients. Serum MDM2 combined with miR-7977 can efficiently diagnose T2DM combined with DPN.
Conclusions: miR-7977 and MDM2 may serve as a novel biomarker for early DPN diagnosis. miR-7977 may promote oxidative stress process by targeting MDM2, suggesting a potential therapeutic pathway.

Keywords: type 2 diabetes mellitus; diabetic peripheral neuropathy; miR-7977; MDM2

Introduction

In the 21st century, diabetes mellitus has emerged as a ubiquitous health challenge worldwide, with projections by the International Diabetes Federation indicating that the global diabetic population will soar to 366 million by 2030 [1]. Type 2 diabetes mellitus (T2DM) is the main type of diabetes. Diabetic Peripheral Neuropathy (DPN), a frequent yet often overlooked complications of T2DM [2]. Epidemiological studies indicate that approximately 50 % of individuals with T2DM develop DPN, predominantly presenting as distal symmetric sensorimotor polyneuropathy characterized by bilateral sensory deficits, hyperalgesia, and paresthesia [3, 4]. Severe patients with DPN are at risk of amputation or even death, which has a greater impact on physical and mental health and quality of life [5, 6]. Recent cohort analyses further highlight its prevalence in early-stage T2DM [7], underscoring the urgency for proactive screening and mechanistic investigations.

MicroRNAs (miRNAs) are not coding for proteins but can induce mRNA degradation or inhibit protein translation by binding to the 3′-UTR region of mRNAs of target genes. The important role of miRNAs in disease development has been gradually revealed in recent years [8]. Various studies have demonstrated that the production and release of miRNAs are affected by the effects of prolonged hyperglycemia [9]. Aberrantly expressed miRNAs have been found to play an important regulatory role in diabetes and its complications [10]. Multiple literature has confirmed the involvement of miRNAs in the development and progression of DPN. Zhang et al. pointed out that miR-25 is a potential diagnostic and therapeutic target for DPN, and what they discovered in DPN model mice was that miR-25 expression level was reduced and

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attenuated oxidative stress-induced cellular damage by inhibiting the expression of reactive oxygen species (ROS) [11]. miR-193a was found to have the potential to be a diagnostic DPN marker and to alleviate diabetic neuropathic pain by inhibiting HMGB1 expression [12, 13]. miRNAs have enriched the idea of prevention and treatment of DPN. However, the mechanism of miRNA involvement in the pathogenesis of DPN is complex, and many miRNAs involved in DPN have not yet been elucidated, so more studies are still needed to elucidate and confirm them and provide new ideas for the diagnosis and treatment of DPN.

miR-7977 is a novel miRNA that has been found to serve as a diagnostic marker for lung adenocarcinoma and is involved in the regulation of cancer cell proliferation and apoptosis [14]. In bone marrow mesenchymal stromal cells, miR-7977 can regulate the development of leukemia by modulating the Yes-associated protein 1 (YAP1) signaling pathway [15]. Among the few reports on miR-7977, one report caught our attention, in which they pointed out that there is a correlation between insulin levels and miR-7977 expression, and miR-7977 regulates kidney injury triggered by high blood glucose levels [16]. From this, it was hypothesized that miR-7977 may be somehow associated with the development of T2DM and DPN.

To preliminarily investigate the role of miR-7977 in DPN and T2DM, healthy volunteers, patients with DPN and T2DM were included in this study, and the clinical baseline data of the subjects were analyzed to investigate the clinical value of miR-7977 in DPN and T2DM.

Methods and materials

Research object

The study was approved by the Affiliated Hospital of Panzhihua University Ethics Committee, and the study subjects all signed an informed consent statement.

Study participants included three groups:

T2DM patients with DPN (n=62) and T2DM patients without DPN (NDPN, n=63), selected from 356 patients with T2DM who attended Affiliated Hospital of Panzhihua University between 2020 and 2022. DPN diagnosis was confirmed by neuromuscular electromyography and clinical criteria [17]. Healthy controls (n=58), recruited from age- and sex-matched individuals undergoing routine health checkups during the same period, with no history of diabetes, neuropathy, or chronic systemic diseases.

Inclusion criteria for T2DM patients: (1) age ≥18 years; (2) meeting the 1999 World Health Organization (WHO) diagnostic criteria for T2DM [18].

Exclusion criteria (applied to all groups): (1) patients with a history of cerebral infarction, intervertebral disc herniation, motor neuron disease, retinal degeneration, and facial neuropathy; (2) Malignancies, acute infections, cardiovascular lesions, or severe hepatic/renal dysfunction.

The sample size was determined through analytic triangulation using G*Power 3.1 (one-way ANOVA). Informed by empirical evidence from Ji et al.'s three-group comparison study [19] where n=150 demonstrated adequate power, we first reverse-calculated an effect size of Cohen's f=0.275. To ensure methodological conservatism [20], we deliberately adopted a smaller effect size (f=0.25) with α=0.05 and power=0.8, yielding a minimum requirement of 159 participants (53/group). Our final enrollment of 183 participants achieved 85 % statistical power for this conservative parameter configuration.

Basic data collection

All participants underwent fasting venous blood collection (10 mL) from the cubital vein in the morning using EDTA-containing anticoagulant tubes. Samples were centrifuged at approximately 1,500×g (relative centrifugal force, rcf) for 15 min at 4 °C within 30 min of collection. Serum was separated, aliquoted into cryotubes, and stored at −80 °C until analysis.

Clinical data of the study subjects were collected, including gender, age, smoking and drinking status, duration of disease; weight, height and calculation of body mass index (BMI); measurement of systolic blood pressure (SBP), diastolic blood pressure (DBP); Serum levels of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured using an automated biochemistry analyzer (Cobas 8000 C702, Roche, Mannheim, Germany) with enzymatic colorimetric assays for TC/TG and homogeneous enzymatic methods for LDL-C/HDL-C. Glycated haemoglobin (HbA1c) was measured by high performance liquid chromatography using a specific analyzer (Bio-Rad D-10 Hemoglobin Testing System, Hercules, CA, USA). The ACCU-CHECK blood glucose meter (Roche, Germany) was applied to detect fasting blood glucose (FPG) by glucose oxidase method.

QRT-PCR

Total RNA was extracted from serum or cell samples with TRIzol Reagent (Invitrogen, USA) and high-quality total RNA was synthesized into cDNA by PrimeScript RT reagent kit

(Vazyme, Nanjing, China). Next, cDNA was then amplified and quantified using SYBR Green PCR kit (Takara, Japan), and the reaction system and conditions were carried out according to the protocol provided in the manual. Primer sequences for miR-7977 (Forward: 5′- CGCGTTCCCAGCCAA C-3′; Reverse: 5′- AGTGCAGGGTCCGAGGTATT-3′) and MDM2 (Forward: 5′-TGTTTGGCGTGCCAAGCTTCTC-3′; Reverse: 5′-CACAGATGTACCTGAGTCCGATG-3′) were synthesized by Sangon Biotech (Shanghai, China). Amplification efficiency was 95–105 % (R^2>0.99) and melt curve analysis confirmed single-product specificity. GAPDH and U6 corresponded to serve as the internal reference genes for MDM2 mRNA and miR-7977, respectively, and the relative expression levels of both were calculated by $2^{-\Delta\Delta Ct}$.

Measurements of the levels of MDA, SOD and GSH

The serum levels of malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione (GSH), which are related to oxidative stress, were measured by ELISA (Enzyme-Linked Immunosorbent Assay). The levels of MDA, SOD and GSH were measured using commercial ELISA kits (Nanjing Jiancheng Bioengineering Institute), following the manufacturers' protocols. The kits had sensitivities of 0.1 nmol/mL, 5 U/mL and 10 nmol/mL respectively, with intra-assay CVs of less than 6 % and inter-assay CVs of less than 10 %. The linear ranges were 0.5–50 nmol/mL, 20–500 U/mL and 50–1,000 nmol/mL respectively. Different concentrations of standards were prepared according to the instructions, and a blank standard curve was set up. After 50 L of sample was added into 96-well plate, the enzyme-labeled antibody was added and incubated at 37 °C for 60 min. Then the colour developer was added to it, and 50 L of termination solution was added after 5 min. The absorbance value of each well was detected at 450 nm using an automatic microplate reader (Beijing Liuyi Biotechnology Co., Ltd., China), and the content of each index was calculated.

Cell culture and double luciferase reporter gene assay

The cryopreserved rat Schwan cells RSC96, retrieved from −80 °C storage, were revived and then propagated in a DMEM-based growth medium supplemented with 10 % FBS and 1 % antibiotic cocktail (penicillin-streptomycin). The culture flasks were incubated in a 37 °C, 5 % CO_2 incubator and passaged when the cell density reached 80 % of the flask.

The predicted 3′ -UTR sequences of mutual binding between MDM2 and miR-7977 were used as a template to design wild-type (WT) and mutant (MT)-MDM2 primers for the dual luciferase gene reporter and synthesized by Sangon Biotech (Shanghai) Co., Ltd. The primers were amplified and inserted into pmirGLO vector respectively. Based on the experimental steps of the instructions of the Lipofectamine2000 transfection kit (Thermo Fisher, Shanghai, China), miR-NC, miR-7977 mimic or miR-7977 inhibitor were mixed with the WT-MDM2 and MT-MDM2 plasmids, respectively, and transfected into the RSC96 cells that had completed the fourth generation of passaging culture. After 48 h of incubation, luciferase activity was measured.

Bioinformatics analysis

The downstream targets of miR-7977 were forecasted utilizing the TargetScan (https://www.targetscan.org/vert_80/), miRDB (https://mirdb.org/), and miRWalk (http://mirwalk.umm.uni-heidelberg.de/) platforms. The selection thresholds were set as follows: (1) a target score exceeding 70 in miRDB; (3) a binding score of at least one in miRWalk. The common genes underwent GO (Gene Ontology) analysis and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment evaluation. The GO categorization encompasses three levels, delineating genes based on their participation in biological processes (BP), molecular functions (MF), and cellular components (CC). The focus of the KEGG analysis was to pinpoint the pathways in which these genes are implicated.

Subsequently, the common genes identified across these databases were integrated into the STRING database (https://string-db.org/) to construct a comprehensive Protein-Protein Interaction (PPI) network.

Statistical analysis

SPSS 24.0 statistical package was used to process the data. Normality was assessed via Shapiro-Wilk test (p>0.05 for all groups), and homogeneity of variance was confirmed using Levene's test (p>0.05). Continuous variables were presented as mean with standard deviation (M±SD). Group comparisons were performed as follows: Independent samples t-test for two groups meeting parametric assumptions) or Mann-Whitney *U* test (non-parametric). One-way ANOVA for (for multi-group comparisons with normality and variance homogeneity) or Kruskal-Wallis test (non-parametric), followed by Tukey's HSD or Dunn's post-hoc tests with Bonferroni correction. For categorical data, the χ^2 test was

employed. The link between serum miR-7977 levels and clinical manifestations of DPN patients was examined through Pearson's correlation analysis. Multiple logistic regression analysis was conducted to identify factors influencing the risk of DPN. Furthermore, the diagnostic efficacy of serum miR-7977 and MDM2 levels in DPN was evaluated by constructing receiver operating characteristic (ROC) curves. The optimal cut-off value for biomarkers in ROC analysis was determined by maximizing the Youden index (sensitivity + specificity − 1). When the p-value is less than 0.05, it indicates that the differences studied are statistically significant.

Results

Comparison of baseline data

Analyzing the baseline data of the study subjects (Table 1), the levels of FBG, HbA1c and MDA were markedly higher in both groups of diabetic patients than in the control group (p<0.001), while there was a significant decrease in the levels of SOD and GSH-PX (p<0.001). In addition, DPN patients showed a significant difference in all the above indicators compared to NDPN patients, and the duration of the disease was also longer (<0.001).

Association of miR-7977 expression with clinical characteristics in DPN patients

We further analyzed the association between miR-7977 expression and these indicators in DPN patients. The serum miR-7977 mean expression value (1.66 ΔCt) derived from the entire DPN cohort was used as the threshold to categorize patients into high- and low-expression groups. As shown in Table 2, significant differences between the two groups were observed in the duration of disease (p=0.020), FBG (p=0.004), HbA1c (p=0.005), MDA (p=0.001), SOD (p=0.002) and GSH-PX (p=0.031).

The logistic regression model was designed to differentiate DPN (yes=1) from NDPN (no=0) groups; healthy controls were excluded to focus on DPN-specific risk factors. Table 3 presents the logistic regression analysis of risk factors for T2DM combined with DPN. miR-7977 was dichotomized into low/high expression groups using a cut-off value of 1.5 (ΔCt) based on its mean expression level in T2DM patients (<1.5=0, ≥1.5=1). Other variables included in the model – duration of diabetes, FBG, HbA1c, MDA, SOD, and GSH-PX – were selected based on their significant differences between DPN and NDPN groups in Table 1 (p<0.05) and their established biological relevance to oxidative stress and diabetic complications in prior studies. As shown in Table 3, elevated miR-7977 expression

Table 1: Comparison of clinical characteristics between groups.

Parameter	Control (n=58)	NDPN (n=63)	DPN (n=62)	Overall p-Value	Control vs. NDPN	NDPN vs. DPN	Control vs. DPN
Age, year	47.97 ± 13.06	49.78 ± 11.54	49.27 ± 13.39	0.712	0.712	0.839	0.901
Gender[a]	30/28	27/36	33/29	0.329	–	–	–
BMI, kg/m^2	24.21 ± 2.16	24.83 ± 2.32	25.18 ± 2.55	0.317	–	–	–
Smoking[a]	24/34	31/32	33/29	0.072	–	–	–
Drinking[a]	37/21	35/28	38/24	0.356	–	–	–
Duration, year[b]	–	8.06 ± 5.52	11.94 ± 4.43	<0.001[b]	–	<0.001	–
FBG, mmol/l	4.73 ± 0.56	7.74 ± 0.81	11.71 ± 2.62	<0.001	<0.001	<0.001	<0.001
HbA1c, %	4.42 ± 0.76	7.79 ± 0.67	10.16 ± 0.99	<0.001	<0.001	<0.001	<0.001
DBP, mmHg	73.78 ± 8.34	74.99 ± 8.41	76.77 ± 9.1	0.725	–	–	–
SBP, mmHg	105.66 ± 9.45	108.09 ± 11.38	107.16 ± 11.45	0.437	–	–	–
TC, mmol/l	3.96 ± 0.66	4.1 ± 0.72	4.12 ± 0.67	0.490	–	–	–
TG, mmol/l	1.2 ± 0.41	1.3 ± 0.41	1.22 ± 0.41	0.379	–	–	–
LDL-C, mmol/l	2.64 ± 0.37	2.77 ± 0.4	2.8 ± 0.38	0.159	–	–	–
HDL-C, mmol/l	1.33 ± 0.1	1.36 ± 0.13	1.38 ± 0.12	0.385	–	–	–
MDA, nmol/l	5.81 ± 1.61	12.51 ± 2.69	18.25 ± 3.73	<0.001	<0.001	<0.001	<0.001
SOD, U/ml	169.25 ± 24.67	134.52 ± 22.64	92.47 ± 17.92	<0.001	<0.001	<0.001	<0.001
GSH-PX, U/ml	365.76 ± 21.59	257.49 ± 23.98	205.41 ± 38.97	<0.001	<0.001	<0.001	<0.001

BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin; MDA, malondialdehyde; SOD, superoxide dismutase; GSH-PX, glutathione peroxidase; DPN, diabetic peripheral neuropathy; NDPN, no diabetic peripheral neuropathy.[a]Categorical variables analyzed by chi-square test. [b]Duration compared between NDPN, and DPN, only using Mann-Whitney *U* test (non-parametric). Overall p-values derived from: ANOVA (parametric, normally distributed data), Kruskal-Wallis test (non-parametric). Post-hoc pairwise comparisons: Tukey's HSD, for ANOVA (parametric). Dunn's test for Kruskal-Wallis (non-parametric). Bonferroni-adjusted significance level: α=0.017. Post-hoc pairwise comparisons were not performed if the overall ANOVA/KW p-value was >0.05 (e.g., LDL-C, TC, HDL-C).

Table 2: Association between miR-7977 expression and clinical characteristics in DPN patients.

Parameter	patients (n=62)	miR-7977 expression		p-Value
		Low (n=28)	High (n=34)	
Duration of disease, year				0.020
≤11.94	32	19 (59.38 %)	13 (40.63 %)	
>11.94	30	9 (30.00 %)	21 (70.00 %)	
FBG, mmol/l				0.004
≤11.71	39	23 (58.97 %)	16 (41.03 %)	
>11.71	23	5 (21.74 %)	18 (78.26 %)	
HbA1c, %				0.005
≤10.16	32	20 (62.50 %)	12 (37.50 %)	
>10.16	30	8 (26.67 %)	22 (73.33 %)	
MDA, mmol/l				0.001
≤18.25	30	20 (66.67 %)	10 (33.33 %)	
>18.25	32	8 (25.00 %)	24 (75.00 %)	
SOD, U/ml				0.002
≤92.47	29	7 (24.14 %)	22 (75.86 %)	
>92.47	33	21 (63.64 %)	12 (36.36 %)	
GSH-PX, U/ml				0.031
≤205.41	27	8 (29.63 %)	19 (70.37 %)	
>205.47	35	20 (57.14 %)	15 (42.86 %)	

FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin; MDA, malondialdehyde; SOD, superoxide dismutase; GSH-PX, glutathione peroxidase; DPN, diabetic peripheral neuropathy.

Table 3: Logistic regression analysis of risk factors for DPN.

Parameters	B	Standard error	p-Value	OR	95 % CI
miR-7977	1.339	0.521	0.01	3.815	1.375–10.585
Duration of diabetes	1.236	0.53	0.02	3.442	1.218–9.728
FBG	1.106	0.537	0.04	3.021	1.054–8.659
HbA1c	1.148	0.552	0.038	3.153	1.068–9.31
MDA	1.118	0.547	0.041	3.06	1.048–8.933
SOD	−1.284	0.525	0.015	0.277	0.099–0.775
GSH-PX	−1.195	0.541	0.027	0.303	0.105–0.874

FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin; MDA, malondialdehyde; SOD, superoxide dismutase; GSH-PX, glutathione peroxidase; DPN, diabetic peripheral neuropathy.

(OR=3.22, p=0.01, 95 % CI: 1.38–10.59), longer diabetes duration (OR=3.44, p=0.02), poor glycemic control (FBG: OR=3.02, p=0.04; HbA1c: OR=3.15, p=0.038), and oxidative stress markers (MDA: OR=3.06, p=0.041; SOD: OR=0.28, p=0.015; GSH-PX: OR=3.03, p=0.027) were independently associated with DPN risk.

Diagnostic role of miR-7977 in DPN

The expression of miR-7977 was remarkably increased sequentially in control, NDPN and DPN (Figure 1A). Using the Youden index, miR-7977 showed an AUC of 0.843, with an optimal cut-off value of 1.505 (specificity 80.65 %, sensitivity 80.90 %) for diagnosing DPN (Figure 1B).

Correlation between the expression of miR-7977 and oxidative stress indexes

Positive correlations were found between miR-7977 expression and serum levels of FBG (Figure 2A, r=0.512, p<0.0001), HbA1c (Figure 2B, r=0.558, p<0.0001) and MDA (Figure 2C, r=0.509, p<0.0001). miR-7977 expression was found to be inversely correlated with GSH-PX (Figure 2D, r=−0.539, p<0.0001) and SOD (Figure 2C, r=−0.561, p<0.0001).

Targeting relationship between miR-7977 and MDM2

The 134 target genes of miR-7977 were obtained by searching the intersection of two databases (Figure S1A). The results of GO function analysis indicate that the primary biological processes encompass the Notch signaling pathway and the positive regulation of vasculogenesis. The cellular components predominantly involve ribonucleoprotein granules and the postsynaptic density membrane. The molecular functions of the relevant targets mainly consist of binding to DNA-binding transcription factors and hormone binding, as illustrated in Figure S1B. The KEGG enrichment pathways are focused on endocrine resistance and the MAPK signaling pathway, as shown in Figure S1C. The overlapping genes were submitted to the STRING database for protein-protein interaction analysis and network construction. The resultant PPI network comprises 134 nodes and 456 edges (p=0.009, Figure S1D). Notably, MDM2 occupies an important hub position in the PPI network. MDM2 was found to be dramatically downregulated in DPN (Figure 3A) and was dramatically negatively related to miR-7977 (r=−0.574, p<0.0001, Figure 3B). In addition, this study verified the targeting relationship between miR-7977 and MDM2 via dual luciferase gene assay report (Figure 3C) and discovered that miR-7977 could considerably negatively regulate the expression of MDM2 (Figure 3D). The ROC analysis revealed that the AUC of MDM2 for the diagnosis of DPN was 0.875, optimal cut-off of 0.625 with sensitivity (80.65 %) and specificity (88.89 %) (Figure 3E). In addition, the combined model integrating miR-7977 and MDM2 exhibited enhanced discriminatory power (AUC=0.921), with sensitivity and specificity of 90.32 and 88.89 %, respectively (Figure 3F).

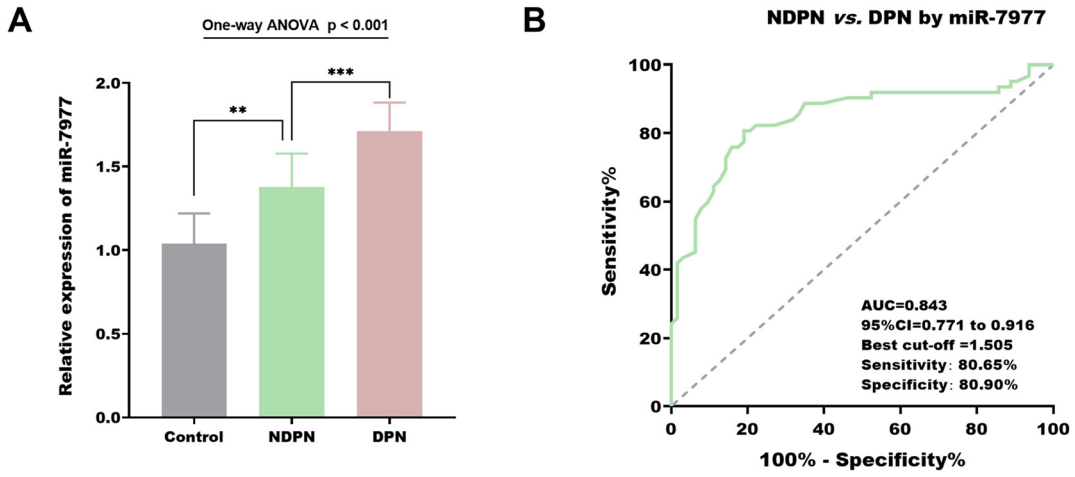

Figure 1: Expression and diagnostic value of miR-7977 in diabetic peripheral neuropathy (DPN). Serum miR-7977 expression (A). One-way ANOVA, p<0.001; Tukey's post-hoc test: Control vs. NDPN, **p<0.01; NDPN vs. DPN, ***p<0.001. ROC evaluated the ability of miR-7977 to distinguish between NDPN and DPN (B).

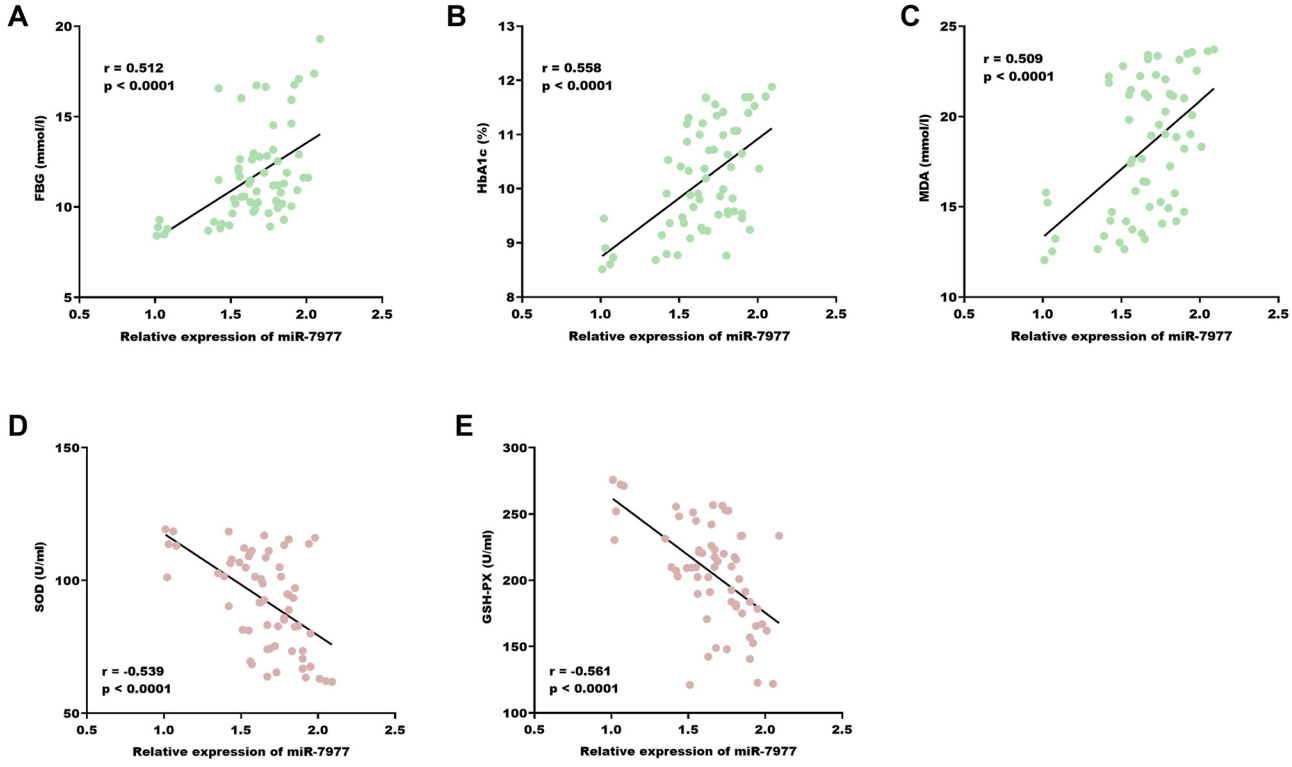

Figure 2: Pearson correlation of miR-7977 expression with FBG (A), HbA1c (B), MDA (C), SOD (D) and GSH-PX in DPN patients.

Discussion

The onset of T2DM combined with DPN is insidious, and the disease is diagnosed at a later stage, which is difficult to be reversed. Currently, most of the diagnostic methods for DPN are traumatic and expensive, which are difficult to be widely used in clinical practice [21]. An efficient and non-invasive method is urgently necessary for the early screening of T2DM combined with DPN. In recent years, miRNA has gradually become an ideal candidate for early disease diagnostic tools. For example, Li et al. found that miR216a and miR377 were abnormally expressed in DPN patients and found that both of them could efficiently predict DPN by ROC analysis [22]. It has been suggested that miR-186-5p can be

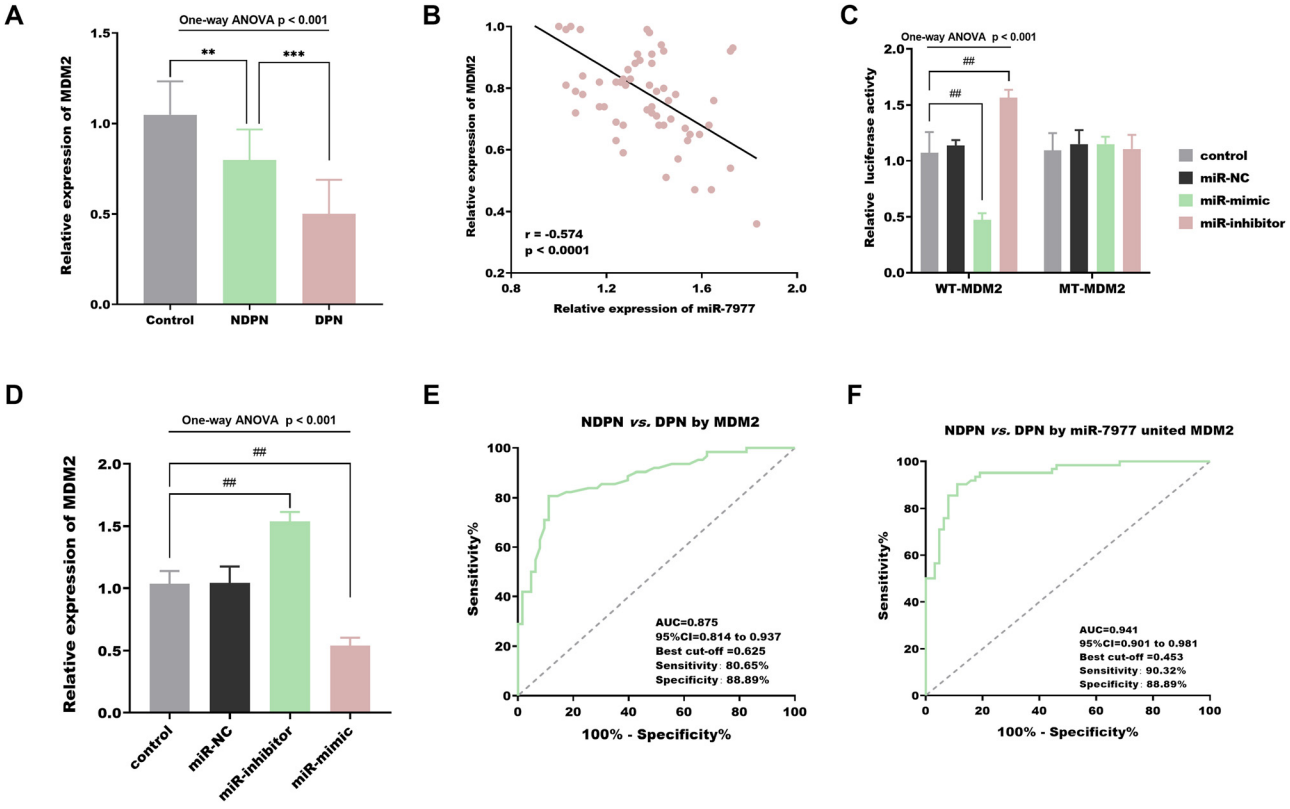

Figure 3: Expression of MDM2 and its interaction with miR-7977 in DPN pathogenesis. Serum MDM2 mRNA expression (A). One-way ANOVA, p<0.001; Tukey's post-hoc test: Control vs. NDPN, **p<0.01; NDPN vs. DPN, ***p<0.001. Pearson correlation of miR-7977 expression with MDM2 (B). Verification of MDM2-miR-7977 binding using a dual-luciferase gene reporter system (C). One-way ANOVA, p<0.001; Tukey's post-hoc test: control vs. miR-mimic, ##p<0.001; control vs. miR-inhibitor, ##p<0.001. The regulation of MDM2 by miR-7977 (D). One-way ANOVA, p<0.001; Tukey's post-hoc test: control vs. miR-mimic, ##p<0.001; control vs. miR-inhibitor, ##p<0.001. ROC curve assessing the diagnostic performance of MDM2 in distinguishing T2DM patients with DPN (E). ROC analysis evaluating the combined diagnostic efficacy of miR-7977 and MDM2 for DPN identification (F).

used as a predictor for the diagnosis of DPN in patients with T2DM. [23]. Therefore, this study focused on the investigation of miRNA. In this study, comparing the expression levels of serum miR-7977 in the healthy control group, NDPN group and DPN group, a statistically significant difference was found, in which miR-7977 expression was significantly higher in DPN patients than in NDPN. Therefore, it was speculated that miR-7977 has a relationship with the occurrence of DPN.

Studies have shown that cells in the hyperglycemic state can release highly expressed miRNAs that inhibit neural axon growth and exacerbate the extent of DPN pathology [13, 24, 25]. Such as, miRNA-29a was found to be significantly up-regulated in response to prolonged hyperglycemia, exacerbating the extent of peripheral nerve damage [26]. In this study, it was shown that the risk of combined DPN was higher with longer duration of T2DM, and there was a significant difference between high and low miR-7977 expression groups in the duration of the disease. In DPN patients miR-7977 expression trend was positively and significantly correlated with FBG and HbA1c. Similarly, Gao et al. discovered that miR-7977 showed significant upregulation under hyperglycemic infiltration [16]. Based on the above evidence, it is speculated that prolonged hyperglycemia induces miR-7977 expression, thereby exacerbating the extent of damage to peripheral nerves.

Studies suggest that hyperglycemia can lead to oxidative stress damage in nerves by activating multiple pathological pathways and inducing the production of large amounts of ROS [27, 28]. There was a finding that SOD was remarkably down-regulated in DPN mice, and miR-106a overexpression significantly inhibited high glucose-induced oxidative stress response and promoted SOD secretion [29]. Lin et al. noted that miR-34a overexpression exacerbated oxidative stress in DPN rats, while miR-34a silencing inhibited MDA release, increased the concentration of promoted GSH and SOD, and exerted a neuroprotective effect [30]. In the present study, it was found that the serum release of SOD and GSH-PX was reduced, and MDA was increased in DPN patients, which was consistent with previous studies, suggesting that nerve injury was closely related to oxidative stress [31]. Further, it found that miR-7977

was significantly correlated with the above oxidative stress indicators, that is, the higher the degree of oxidative stress, the higher the expression of miR-7977 in the serum of DPN patients. Ji et al. also discovered that rising insulin levels were accompanied by an increase in miR-7977, which activated oxidative stress response in epithelial cells by inhibiting SIRT3 [32]. From this, it can be speculated that miR-7977 may be involved in the development of T2DM combined with DPN by regulating oxidative stress mechanisms, causing degeneration and necrosis of peripheral nerve tissue.

Bioinformatics analysis has uncovered that the downstream target genes of miR-7977 are implicated in diabetes mellitus-related peripheral neuropathy, for example involving the positive regulation of vasculogenesis and the MAPK signaling pathway. Research indicates that hyperglycemia-triggered microangiopathy disrupts neural blood flow and nutrient supply, with abnormal neovascularization being a crucial factor contributing to neurological impairments [33, 34]. The MAPK pathway plays a pivotal role in the development of DPN by modulating inflammatory responses, oxidative stress, and apoptotic processes in nerve cells [35]. These findings imply a significant role for miR-7977 and its downstream targets in DPN progression. In this study, MDM2, which occupies a key pivotal position in PPI, was taken as an example, and its role in DPN was preliminarily investigated. It was investigated that MDM2 was significantly decreased in DPN and miR-7977 could target and regulate MDM2 in DPN. In addition, miR-7977 combined with MDM2 significantly improved the discrimination of DPN. Zheng et al. observed that the expression pattern of MDM2 in T2DM mirrors the findings of our study [36]. Additionally, research has shown that MDM2 is implicated in ovarian dysfunction through its regulation of oxidative stress in granulosa cells [37]. Similarly, in the context of diabetic cataracts, MDM2 has been demonstrated to reduce oxidative stress in human lens epithelial cells exposed to a high-glucose environment [38]. These observations underscore the close relationship between MDM2 and the progression of T2DM, as well as its role in modulating cellular oxidative stress responses. Consequently, it is speculated that miR-7977 may contribute to the development of T2DM-associated neuropathy by targeting MDM2, leading to the activation of intracellular oxidative stress.

This study has several limitations. First, the single-center design with a limited cohort size may restrict the generalizability of findings. Second, while clinical correlations and *in vitro* experiments were conducted, the absence of *in vivo* validation in animal models limits mechanistic insights into the miR-7977/MDM2 pathway in diabetic neuropathy. Additionally, potential synergistic effects of other regulatory pathways on DPN progression remain unexplored. Future work should prioritize multicenter validation with larger cohorts and establish diabetic neuropathy animal models to delineate miR-7977's molecular interplay. Further investigations could also explore therapeutic interventions targeting miR-7977 and integrate oxidative stress biomarkers into predictive algorithms for early DPN detection, advancing translational applications.

In conclusion, this study found that miR-7977 may be an independent risk factor for T2DM combined with DPN and may be involved in peripheral nerve injury by targeting MDM2 to regulate oxidative stress. miR-7977 in combination with MDM2 may be a new serum biomarker for early screening of T2DM combined with DPN. However, the specific mechanism needs to be further elaborated by expanding the sample size or through animal studies.

Research ethics: The research protocol was approved by the Ethics Committee of Affiliated Hospital of Panzhihua University (No. 2019-231).
Informed consent: Informed consent was obtained from all individuals included in this study.
Author contributions: All authors designed this study. Z.L. and L.L. conducted the experiment and analyzed the data. Z.L. wrote the manuscript. L.L. revised the manuscript. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
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