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Integrating exosome wide associations study and Mendelian randomization identified causal miRNAs for type 2 diabetes mellitus and its complications

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Abstract

Background Type 2 diabetes mellitus (T2DM) and its complications, including diabetic lower extremity arterial disease (DLEAD) and diabetic foot (DF), impose significant health burdens worldwide. However, the differential expression of microRNAs (miRNAs) between T2DM and its complications and its causal effects remain poorly understood.

Methods We conducted an exosome-wide association study (EWAS) comparing miRNA profiles between T2DM and its complications, including DLEAD and DF, without healthy controls. The significant miRNAs identified between DM and its complications were further validated by integrating cis-miRNA expression quantitative trait loci (cis-miR-eQTLs) and genome-wide association study (GWAS) summary data of T2DM and peripheral arterial disease (PAD) through two-sample Mendelian randomization (MR) analysis.

Results We identified several differential expressions of miRNAs between T2DM, DLEAD, and DF, such as hsa-miR-409-3p between T2DM and DLEAD, hsa-miR-543 between T2DM and DF and hsa-miR-206 between DLEAD and DF. The two sample MR analysis revealed potential causal relationships between dysregulated miRNAs and T2DM and its complications, such as hsa-miR-30b-3p and hsa-miR-30b-5p showed causal associations with T2DM and PAD respectively.

Conclusions Our study elucidates the miRNA signatures associated with T2DM and its complications. These findings provide insights into the pathogenesis of T2DM and its complications and suggest potential therapeutic targets for intervention.

Keywords Exosome, Type 2 diabetes mellitus, Diabetic lower extremity arterial disease, Diabetic foot, Causal inference

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Background

Type 2 diabetes mellitus (T2DM) is one of the leading causes of death and disability worldwide, and affects people regardless of country, age group, or sex [1]. In 2021, there were about 507.84 million people living with T2DM worldwide, and the global age-standardized total T2DM prevalence was 5.49% [2]. Peripheral arterial disease (PAD) is a major cause of nonhealing ulcers, lower limb amputation and mortality, especially in people with T2DM [3]. Lower extremity arterial disease (LEAD), as a kind of PAD, is a serious vascular complication of T2DM, associated with important disability, cardiovascular risk, and socio-economic burden [4]. China-DiaLEAD has declared that the severity of diabetic LEAD (DLEAD) increases with age and the duration of diabetes, leading to a higher risk of DLEAD patients with diabetic foot (DF) and amputation than in nondiabetic individuals [5]. The underlying pathophysiology of diabetic vascular complications is complex, involving endothelial dysfunction, chronic inflammation, oxidative stress, and impaired angiogenesis [6–8]. These processes contribute to vascular damage and hinder tissue repair, ultimately increasing the risk of foot ulceration and limb loss. Thus, it is urgent to develop biomarkers for earlier diagnosis and treatment of T2DM and its complications.

T2DM and its vascular complications are complex, multifactorial conditions with both major environmental and genetic components. The environmental factors include being overweight, unhealthy diet, not getting enough exercise, and smoking [9]. Recently, benefit from the explosion of new genomic datasets, both in terms of biobanks and aggregation of worldwide cohorts has more than doubled the number of genetic discoveries for both T2DM and its complications [10–12]. Together these top genome-wide association studies (GWAS) signals explain > 17% of the phenotypic variance in T2DM [11]. In addition, a sequencing study interrogated multi-ethnic exome sequencing of ~ 21,000 cases and ~ 24,000 non-diabetic controls identified four exome-wide significant gene-level associations of T2DM, such as *SLC30A8*, *PAM* and *MC4R* [13]. Gene-level analysis identified *KCNJ11* and *ANK1* as associated with the cross-trait of T2D and PAD in both sexes, while multi-trait GWAS revealed two novel European-specific SNPs (rs927742 and rs1734409) linked to their shared genetic basis [14, 15]. However, the etiology of T2DM and the progress of its complications remain elusive.

miRNAs are small non-coding RNAs that are widespread and highly conserved, accounting for about 1–2% of non-protein-coding genes [16, 17]. They block mRNA expression by pairing with mRNA, which in turn inhibits and degrades mRNA [16, 18]. miRNAs have been found to be important regulatory molecules in the development of T2DM through multiple pathways, such as the regulation of glycolipid metabolism, liver glycogen metabolism, and insulin secretion [19–21]. It has been demonstrated that mir-21 is a promising biomarker that can diagnose DF. The re-epithelization of the wound is significantly delayed due to the reduction in the expression of miR-21 by inhibiting the fibroblasts and keratinocytes migration [22]. However, there is a lack of studies comparing differential miRNAs between T2DM and its complications, such as DLEAD and DF, as well as studies exploring causal associations between and miRNAs and those complex conditions.

In this study, to evaluate the potential of circulating miRNAs as biomarkers for T2DM and its complications, we first conducted an exosome wide association study of T2DM and its complications, including DLEAD and DF. Two-sample Mendelian randomization (MR) analysis were further used to explore the causal relationship between those identified miRNAs and the risk of T2DM and its complications. Using pooled data from GWAS, we identified several putative causal miRNAs that may be associated with T2DM and its complications which may guide the treatment for clinical practice.

Methods

Participants

The subjects included in current study were collected from Nangfang hospital from 2022.06 to 2023.06. Briefly, fifteen individuals were enrolled in this study with five patients in each of the T2DM, DLEAD and DF group respectively. The inclusion criteria for the T2DM group were based on the 1999 WHO diagnostic criteria for diabetes, defined as: [1] fasting plasma glucose (FPG) ≥ 7.0 mmol/L; [2] 2-hour plasma glucose ≥ 11.1 mmol/L after an oral glucose tolerance test (OGTT); or [3] symptoms of diabetes with random plasma glucose ≥ 11.1 mmol/L [23]. The DLEAD and DF groups were also required to meet the above diagnostic criteria for diabetes, along with additional criteria: DLEAD group required an ankle-brachial index (ABI) < 0.9; DF group required ABI < 0.9 and concurrent foot ulcer infection. Additionally, all participants had HbA1c levels greater than 6.5%, consistent with current diagnostic standards. The mean HbA1c levels for each group are provided in Table 1. Exclusion criteria: [1] individuals with severe acute complications of diabetes; [2] individuals with severe dysfunction of important organs such as heart, lungs, liver, and kidneys; [3] patients with malignant tumors, connective tissue diseases, blood system disorders, and similar conditions; [4] individuals with severe infections in areas other than the foot. The study was approved by the local ethics committee and participants provided their written informed consent.

Table 1 Basic characteristics of individuals included in exosome wide association study

Study variables	T2DM (*n* = 5)	DLEAD (*n* = 5)	DF (*n* = 5)	*P* value
Age (years)	39.60 (14.74)	73.60 (10.41)	69.80 (7.69)	< 0.01
Males (%)	80	60	80	0.71
BMI	24.84 (3.79)	23.67 (3.29)	24.20 (1.80)	0.83
FBG (mmol/L)	9.20 (3.50)	9.64 (3.69)	7.74 (2.62)	0.64
2-hour plasma glucose (mmol/L)	17.62 (4.27)	13.36 (3.60)	13.10 (5.65)	0.45
HbA1c (%)	11.52 (1.87)	9.49 (2.14)	10.46 (2.97)	0.43
ABI (left)	1.07 (0.04)	0.86 (0.09)	0.87 (0.18)	0.02
ABI (right)	1.09 (0.03)	0.82 (0.16)	0.85 (0.11)	< 0.01

* Age, BMI, FBG and ABI were described as Mean (standard deviation). T2DM, type 2 diabetes mellitus; DLEAD, diabetic lower extremity arterial disease; DF, diabetic foot; BMI, body mass index; FBG, fasting blood glucose; ABI, ankle-brachial index

RNA isolation, library preparation and sequencing

Serum samples were collected to obtain the exosomes by the exoRNeasy Maxi Kit (Qiagen, 77164). RNA degradation and contamination were monitored on 1% agarose gels. RNA purity was checked using the NanoPhotometer® spectrophotometer (IMPLEN, CA, USA). RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA). RNA concentration was measured using Qubit® RNA Assay Kit in Qubit® 2.0 Flurometer (Life Technologies, CA, USA). The small RNA libraries were prepared from a total of 3 μg total RNA isolated from each sample using NEBNext® Multiplex Small RNA Library Prep Set for Illumina ® (NEB, USA.). At last, library quality was assessed on the Agilent Bioanalyzer 2100 system using DNA High Sensitivity Chips. The clustering of the index-coded samples was performed on a cBot Cluster Generation System using TruSeq SR Cluster Kit v3-cBot-HS (Illumina). After cluster generation, the library preparations were sequenced on an Illumina Hiseq 2500/2000 platform and 50 bp single-end reads were generated.

Identification of miRNA

The processed small RNA reads were used in Bowtie [24] for read mapping to reference sequence. The unique sequences that aligned to the known miRNA sequences in miRBase 22.0 were identified as known miRNA. The characteristics of hairpin structure of miRNA precursor can be used to predict novel miRNA. The available software miREvo [25] and mirdeep2 [26] were integrated to predict novel miRNA through exploring the secondary structure, the Dicer cleavage site and the minimum free energy of the small RNA reads unannotated in the former steps.

Differential expression miRNA analysis and target gene prediction

miRNA expression levels were estimated by transcript per million (TPM) through the following criteria: Normalization formula: Normalized expression = mapped read count/Total reads*1,000,000 [27]. Differential expression analysis of three groups was performed using the DESeq R package (3.0.3), with age included as a covariate in the model design to adjust for potential confounding effects [28]. The P-values was adjusted using the Benjamini& Hochberg method. Corrected P-value < 0.05 was set as the threshold for significantly differential expression by default. The target gene prediction of miRNA was performed by miRanda [29].

Target gene functional enrichment analysis

Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis were used on the target gene candidates of differentially expressed miRNAs for biological analysis to determine whether the differentially expressed miRNA target genes were part of any particular pathway. GOseq [30] based Wallenius non-central hypergeometric distribution, which could adjust for gene length bias, was implemented for GO enrichment analysis. We used KOBAS [31] software to test the statistical enrichment of the target gene candidates in KEGG pathways.

miRNA eQTL data

The miRNA expression quantitative loci (eQTLs) are genome-wide analyses of variants associated with the expression level of miRNAs. We obtained miRNA eQTL data from 141 Asians [32]. Briefly, the researchers quantified 343 autosomal mature miRNAs that were expressed in > 70 individuals for subsequent miRNA-eQTL mapping by using 7,170, 825 variants with a minor allele frequency (MAF) ≥ 0.01. The cis-miRNA-eQTL mapping were performed by using linear regression and permutation-based multiple testing correction. The *cis*-window was defined as 1 Mb up- and down-stream of the mature miRNA coding region. Finally, 1,011 *cis*-miRNA-eQTL variants for 25 miRNAs were significant identified at false discovery rates < 0.1. Specifically, cis-eQTL was selected for MR analysis to minimize the potential horizontal pleiotropic effects of the candidate SNPs.

GWAS summary statistics of T2DM and peripheral artery disease (PAD)

The GWAS summary statistics of T2DM were derived from a meta-analysis of 122 GWAS including 180,834 individuals with T2DM and 1,159,055 controls (effective sample size, 492,191) across five ancestry groups [33]. Briefly, we used the ancestry group of East Asian

(Chinese, Japanese and Korean ancestry), which included 56,268 T2DM cases and 227,155 controls in this study. A total of 100 loci attaining genome-wide significance ($P < 5 \times 10^{-8}$) in East Asian were identified by the ancestry-specific meta-analyses. The association summary statistics across GWAS were aggregated via fixed-effects meta-analysis by using METAL [34] based on inverse-variance weighting of allelic log ORs to obtain effect-size estimates.

The GWAS summary statistics of 3,593 PAD cases and 208,860 controls were derived from the BioBank Japan Project [35]. Briefly, the authors reported a GWAS of 42 common diseases consisting of around 200,000 individuals. After imputing the genotypes with reference data from the 1000 Genomes Project Phase 3, a total of 8,712,794 autosomal variants were included for association analysis with 42 diseases. The generalized linear mixed model was employed in the association analysis. Detailed description of sample characteristics, experimental design, and statistical analysis can be found in the published study [35].

Mendelian randomization

Two-sample MR analysis was then conducted to assess the causal relationship between miRNA and T2DM and its complications respectively. Cis-miR-eQTLs for miRNAs that with P value < 0.05 were selected as instrument variables (IVs) to amass a larger number of SNPs for sensitivity analyses. We exclusively retained independent SNPs characterized by an r^2 value of less than 0.01 and situated within a 10,000 kb range, according to the 1000 Genomes East Asian data implemented in the "TwoSampleMR" package. An F statistic was estimated to evaluate the strength of these selected instrumental variables miRNA [36]. Generally, an F statistic > 10 was considered as a typical threshold for the selection of strong instrumental variables [37]. The same set of SNPs was retrieved from GWAS of T2DM and PAD. Then the exposure and outcome data were harmonized to ensure common effect alleles. To ensure robust causal inference, we employed multiple MR methods. In addition to the inverse variance weighted (IVW) methods, which were used as the primary approach, we further included the MR-Egger, weighted median, simple mode, weighted mode, and MR-PRESSO approaches. These additional methods are less sensitive to invalid instruments and pleiotropy, and allow for more conservative but reliable estimation of causal effects. Heterogeneity, horizontal pleiotropy, and leave-one-out sensitivity tests were also performed. All MR analyses were conducted using R version 4.1.2 and the "TwoSampleMR" and "MRPRESSO" packages.

Results

Basic characteristics of the participants

The clinical characteristics of the participants are provided in Table 1, from which it is evident that sex, body mass index and fasting blood glucose did not differ between the three groups. Further, the ABI in left and right was significantly lower for patients with DLEAD and DF.

miRNA expression profiles

Differential analysis identified multiple miRNAs that significantly differed between each of the two groups (T2DM vs. DLEAD, T2DM vs. DF and DLEAD vs. DF; $P < 0.05$). The overall distribution of differentially expressed miRNAs was shown in volcano plots using log2(fold change) as the horizontal coordinate and -log10 (P-value) as the vertical coordinate (Fig. 1A); hierarchical clustering heat map uncovered the similarity of miRNA expression profiles in the discovery group by clustering the dysregulated miRNAs (Fig. 1B). Among these, 2 common miRNAs were detected in each of the two groups (Fig. 1C). The top 5 differentially expressed miRNA between T2DM and its complications were summarized in Table 2. The full results of differentially expressed miRNA between T2DM and its complications were summarized in Supplementary Table 1.

GO enrichment and KEGG annotation of differentially expressed MiRNAs target genes

GO analysis indicated that the differentially expressed miRNAs in each of the two groups were primarily involved in biological processes, cellular components, and molecular functions. The enriched miRNAs related to biological processes were involved in metabolic process and regulation of cell communications. Further, the enriched miRNAs related to cellular components were involved in cells, cell parts, and extracellular regions. Finally, the enriched miRNAs related to molecular function were primarily involved in binding, nucleic acid-binding transcription factor activities, suggesting that they play key roles in regulating the development of T2DM and its complications (Fig. 2A-C).

The KEGG pathway enrichment analysis indicated that AMPK signaling pathway, vasopressin-regulated water reabsorption, insulin secretion, inflammatory mediator regulation of TRP channels and platelet activation were related to the target genes of the differentially expressed miRNAs in each of the two groups (Fig. 2A-C).

Differentially expressed miRNAs show potential causal associations with T2DM and PAD

All heterogeneity tests for the IVW model yielded non-significant results ($P > 0.05$), indicating consistency among the instrumental variables. Furthermore, no

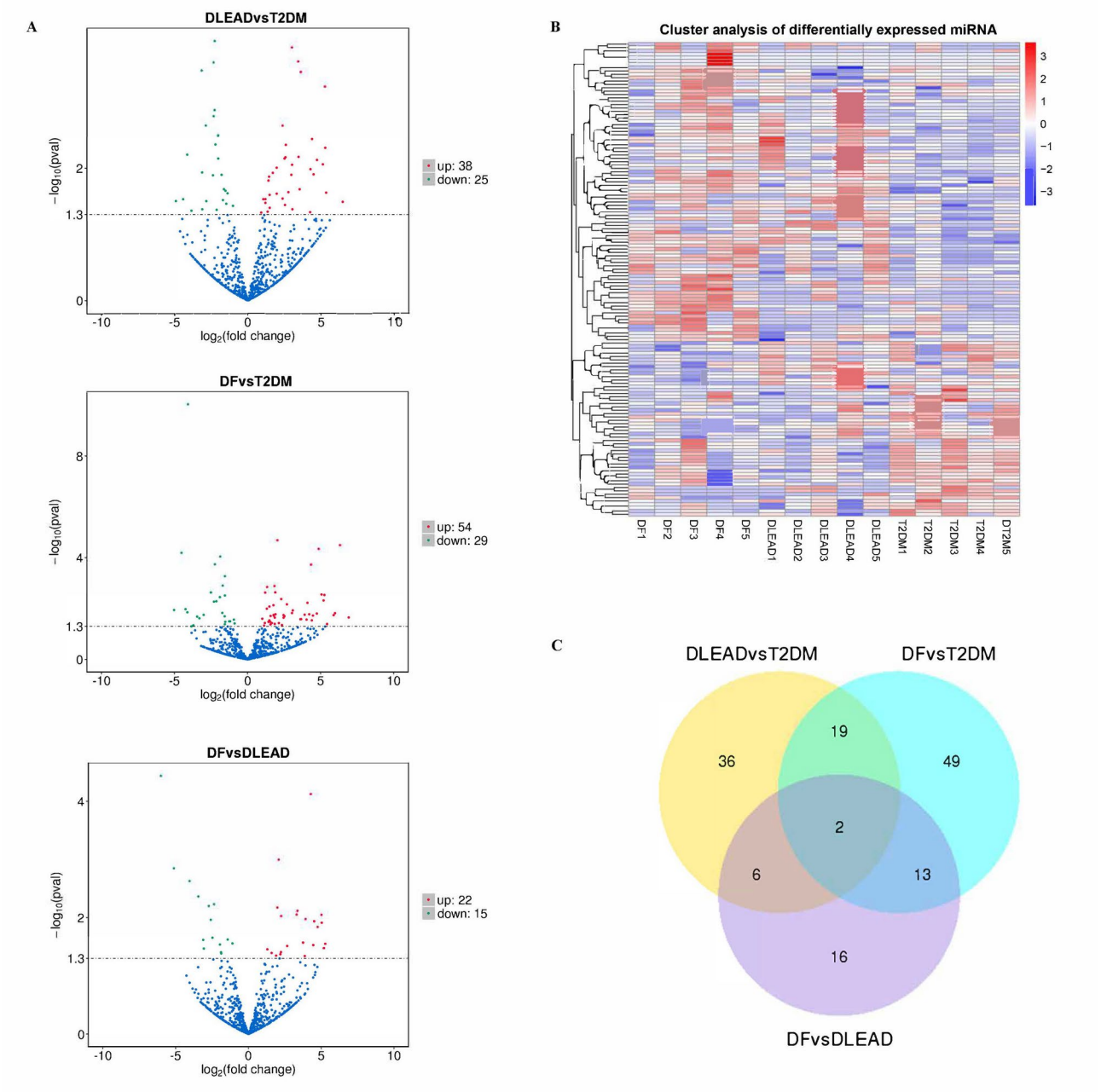

Fig. 1 Differential expression analysis of exosomal miRNAs among T2DM, diabetic foot (DF), and diabetic lower extremity arterial disease (DLEAD). (**A**) Volcano plots showing the differentially expressed miRNAs between groups: DLEAD vs. T2DM (top), DF vs. T2DM (middle), and DF vs. DLEAD (bottom). The horizontal dashed line represents the significance threshold ($P < 0.05$). Red and green dots indicate significantly upregulated and downregulated miRNAs, respectively. (**B**) Heatmap of the differentially expressed miRNAs across all samples. Samples are hierarchically clustered, and the color scale represents normalized expression values (Z-scores). (**C**) Venn diagram illustrating the overlap of differentially expressed miRNAs among the three pairwise comparisons: DLEAD vs. T2DM, DF vs. T2DM, and DF vs. DLEAD

evidence of directional pleiotropy was detected using MR-Egger regression, and the MR-PRESSO global test did not identify any horizontal pleiotropy. Overall, findings from the sensitivity analyses were consistent with those of the primary analyses. Using IVW regression, we identified three miRNAs showing causal associations with T2DM and PAD risk, including hsa-miR-760, which differentially expressed between T2DM and DLEAD, hsa-miR-30b-3p, which differentially expressed between T2DM and DF, and hsa-let-7f-1-3p, which differentially expressed between DLEAD and DF (Fig. 3 and Supplementary Table 2).

However, the causal miRNA identified for PAD showing no overlap with the differentially miRNAs between

Table 2 The top five significantly expressed MiRNA between T2DM and its complications

Group	miRNA	Log2 (Fold change)	*P* value
DLEAD versus T2DM	hsa-miR-409-3p	-2.26	1.19E-04
	hsa-miR-1268a	3.02	1.49E-04
	hsa-miR-134-5p	-2.33	2.52E-04
	hsa-miR-431-5p	-3.15	3.33E-04
	hsa-miR-203a-3p	3.63	3.51E-04
DF versus T2DM	hsa-miR-543	-4.08	9.27E-11
	hsa-miR-629-5p	2.04	2.07E-05
	hsa-miR-6734-5p	6.33	3.20E-05
	hsa-miR-30c-1-3p	4.87	4.50E-05
	hsa-miR-151a-3p	-1.87	8.99E-05
DF versus DLEAD	hsa-miR-206	-6.01	3.64E-05
	hsa-miR-2355-3p	4.28	7.51E-05
	hsa-miR-146a-5p	2.08	1.01E-03
	hsa-miR-34c-5p	-3.4	4.32E-03
	hsa-miR-218-5p	-2.36	5.87E-03

T2DM and its complications. In addition, we also identified two common miRNAs showing causal association with T2DM and PAD, including hsa-miR-30b-5p and hsa-miR-26b-5p (Fig. 4 and Supplementary Table 3).

Discussion

In this study, we conducted an exosome-wide association study to investigate the differential expression of miRNAs associated with T2DM, DLEAD, and DF. Unlike previous studies that typically compare patients with diabetes to healthy controls, we chose to focus solely on individuals with diabetes and its complications, allowing us to elucidate the unique miRNA signatures associated with the progression of diabetic PAD and foot complications. Our findings reveal a subset of miRNAs that exhibit differential expression patterns between diabetic patients with and without complications. Furthermore, we conducted two-sample MR analysis to infer causal relationships between the identified miRNAs and diabetic outcomes. Through MR analysis, we identified miRNAs that exhibit causal associations with diabetes and its complications, suggesting their potential roles as biomarkers or therapeutic targets for intervention.

Our findings reveal several miRNAs that exhibit significant differences in expression levels between diabetic individuals and those with complications. For example, we found that hsa-miR-30b-3p is a differentially expressed miRNA between T2DM and DF, which also showing causal associations with T2DM. Meanwhile, hsa-miR-30b-5p was identified to be causally associated with T2DM and PAD. hsa-miR-30b-3p and hsa-miR-30b-5p belong to the miR-30 family, which is conserved across species and plays important roles in various biological processes, including cell proliferation, differentiation, apoptosis, and metabolism. Yu et al. performed a whole transcriptome sequencing analysis and created a competitive endogenous RNA network for T2DM [38]. The researchers suggested that hsacirc_013887—hsa-miR-6785-5p/hsa-miR-4763-5p/hsa-miR-30b-3p-RAB37, MIR4435-1HG—hsa-miR-30b-3p—RAB37, and GAS5—hsa-miR-30b-3p—RAB37 are potential RNA regulatory pathways that regulate the progression of T2DM [38]. In addition, Zang et al. showed reduced expression of miR-30b in the urinary exosomes of patients with T2DM and diabetic kidney disease (DKD) compared with subjects with T2DM without DKD [39]. Those evidence suggesting their potential roles as biomarkers or mediators of vascular dysfunction and diabetic complications.

The miRNAs identified in our study have diverse biological functions and regulatory roles in pathways implicated in diabetes and vascular dysfunction, including insulin secretion, insulin signaling, AMPK signaling, inflammation, and platelet activation. According to previous studies, the metabolic milieu of T2DM, including insulin resistance, hyperglycemia and release of excess free fatty acids, along with other metabolic abnormalities affects vascular wall by a series of events including endothelial dysfunction, platelet hyperactivity, oxidative stress and low-grade inflammation [40]. The AMPK is an energy sensor with aberrant expression in various diseases including cancer, cardiovascular diseases and T2DM [41]. It can improve neuropathy, nephropathy, liver diseases and reproductive alterations occurring during T2DM [41]. Understanding the mechanistic underpinnings of these miRNAs may offer insights into novel therapeutic targets and strategies for mitigating the risk of diabetic complications.

Our study has several strengths, including the use of exosome-based miRNA profiling, which offers a minimally invasive and highly sensitive approach for biomarker discovery in diabetes and its complications. Additionally, the integration of cis-miR-eQTLs and

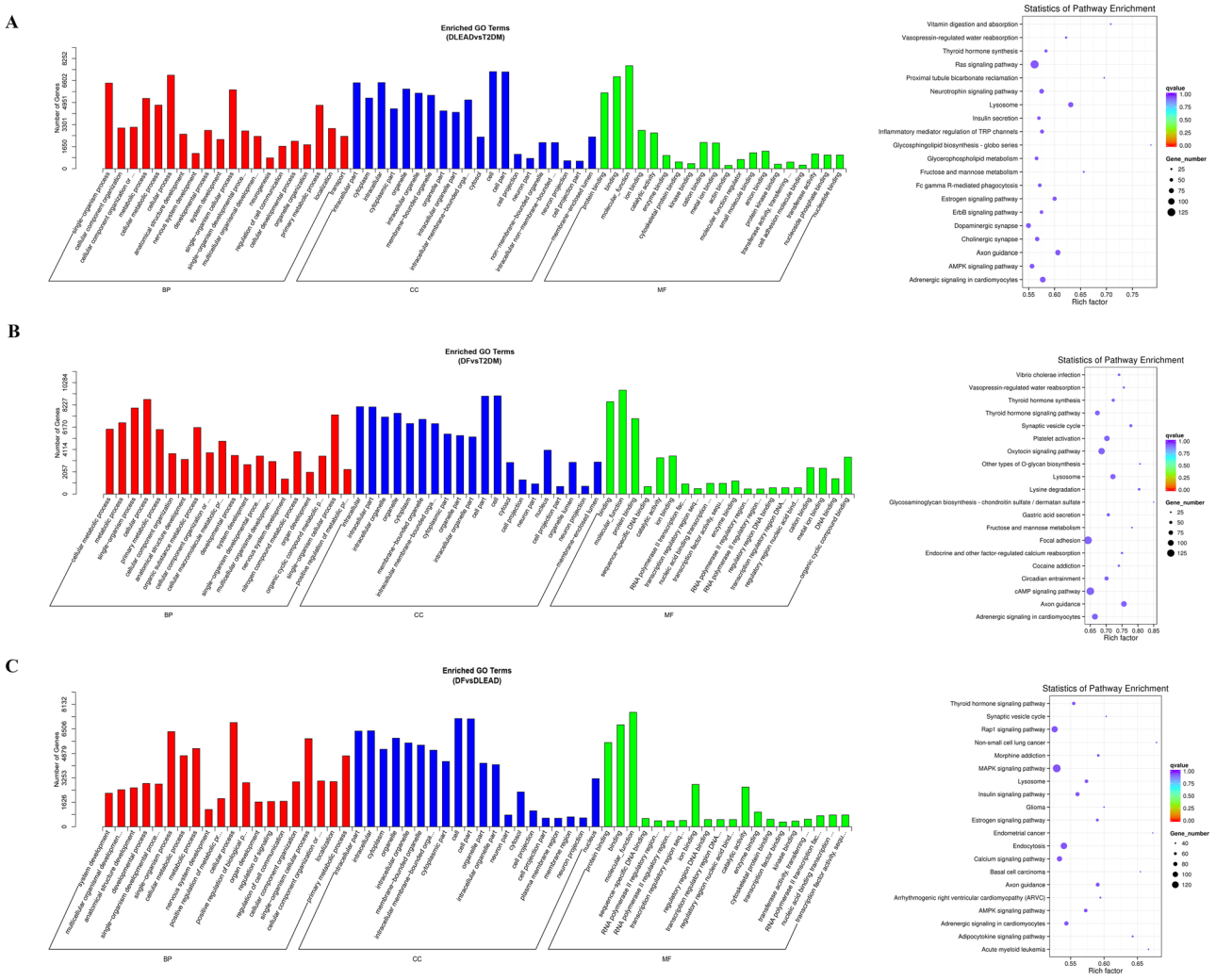

Fig. 2 Functional enrichment analysis of differentially expressed miRNAs among T2DM, diabetic foot (DF), and diabetic lower extremity arterial disease (DLEAD). (**A**–**C**) Gene Ontology (GO) and KEGG pathway enrichment analyses for predicted target genes of differentially expressed miRNAs in the comparisons: DLEAD vs. T2DM (**A**), DF vs. T2DM (**B**), and DF vs. DLEAD (**C**). The left panels show GO enrichment results categorized into three domains: biological processes (BP, red), cellular components (CC, blue), and molecular functions (MF, green). The right panels display the KEGG pathway enrichment results. The bubble plots illustrate the top enriched pathways with dot size representing the number of enriched genes and color indicating the adjusted P value

MR analysis provides robust evidence for the causal relationships between dysregulated miRNAs and disease phenotypes. Despite the strengths of our study, several limitations should be acknowledged. First, the sample size for the exosomal miRNA sequencing was relatively small ($n = 5$ per group), which may reduce the statistical power to detect subtle expression differences and increase the risk of false-positive findings. Second, the study design did not include healthy controls, limiting our ability to distinguish miRNA changes that are specific to diabetes progression from those related to diabetes itself. Third, due to the limitations of currently available cis-miR-eQTL datasets, our Mendelian randomization analysis was unidirectional, focusing only on the causal effects of miRNAs on diabetes and its complications, without assessing potential reverse causality. Lastly, the lack of in vitro or in vivo functional validation means that the biological mechanisms

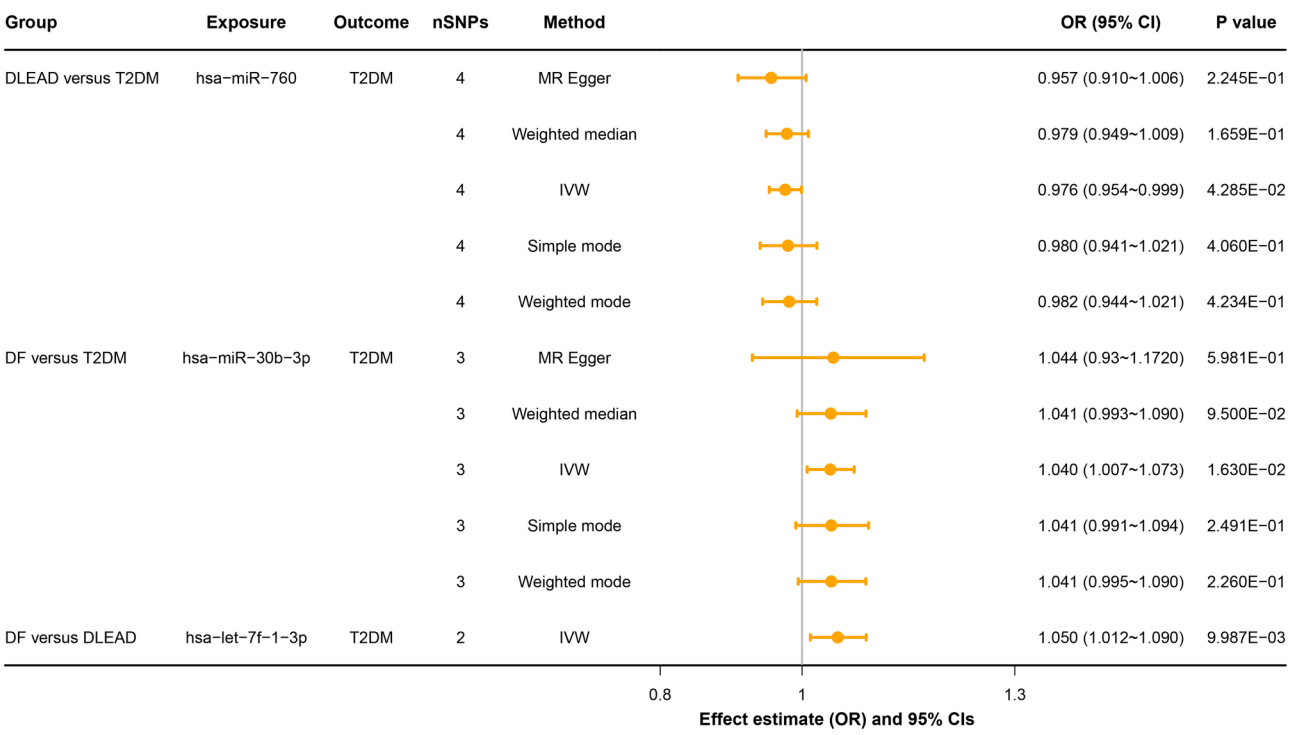

Fig. 3 Mendelian randomization analysis of differentially expressed miRNAs in relation to type 2 diabetes mellitus (T2DM). Forest plot showing the causal associations between selected differentially expressed miRNAs and T2DM, based on various Mendelian randomization methods including MR Egger, weighted median, inverse variance weighted (IVW), simple mode, and weighted mode

underlying the identified miRNA-disease associations remain speculative. Future studies with larger cohorts and inclusion of healthy individuals are warranted to validate and extend our findings. Moreover, functional experiments will be essential to elucidate the mechanistic roles of candidate miRNAs in the pathogenesis of diabetic vascular complications. These efforts will help translate exosomal miRNA profiles into clinically actionable biomarkers or therapeutic targets for diabetes and its complications.

Conclusions

In conclusion, our study highlights the utility of exosome-based miRNA profiling combined with cis-miR-eQTLs and MR analysis for identifying causal biomarkers and therapeutic targets in T2DM and its complications. These findings contribute to our understanding of the molecular mechanisms underlying diabetic vascular disease and may inform the development of precision medicine approaches for the prevention and treatment of diabetic complications.

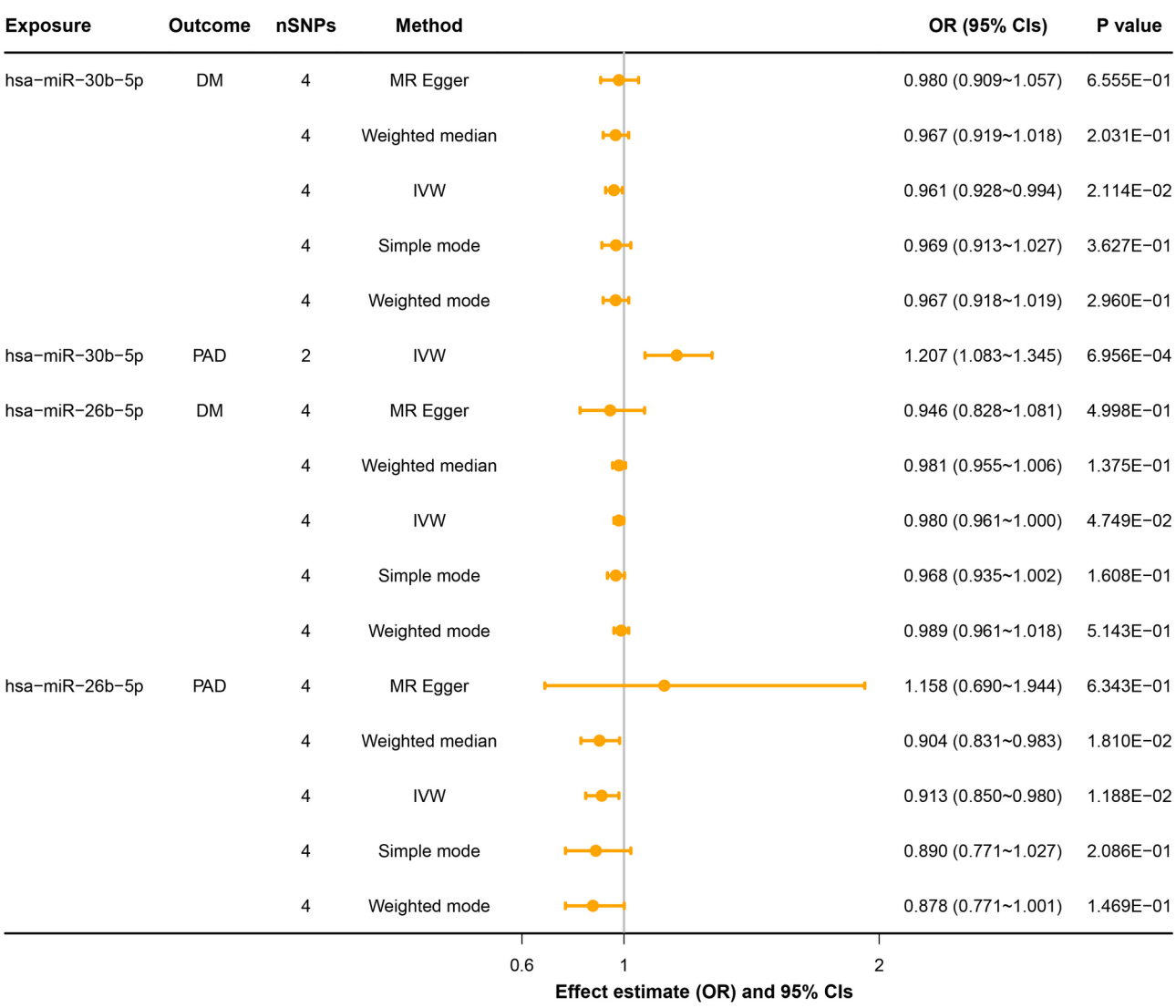

Fig. 4 Common significant causal miRNAs associated with both type 2 diabetes mellitus (T2DM) and peripheral artery disease (PAD) identified through Mendelian randomization analysis. Forest plot illustrating the causal effects of overlapping differentially expressed miRNAs on T2DM and PAD, based on multiple Mendelian randomization approaches: MR Egger, weighted median, inverse variance weighted (IVW), simple mode, and weighted mode

Abbreviations

DM	Diabetes mellitus
DLEAD	Diabetic lower extremity arterial disease
DF	Diabetic foot
miRNAs	microRNAs
EWAS	Exosome-wide association study
cis-miR-eQTLs	cis-miRNA expression quantitative trait loci
GWAS	Genome-wide association study
PAD	Peripheral arterial disease
MR	Mendelian randomization
T2DM	Type 2 diabetes mellitus
ABI	Ankle-brachial index
TPM	Transcript per million
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes KEGG
MAF	Minor allele frequency
IVs	Instrument variables
IVW	Inverse variance weighted
DKD	Diabetic kidney disease

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13098-025-01725-5.

Supplementary Material 1

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None.

Author contributions
All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by MZ, DL, XD, QH and WZ. The first draft of the manuscript was written by JL, MH, JZ, QZ, NL and YC and all authors commented on previous versions of the manuscript.

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Data availability
The datasets generated during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study was approved by the local ethics committee and participants provided their written informed consent.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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