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Received: 7 May 2024 | Revised: 18 June 2024 | Accepted: 26 June 2024

DOI: 10.1111/dom.15786

ORIGINAL ARTICLE

WILEY

Circulating small non-coding RNAs are associated with the insulin-resistant and obesity-related type 2 diabetes clusters

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Funding information
Innovative Medicines Initiative 2 Joint Undertaking (RHAPSODY), Grant/Award Number: 115881; ZonMW Priority Medicine Elderly, Grant/Award Number: 113102006; BBMRI-NL, Grant/Award Number: CP2013-69; Swiss State Secretariat for Education, Research and Innovation (SERI), Grant/Award Number: 16.0097-2

Abstract

Aim: To uncover differences in small non-coding RNAs (sncRNAs) in individuals with type 2 diabetes (T2D) categorized into five clusters based on individual characteristics, which may aid in the identification of those prone to rapid progression.

Materials and Methods: In the Hoorn Diabetes Care System (DCS) cohort, participants were clustered by age, body mass index (BMI), and glycated haemoglobin, C-peptide and high-density lipoprotein (HDL) cholesterol levels, yielding severe insulin-deficient diabetes, severe insulin-resistant diabetes (SIRD), mild obesity-related diabetes (MOD), mild diabetes, and mild diabetes with high HDL cholesterol clusters ($n = 412$). Utilizing plasma sncRNA-sequencing, we identified distinct cluster-specific sncRNAs. Validation was performed in a smaller DCS Hoorn dataset ($n = 138$). To elucidate their potential functions, we examined tissue expression, identified potential targets or (co-)regulated proteins, conducted gene set enrichment analyses on the targets through Reactome, and examined tissue expression of the (co-)regulated proteins.

Results: The insulin-resistant cluster exhibited aberrant expression of 10 sncRNAs, while the high BMI cluster featured eight differentially expressed sncRNAs. Multiple (co-)regulated proteins were identified for sncRNAs associated with both clusters. Proteins associated with both clusters showed enrichment for metabolism. Proteins that specifically and only associated with the SIRD cluster showed enrichment for immune-related signalling. Furthermore, MOD cluster-specific associated proteins showed enrichment for the complement system.

Conclusions: Our research showed differential sncRNA levels among type 2 diabetes clusters. This may reflect and could deepen our understanding of molecular mechanisms, in development, progression, and risk factors for each cluster.

KEYWORDS
clusters, metabolism, *small non-coding RNAs*, type 2 diabetes

LM 't Hart and Roderick Slieker are joint senior author.

1 | INTRODUCTION

Type 2 diabetes is a heterogenous disease resulting in different risks of complications at diagnosis.[1,2] Individuals with type 2 diabetes can be clustered at type 2 diabetes diagnosis based on five clinical variables: age at diagnosis, body mass index (BMI), glycated haemoglobin (HbA1c) level, high-density lipoprotein (HDL) cholesterol level and C-peptide level, resulting in five clusters: severe insulin-deficient diabetes (SIDD), severe-insulin resistant diabetes (SIRD), mild obesity-related diabetes (MOD), mild diabetes (MD) and mild diabetes with high HDL cholesterol (MDH).[3] This clustering method could improve identification of those at greater risk of certain complications, which could help to prolong or prevent type 2 diabetes complications.[2] For example, individuals in the SIRD cluster have been shown to have a higher risk of developing chronic kidney disease (CKD).[2] It has been shown that clusters differ in terms of lipids, metabolites, and protein levels in line with their respective clinical characteristics.[4] This difference also occurs in blood cells, where we demonstrate an aberrant gene expression profile in the obese type 2 diabetes cluster and that these genes have a suggested causal role on multiple diabetes-relevant traits.[5] Small non-coding RNAs (sncRNAs), including, among others, microRNAs (miRNAs), Piwi-interacting RNAs (piRNAs) and small nucleolar RNAs (snoRNAs), are small (50–200 nt) RNA molecules not transcribed into protein. The sncRNAs have diverse functions, including inhibition of translation of mRNAs by binding to their 3′ regions and stabilizing other RNA species.[5] Consequently, during disease progression, their expression can alter. In type 2 diabetes, multiple miRNAs are associated with the pathogenesis of the disease.[6] Although clusters differ in terms of clinical outcomes, multi-omics signatures and diabetes progression, whether these differences are also reflected in the sncRNA expression profile in plasma has not been investigated. We hypothesize that the clusters also differ in their plasma sncRNA expression, as sncRNAs are involved in the complex interplay between genes and their products in the disease state. The aim of the present study was to find differences in sncRNAs in plasma of individuals with type 2 diabetes assigned to one of the five previously identified clusters.

2 | MATERIALS AND METHODS

2.1 | Participants

The Hoorn Diabetes Care System (DCS) cohort, initiated in 1998, comprises individuals with type 2 diabetes residing in the northwest region of the Netherlands. Participants attended annual DCS visits for ongoing monitoring of their type 2 diabetes. These visits included regular measurements, encompassing both anthropometric and laboratory assessments as part of their standard healthcare routine. Within this cohort, individuals were asked to join the Hoorn DCS Biobank, where, after informed consent had been obtained, blood samples were collected and stored for future research. All laboratory measurements were carried out on samples taken in a fasted state. Details of these laboratory procedures can be found in van der Heijden et al.[7] This study was performed in line with the principles of the Declaration of Helsinki. The study was approved by the Ethics Committee of the Amsterdam University Medical Center, location VUmc (approval number 2007/57; NL14692.029.07).

2.2 | RNA isolation and sequencing

As described previously, sncRNAs were extracted from 800 μL of plasma for 475 participants of the Hoorn DCS study using the QuickcfRNA Serum and Plasma Kit from Zymo Research (Irvine, CA, USA).[8] Subsequently, library preparation was accomplished using the NEBNext® Multiplex Small RNA Library Prep Set for Illumina from New England Biolabs (Ipswich, MA, USA). Size selection of fragments ranging from 120 to 200 nucleotides, aiming to enhance sncRNAs within the size of approximately 20 to 100 nucleotides, was conducted using the Pippin Prep instrument from Sage Science (Beverly, MA, USA). Sequencing was carried out on the Illumina NextSeq500 sequencer using version 2.5 sequencing reagent kits with 35-cycle paired-end reads (PE35). To process the data and generate estimates of RNA abundance for various sncRNA species, such as miRNA, piRNA, long non-coding RNA (lncRNA), transfer-RNAs (tRNA), Y RNA, small nuclear RNA (snRNA), snoRNA, small cajal body-specific RNA, and others, the exceRpt pipeline developed by the NIH Extracellular RNA Communication Consortium was employed.[9] The respective databases used include miRBase version 22,[10] piRNABank version 1,[11] Gencode (version 38),[12] circBase[13] and GtRNAdb.[14]

2.3 | Clusters

Individuals within the DCS cohort diagnosed with type 2 diabetes were clustered based on five clinical variables: age at initial visit (years); BMI (kg/m^2); HbA1c (mmol/mol); C-peptide (nmol/L); and HDL cholesterol (mmol/L; $n = 2953$). Clustering was performed stratifying by sex and using the k-means method.[3] This resulted in the identification of five distinct clusters: a cluster characterized by severe insulin deficiency (SIDD); a cluster exhibiting severe insulin resistance (SIRD); a cluster associated with obesity (MOD); a cluster with mild diabetes and low HDL cholesterol levels (MD), and a cluster with mild diabetes and high HDL cholesterol levels (MDH). From the clustering in the larger set,[3] we obtained the cluster allocation for those with sncRNA-sequencing data, which resulted in a dataset of 412 individuals.

2.4 | Statistical methods

Lowly expressed sncRNAs were filtered out (threshold of mean ≥5 copies). The edgeR R-package (version 3.18) was employed for conducting differential expression analysis utilizing a quasi-likelihood *F*-test. Comparisons involved one specific cluster versus the remaining four clusters. The model remained unadjusted, given that age, sex, BMI, HbA1c, C-peptide and HDL-cholesterol had already been used

to define the clusters. sncRNAs were regarded as differentially expressed if the observed contrast between two conditions demonstrated statistical significance, that is, a false-discovery rate (FDR)-adjusted *p* value below 0.05.

2.5 | Independent validation

Additional samples from the DCS Hoorn cohort were used to validate the results of the original dataset. A total of 138 individuals with cluster and small RNA-sequencing data were available. A different read length was used for size selection of fragments ranging from 120 to 200 nucleotides to further enrich for sRNAs with a size of ~20 to 100 nucleotides. Differential expression analysis was performed in the same way as described above. sncRNAs were regarded as significant based on a nominally significant *p* value below 0.05.

2.6 | Tissue expression

We acquired publicly accessible raw sncRNA-sequencing data from various metabolic tissues through the Gene Expression Omnibus (GEO)-encompassed samples from the kidney, thyroid, brain, liver, muscle, heart, colon, pancreas, subcutaneous white adipose tissue, whole blood, and urine. GEO accession numbers are provided in Table S1. Data were processed using the exceRpt pipeline, according to the aforementioned methodology. Data were z-scaled in figures.

2.7 | Plasma proteomics

In the Hoorn DCS study, the plasma proteomic data of 1195 proteins (SomaLogic SOMAscan platform in Boulder, CO, USA) were accessible for 589 participants, as detailed in our previous work.[4] Additionally, for 189 participants (Table S2), corresponding sncRNAseq data from the same sampling date were available (SIDD = 13, SIRD = 34, MOD = 40, MD = 67, MDH = 35). A linear regression model was used to test for a correlation between the sncRNA transcriptome and plasma proteins. The proteomic measurement was log-transformed. To account for multiple testing, the Benjamini–Hochberg procedure was applied, considering results with an FDR below 0.05 as significant. Furthermore, gene set enrichment analysis was conducted on the identified targets using the R-package ReactomePA (version 1.44.0). The examination of tissue expression for proteins was carried out using the genotype–tissue expression (GTEx) dataset.[15]

3 | RESULTS

3.1 | Participant characteristics

Table 1 outlines the key characteristics of the individuals involved in this study. The mean (standard deviation [SD]) age of the participants was 61.4 (10.0) years, and 45.4% of the population was female. The study population was defined as obese, indicated by a mean (SD) BMI of 30.5 (5.7) kg/m^2. Participants' HbA1c levels were well controlled, with a mean (SD) HbA1c level of 47.6 (8.9) mmol/mol (equivalent to 6.5% [3.0%]). The participants were typically diagnosed with type 2 diabetes at a mean (SD) age of approximately 59.3 (8.9) years. The median (interquartile range) time since diagnosis was 2.1 (1.2–3.0) years, and 18.4% of the study group were smokers. In terms of medication, the majority (71.4%) were undergoing treatment with metformin, while 78.9% were on oral hypoglycaemic medication, as anticipated within this cohort.

3.2 | Clustering

The characteristics of the subgroup used in this RNA-sequencing study ($n = 412$) matched those of the larger group.[3] Notably, HbA1c levels were marginally higher in the MOD cluster than in the SIRD cluster (Figure 1). Our analysis identified that the SIDD cluster comprised 7% ($n = 28$) of the overall study population (Figure 1), while the SIRD cluster comprised 22%, the MOD cluster 22%, the MD cluster 33%, and the MDH cluster 17%.

3.3 | sncRNAs are differentially expressed between clusters

A total of 938 sncRNAs were included, predominantly comprising miRNAs, followed by snoRNAs, lncRNAs, miscRNAs, piRNAs, tRNAs, snRNAs, and various other RNA types (Figure S1 and Table S3). Among these, 14 sncRNAs exhibited differential expression within the insulin-resistant (SIRD) cluster (Figure 2A, Table S4). These included *VTRNA2* (Figure 2D), *Vault*, *Gln*, and several miRNAs: *hsa-miR-490-3p*, *hsa-miR-148a-5p*, *hsa-miR-378a-3p*, *hsa-miR-148a-3p*, *hsa-miR-548aq-3p*, *hsa-miR-296-3p*, *hsa-miR-22-3p*, *hsa-let-7f-2-3p*, *hsa-miR-378f*, *hsa-miR-27a-5p* and *hsa-miR-378i*. Expression of these sncRNAs ranged from scarce to abundant in plasma (Figure S2). Notably, all these sncRNAs demonstrated upregulation within the SIRD cluster in comparison to the other clusters. It is worth mentioning that *VTRNA2* and *Vault* refer to the same RNA gene associated with the vault RNA class (Figure S3). Characteristics of the validation study matched those of the discovery set (Figure S4, Table S5). The validation dataset revealed that four sncRNAs exhibited nominally significant replication: *hsa-miR-378f*, *hsa-miR-27a-5p*, *hsa-miR-378a-3p* and *miR-378i*. Another six sncRNAs exhibited a similar direction of effect: *hsa-miR-296-3p*, *Gln*, *hsa-miR-148a-3p*, *hsa-miR-148a-5p*, *hsa-miR-22-3p* and *VTRNA2* (Figure S5). In the downstream analysis we proceeded with these 10 sncRNAs. The upregulated sncRNAs in the SIRD cluster weakly correlated with C-peptide (mean $r = 0.17$; Figure 2G) and some of these sncRNAs also correlated with each other (Figure 3A).

In the MOD cluster, 10 microRNAs displayed differential expression patterns (Figure 2B, Table S4). Eight of these were upregulated,

TABLE 1 Characteristics of the individuals in the five clusters.

Patient characteristics						
Cluster	**SIDD ($n = 28$)**	**SIRD ($n = 89$)**	**MOD ($n = 90$)**	**MD ($n = 135$)**	**MDH ($n = 70$)**	**Total ($n = 412$)**
Age, years	60.5 (±7.6)	68.5 (±6.7)	56.2 (±7.7)	57.4 (±8.0)	67.3 (±5.4)	61.4 (±10.0)
Female, %	60.7 ($n = 17$)	32.6 ($n = 29$)	56.7 ($n = 51$)	45.2 ($n = 61$)	41.4 ($n = 29$)	45.4 ($n = 187$)
BMI, kg/m^2	28.7 (±5.3)	30.2 (±3.3)	38.2 (±5.1)	28.3 (±3.1)	26.1 (±3.2)	30.5 (±5.7)
HbA1c, mmol/mol	54.3 (±16.5)	48.2 (±8.7)	48.2 (±7.5)	46.8 (±8.1)	45.2 (±6.4)	47.6 (±8.9)
HbA1c, %	7.1 (±3.7)	6.6 (±2.9)	6.6 (±2.8)	6.4 (±2.9)	6.3 (±2.7)	6.5 (±3.0)
Fasting glucose, mmol/L	8.4 (±2.2)	8.1 (±1.5)	8.1 (±1.9)	7.7 (±1.3)	7.7 (±1.2)	7.9 (±1.5)
Age at diagnosis, years	58.5 (±7.8)	66.3 (±6.5)	54.0 (±7.7)	55.4 (±7.8)	65.2 (±5.6)	59.3 (±8.9)
Diabetes duration, years	2.1 (1.1–3.0)	2.3 (1.2–3.0)	2.1 (1.2–3.1)	2.0 (1.2–2.8)	2.1 (1.3–2.9)	2.1 (1.2–3.0)
Smoking, %	28.6 ($n = 8$)	13.5 ($n = 12$)	14.4 ($n = 13$)	26.7 ($n = 36$)	10.0 ($n = 7$)	18.4 ($n = 76$)
HDL, mmol/L	1.3 (±0.3)	1.1 (±0.2)	1.1 (±0.3)	1.1 (±0.2)	1.5 (±0.4)	1.2 (±0.3)
LDL, mmol/L	2.7 (±1.2)	2.6 (±0.9)	2.7 (±0.9)	2.8 (±1.1)	2.7 (±1.0)	2.7 (±1.0)
Total cholesterol, mmol/L	4.7 (±1.4)	4.6 (±1.1)	4.8 (±1.0)	4.7 (±1.3)	4.8 (±1.1)	4.7 (±1.2)
Triglycerides, mmol/L	1.7 (±1.0)	2.2 (±1.3)	2.2 (±1.1)	1.8 (±0.9)	1.2 (±0.5)	1.9 (±1.1)
eGFR, mL/min/1.73 m^2	88.6 (±16.1)	74.1 (±16.3)	88.9 (±14.8)	90.5 (±13.2)	79.0 (±12.9)	84.6 (±15.8)
Creatinine blood, µmol/L	70.6 (±20.0)	88.4 (±27.3)	73.3 (±14.4)	73.2 (±13.3)	79.1 (±18.3)	77.3 (±19.6)
C-peptide	1.0 (±0.4)	1.5 (±0.4)	1.4 (±0.4)	0.9 (±0.2)	0.8 (±0.3)	1.2 (±0.4)
Medication treatment, %						
Insulin	46.4 ($n = 13$)	29.2 ($n = 26$)	36.7 ($n = 33$)	27.4 ($n = 37$)	11.4 ($n = 8$)	28.4 ($n = 117$)
Metformin	100.0 ($n = 28$)	66.3 ($n = 59$)	70.0 ($n = 63$)	68.1 ($n = 92$)	74.3 ($n = 52$)	71.4 ($n = 294$)
Oral hypoglycaemic medications	100.0 ($n = 28$)	76.4 ($n = 68$)	78.9 ($n = 71$)	74.8 ($n = 101$)	81.4 ($n = 57$)	78.9 ($n = 325$)

Note: Data are presented as mean (standard deviation), median (interquartile range) or *n* (%).
Abbreviations: eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MD, mild diabetes; MDH, mild diabetes with high high-density lipoprotein cholesterol; MOD, mild obesity-related diabetes; SIDD, severe insulin-deficient diabetes; SIRD, severe-insulin resistant diabetes.

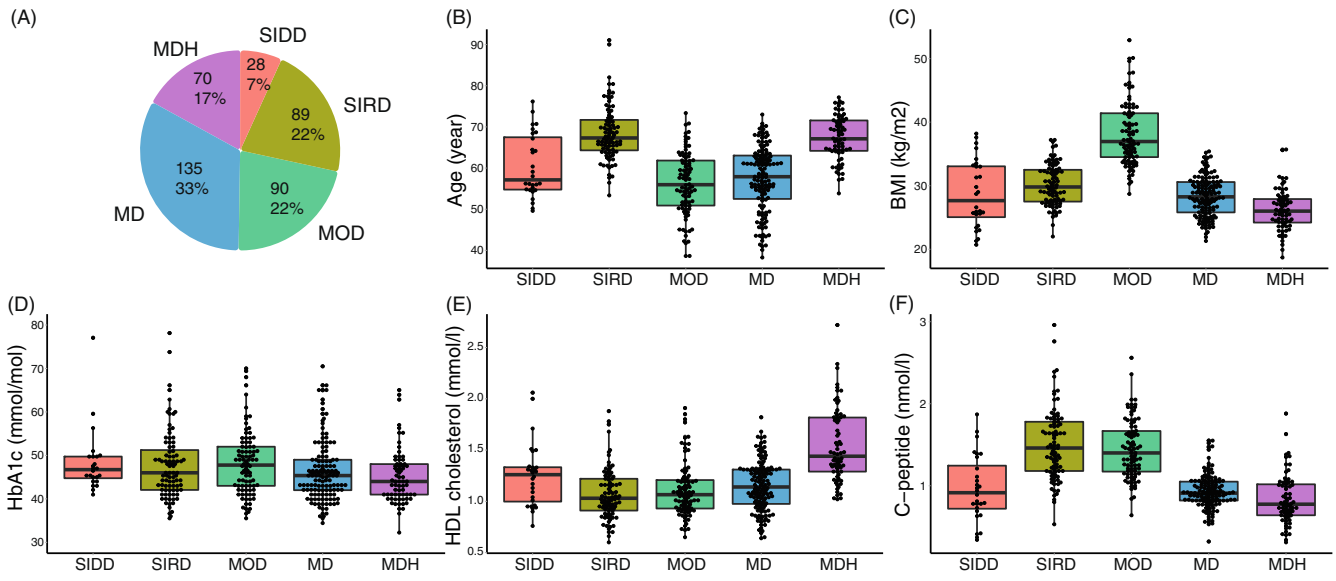

FIGURE 1 Characteristics of the five type 2 diabetes clusters. (A) Pie chart of five clusters in the Diabetes Care System Hoorn study ($n = 412$). (B–F) Age, body mass index (BMI), glycated haemoglobin (HbA1c), high-density lipoprotein (HDL) cholesterol and C-peptide were used to cluster type 2 diabetes patients in five clusters: severe insulin-deficient diabetes (SIDD), severe-insulin resistant diabetes (SIRD), mild obesity-related diabetes (MOD), mild diabetes (MD) and mild diabetes with high HDL cholesterol (MDH). In total 412 individuals with type 2 diabetes participated in this study. Boxplot shows median, 25th percentile and 75th percentile.

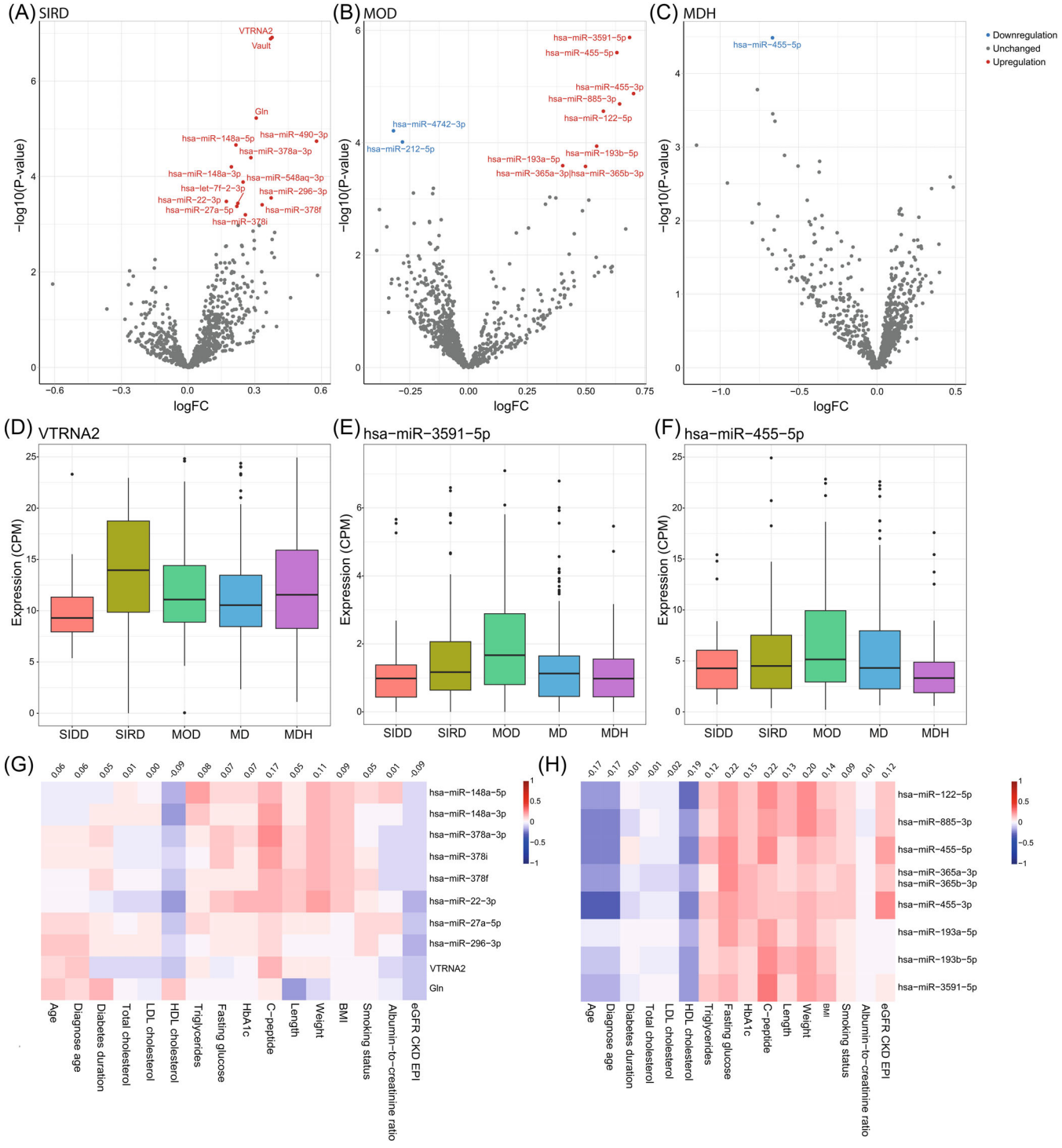

FIGURE 2 Differentially expressed small non-coding RNAs (sncRNAs) in the clusters. (A–C) Volcano plot of sncRNAs in the severe-insulin resistant diabetes (SIRD) (A), mild obesity-related diabetes (MOD) (B) and mild diabetes with high HDL cholesterol (MDH) (C) cluster. (D–F) Boxplot of expression (CPM) of the most significant signal in the SIRD cluster: VTRNA2 (D), MOD cluster: *Hsa-miR-3591-5p* (E) and the MDH cluster: *hsa-miR-455-5p* (F). (G, H) Correlation heatmap of differentially expressed sncRNAs and clinical and biochemical characteristics. BMI, body mass index; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

including *hsa-miR-3591-5p*, *hsa-miR-455-5p*, *hsa-miR-455-3p*, *hsa-miR-885-3p*, *hsa-miR-122-5p*, *hsa-miR-193b-5p*, *hsa-miR-193a-5p* and *hsa-miR-365a-3p*|*hsa-miR-365b-3p* (Figure 2E). Two miRNAs, *hsa-miR-4742-3p* and *hsa-miR-212-5p*, were downregulated in the MOD cluster. *Hsa-miR-122-5p* was expressed abundantly (Figure S6). *Hsa-miR-455-5p* was upregulated in the MOD cluster,

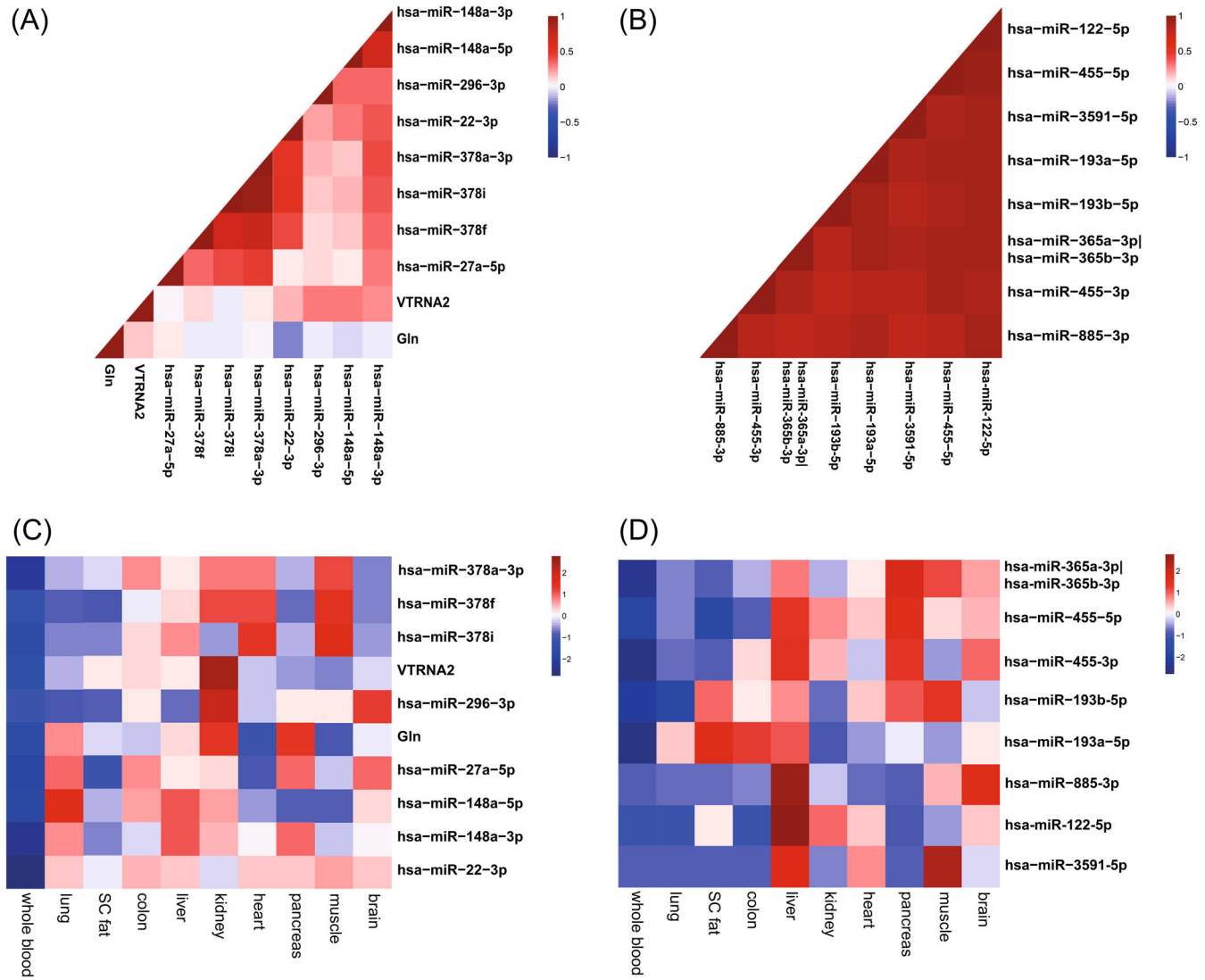

FIGURE 3 Small non-coding RNA (sncRNA) correlation and tissue expression. (A) Correlation heatmap of differentially expressed sncRNAs in the severe-insulin resistant diabetes (SIRD) cluster. (B) Correlation heatmap of differentially expressed sncRNAs in the mild obesity-related diabetes (MOD) cluster. (C) Tissue expression of the sncRNAs in the SIRD cluster. (D) Tissue expression of the sncRNAs in the MOD cluster.

but downregulated in the MDH cluster (Figure 2B,C,F). In the validation dataset, four sncRNAs exhibited nominally significant replication: *hsa-miR-455-5p* (not validated for MDH), *hsa-miR-885-3p*, *hsa-miR-193b-5p* and *hsa-miR-455-3p*. Another four sncRNAs exhibited a similar direction of effect: *hsa-miR-122-5p*, *hsa-miR-365a-3p|hsa-miR-365b-3p*, *hsa-miR-3591-5p* and *hsa-miR-193a-5p* (Figure S5). In the downstream analysis we proceeded with these eight sncRNAs. The upregulated sncRNAs in the MOD cluster positively and weakly correlated with C-peptide (mean $r = 0.22$), fasting glucose (mean $r = 0.22$) and weight (mean $r = 0.20$), and negatively with HDL cholesterol (mean $r = -0.19$), age (mean $r = -0.17$) and diagnosis age (mean $r = -0.17$; Figure 2H). This is in line with the high BMI and relatively young age in this cluster. We observed that the upregulated miRNAs in the MOD cluster strongly correlated with each other (Figure 3B).

3.4 | sncRNAs associated with the MOD cluster primarily expressed in the liver

Next, to gain insight into their possible source and function, we explored the tissue-specific expression patterns of the identified sncRNAs by leveraging publicly available expression data across 12 diverse tissues and body fluids. This highlighted distinct tissue-enriched expression profiles among these sncRNAs. Notably, the sncRNAs within the SIRD cluster demonstrated a widespread distribution across multiple tissues, encompassing the kidneys, heart, pancreas, muscles, thyroid, lungs, and brain (Figure 3C, Figure S7). Interestingly, VTRNA2 showed a high expression in the kidneys, while the SIRD cluster is known to associate with development of diabetic kidney disease. Conversely, within the MOD cluster, the sncRNAs exhibited expression primarily in the liver (Figure 3D). However, these

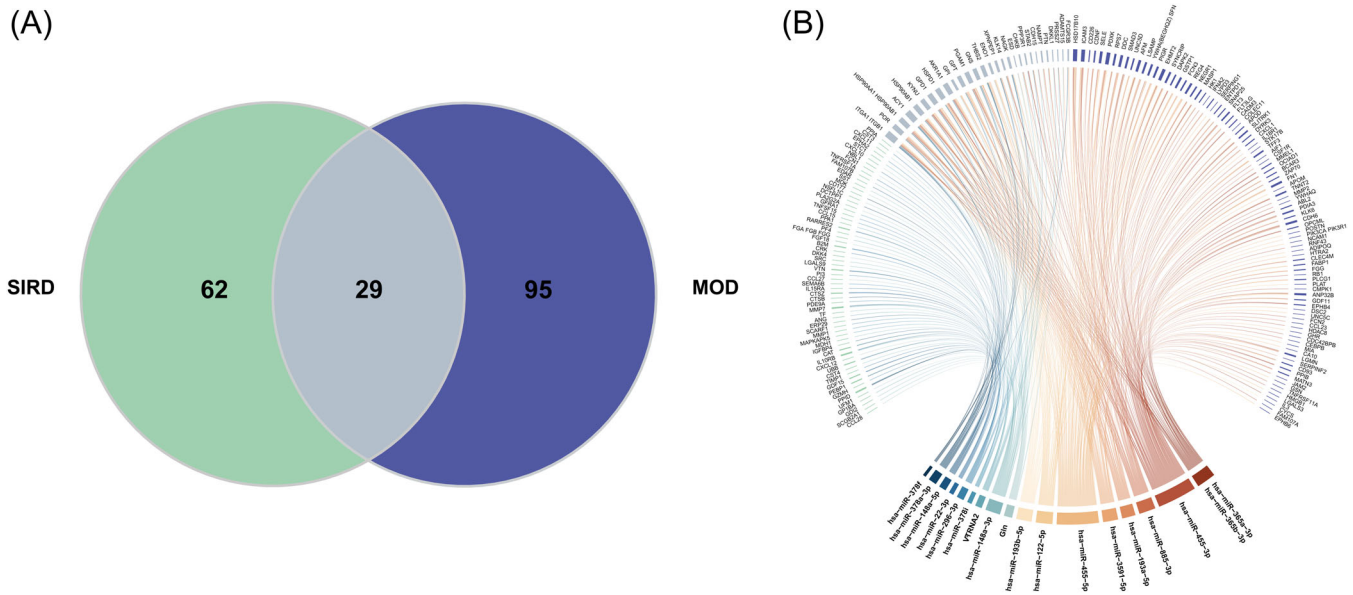

FIGURE 4 Small non-coding RNA (sncRNA)-associated or (co-)regulated proteins. (A) Venn diagram of the proteins associated with the sncRNAs differentially expressed in the severe-insulin resistant diabetes (SIRD) and mild obesity-related diabetes (MOD) cluster. (B) Chord diagram of the sncRNAs and associated or (co-)regulated proteins.

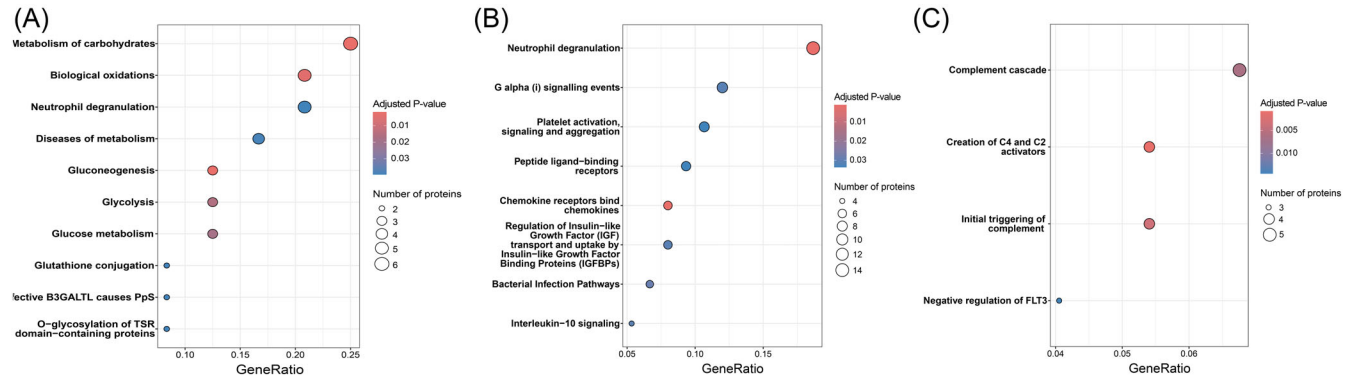

FIGURE 5 Pathway enrichment of the associated or (co-)regulated proteins. (A) Pathway enrichment performed on the 29 proteins associated with both sncRNAs in the severe-insulin resistant diabetes (SIRD) and mild obesity-related diabetes (MOD) cluster. (B) Pathway enrichment performed on the 62 proteins associated with sncRNAs in the SIRD cluster. (C) Pathway enrichment performed on the 95 proteins associated with sncRNAs in the MOD cluster.

sncRNAs were also detected in other pivotal metabolic tissues such as the muscles, brain, and pancreas (Figure S8).

3.5 | Associated or (co-)regulated proteins in plasma

In our study, we identified a significant number of sncRNAs that were specifically associated with the clusters, but their specific functions remain largely unknown. To shed light on their potential role, we compared the expression levels of these sncRNAs with the levels of 1195 proteins in plasma, aiming to identify potential targets or (co-)regulated proteins or pathways. This analysis revealed that 91 proteins were significantly (p_{FDR} <0.05) linked to the expression of 10 sncRNAs within the SIRD cluster (Table S6), of which five proteins have been linked previously to the SIRD cluster (Table S7).[4] For the MOD cluster, 124 proteins were significantly associated with the eight upregulated miRNAs (Table S8), of which 25 proteins have previously been linked to the MOD cluster (Table S7).[4] Notably, 29 proteins were found to be associated with sncRNAs associated with both the SIRD and MOD cluster, suggesting their potential significance in the context of type 2 diabetes (Figure 4A,B). Enriched pathways for these 29 proteins included gluconeogenesis, metabolism of carbohydrates, biological oxidation, glycolysis and disease of metabolism (Figure 5A, Table S9). These pathways included multiple key proteins, including *ENO1*, *PGAM1* and *GPI*. *ENO1*, or *Enolase 1*, is a glycolytic enzyme that acts

as a catalyser of the conversion of 2-phosphoglycerate to phosphoenolpyruvate.[16] *PGAM1* is a catalyser of the reversible reaction of 3-phosphoglycerate (3-PGA) to 2-phosphoglycerate (2-PGA).[17] *GPI* interconverts glucose-6-phosphate and fructose-6-phosphate.[18] Further exploration of the proteins specifically linked to the SIRD cluster (proteins associated with sncRNAs in the SIRD and MOD cluster were excluded) showcased their expression across various tissues (Figure S9). Connections between the identified associated or (co-) regulated proteins within the SIRD cluster were also observed (Figure S10). In addition, there was an enrichment for chemokine receptors bind chemokines, neutrophil degranulation, bacterial infection pathways, regulation of insulin-like growth factor transport and uptake by insulin-like growth factor binding proteins and interleukin-10 signalling (Figure 5B, Table S9). Proteins associated with the SIRD cluster included multiple *CXCL* family proteins. On the other hand, proteins associated with the MOD cluster (proteins associated with sncRNAs in the SIRD and MOD cluster were excluded) also exhibited expression in diverse tissues (Figure S11) and we found evidence for connections between the identified associated or (co-)regulated proteins within the MOD cluster (Figure S12). Additionally, enrichment for complement activation, including the creation of C4 and C2 activators, initial triggering of complement and complement cascade, along with negative regulation of FLT3, was found (Figure 5C, Table S9). The proteins associated with the MOD cluster included among others, *FCN2*, *FCN3* and *MASP1*. Moreover, all the upregulated miRNAs in the MOD cluster were found to be associated with aminoacylase-1 (*ACY1*) and 11 other proteins, predominantly expressed in the liver (Table S9, Figure S13).

4 | DISCUSSION

In this study, we aimed to identify differentially expressed sncRNAs in the plasma of individuals diagnosed with type 2 diabetes in the five clusters previously defined. Our findings revealed noteworthy differences in the expression of these sncRNAs, particularly microRNAs, within the insulin-resistant and obesity-associated clusters, whereas such differences were not evident in the remaining clusters. Ten sncRNAs, including one vault RNA, one tRNA and eight miRNAs, were differentially expressed in the SIRD cluster compared to the other clusters. Furthermore, within the MOD cluster, eight sncRNAs, which were all miRNAs, were differentially expressed. Overall, our study sheds light on the distinct expression profiles of sncRNAs, particularly miRNAs, in specific type 2 diabetes clusters.

The SIRD cluster was associated with 10 sncRNAs, comprising one vault RNA (*VTRNA2*), one transfer RNA (*glutamate*), and eight miRNAs (*hsa-miR-148a-5p*, *hsa-miR-378a-3p*, *hsa-miR-148a-3p*, *hsa-miR-296-3p*, *hsa-miR-22-3p*, *hsa-miR-378f*, *hsa-miR-27a-5p*, and *hsa-miR-378i*). Previous studies linked tRNA glutamate with diabetes, particularly in connection with the *TRMT10A* gene.[19] Mutations in this gene lead to monogenic diabetes by causing insulin deficiency and the fragmentation of tRNA *Gln*. These tRNA *Gln* fragments play a crucial role in the decline of β cells. Nonetheless, the precise mechanisms through which tRNAs impact insulin resistance and β-cell function remain largely unknown. This study shows the association of tRNA glutamate with insulin resistance in type 2 diabetes. Further studies, beyond the goal of the present study, are needed to explore this in further detail. sncRNAs associated with the insulin resistance cluster are expressed in a variety of tissues. Similar tissue expression was observed for the proteins associated with the sncRNAs in this cluster. In addition, *VTRNA2* is highly expressed in kidney tissue. Others have shown that *VTRNA2* contributes to intestinal epithelial barrier dysfunction by reducing the expression of *claudin1*, *occludin* and *E-cadherin*.[20] We hypothesize that *VTRNA2* might also impact the vascular system by dysregulating the endothelial barrier, which is also regulated by *claudin1* and *occludin*.[21] In the kidneys, *VTRNA2* might impact *VE-cadherin* instead of *E-cadherin*. Endothelial barrier dysfunction is a major contributor to diabetic kidney disease and it has been shown that individuals in the SIRD cluster are more likely to develop cardiovascular complications such as diabetic kidney disease, stroke, cardiovascular disease, and diabetic peripheral vascular disease.[2,22] Further studies are needed to examine the function of *VTRNA2* in endothelial cells and the cardiovascular system.

Our analysis identified 29 proteins associated with sncRNAs in both the SIRD and MOD clusters. These proteins exhibited enrichment in essential metabolic processes, including metabolism of carbohydrates, gluconeogenesis, biological oxidations, glycolysis and glucose metabolism, as well as diseases related to metabolism. This suggests their involvement in broader dysregulated processes associated with type 2 diabetes. For instance, *ACY1*, a gene linked with four sncRNAs in the SIRD cluster and eight sncRNAs in the MOD cluster, has previously been associated with increased risk of future type 2 diabetes.[23] Additionally, *Eno1*, associated with one sncRNA in the SIRD cluster and four sncRNAs in the MOD cluster, plays a crucial role in regulating glucose metabolism and insulin secretion.[24] Furthermore, multiple glycolytic enzymes were found to be associated with the SIRD and MOD cluster: *PGAM1*, *ENO1* and *GPI*, all involved in the glycolytic pathway which converts glucose into pyruvate.[25] These findings underscore the potential significance of these proteins in the pathogenesis of type 2 diabetes.

Proteins specifically associated with sncRNAs in the SIRD cluster showed enrichment for immune-related signalling events, including signalling by interleukins, platelet activation, signalling and aggregation, G alpha (i) signalling events, peptide ligand-binding receptors and chemokine receptors bind chemokines. This enrichment is particularly significant given that altered cytokine production, including interleukins, is associated with insulin resistance.[26] For instance, interleukin-6, a proinflammatory cytokine, decisively contributes to the development of insulin resistance.[27] Also, different members of the *CXCL* family are associated with metabolic inflammation and insulin resistance, including *CXCL10* and *CXCL11*.[28] Additionally, insulin resistance may initiate or exacerbate low-grade inflammation, which is a known contributor to various complications such as CKD and cardiovascular problems.[29,30] As shown previously, individuals in this cluster had a significantly higher risk of CKD.[2,22,31]

Consistent with previous research, our study revealed that the cluster linked with obesity displays an unusual expression pattern compared to the remaining clusters. Previously, we noted a significant association between several long non-coding RNAs and mRNAs with the MOD cluster.[5] Consistent observations indicate that the SIRD and MOD clusters exhibit the most significant divergence in multi-omics signatures compared to other clusters.[2–4,32] In our investigation, we identified eight upregulated miRNAs in the MOD cluster: *hsa-miR-3591-5p*, *hsa-miR-455-5p*, *hsa-miR-455-3p*, *hsa-miR-885-3p*, *hsa-miR-122-5p*, *hsa-miR-193b-5p*, *hsa-miR-193a-5p*, and *hsa-miR-365a-3p|hsa-miR-365b-3p*. Remarkably, some of these miRNAs are well known for their involvement in type 2 diabetes, obesity, and liver specificity. For instance, *miR-122* is widely known for its liver-specific expression. *miR-122* maintains the hepatic cell phenotype in the liver, accounting for 80% of the liver's mass.[33] *miR-122* has been shown to regulate genes involved in cholesterol and fatty acid metabolism.[34] Suppressing *miR-122* in mice led to a prolonged decrease in overall plasma cholesterol levels, evident in both the low-density lipoprotein and HDL fractions.[35,36] Furthermore, *miR-455* has been shown to inhibit brown adipose tissue development and thermogenesis by targeting key genes involved in brown adipocyte differentiation and function.[37] Conversely, *miR-193b* and *miR-365* have also been found to promote brown adipose tissue activity by targeting the same adipogenic suppressor as *miR-455*; *Runx1t1*.[38] Our findings also indicate that these miRNAs are predominantly expressed in liver tissue, suggesting liver involvement within this cluster. Additionally, the miRNAs exhibited strong correlations among themselves, hinting at a potential miRNA family with closely related sequences and shared targets. As such, we identified 124 proteins associated with the upregulated miRNAs in the MOD cluster, with 12 proteins associated with all miRNAs. These 12 proteins were also primarily expressed in liver tissue, further supporting liver involvement in this cluster. Previous studies have shown that individuals in the SIRD cluster have the highest prevalence of nonalcoholic fatty liver disease (NAFLD) at diagnosis, with a slightly lower prevalence in the MOD cluster.[2,39,40] Multiple miRNAs associated with the MOD cluster in our study are also linked with NAFLD in other studies.[41] Also, multiple proteins found in this study have previously been found in relation to NAFLD, such as *FCN2* and *MASP1*. *FCN2* has been described as a biomarker for liver fibrosis in NAFLD,[42] and *MASP1*-enriched small extracellular vesicles have even been found to promote liver fibrosis.[43,44] Furthermore, proteins associated with sncRNAs specifically associated with the MOD cluster showed enrichment for the complement system. Most of the proteins that make up the body's complement system are created in the liver and are then transported to other tissues via the bloodstream.[45]

This study has several strengths and limitations worth considering. One notable strength lies in the utilization of untargeted RNA sequencing, enabling the investigation not only of miRNAs but also other types of sncRNAs. We found, besides miRNAs, that one vault RNA and one tRNA were differentially expressed in the insulin-resistant type 2 diabetes cluster. Additionally, the study benefits from the utilization of a well-phenotyped cohort, which enhances the robustness of our findings. Also the use of an independent validation strengthens the robustness of this study. Moreover, while numerous papers have explored the multi-omics variances among clusters over the years, this study represents the pioneering attempt to explore sncRNAs in relation to type 2 diabetes clusters. However, a notable limitation pertains to the limited number of samples available for the plasma proteomic correlation analysis. Nevertheless, the available samples for plasma proteomics exhibited a similar cluster distribution as the entire small RNA-sequencing set and showed some significant associations. We believe the entire small RNA-sequencing set ($n = 412$) is sufficient to detect differences between the clusters as we did not see a relation between the number of individuals in a cluster and the number of differentially expressed sncRNAs. Lastly, this study highlights cross-sectional differences and not causal relationships.

In conclusion, our study reveals an aberrant expression profile of sncRNAs within the insulin-resistant and obesity-associated type 2 diabetes cluster. These findings may reflect the different phenotypes of the clusters and contribute to our understanding of the diverse molecular mechanisms underlying the five clusters, providing insights into potential mechanistic variations in the development, progression, and associated risk factors of type 2 diabetes within each cluster. However, further research is warranted to elucidate the specific functions of these sncRNAs and their implications in the pathophysiology of type 2 diabetes.

AUTHOR CONTRIBUTIONS

Juliette A. de Klerk, L. M. 't Hart and Roderick Slieker designed the study and drafted the manuscript. Juliette A. de Klerk and Roderick Slieker performed the analyses. Joline W. J. Beulens, Petra J. M. Elders and Leen M. 't Hart contributed to the data acquisition and project logistics. Juliette A. de Klerk, Joline W. J. Beulens, Roel Bijkerk, Anton Jan. van Zonneveld, Petra J. M. Elders, Leen M. 't Hart and Roderick Slieker contributed to the data interpretation. All authors critically revised the manuscript and approved the final version. Juliette A. de Klerk and Roderick Slieker are the guarantors of the work.

ACKNOWLEDGEMENTS

We extend our sincere gratitude to all the staff and participants involved in the Hoorn Diabetes Care System for their invaluable support and participation.

FUNDING INFORMATION

This work has been funded by BBMRI-NL (Complementary project, CP2013-69), ZonMW Priority Medicine Elderly (grant no. 113102006 to LMtH). Roderick Slieker, L. M. 't Hart and J. W. J. Beulens received support from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement no. 115881 (RHAPSODY). This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and European Federation of Pharmaceutical Industries and Associations (EFPIA) and is supported by the Swiss State Secretariat for Education, Research and Innovation (SERI) under contract no. 16.0097–2. R. Bijkerk and A. J. van Zonneveld were supported by funding from the European Foundation for

the Study of Diabetes. The opinions expressed and arguments employed herein do not necessarily reflect the official views of these funding bodies.

CONFLICT OF INTEREST STATEMENT

Nothing to declare.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/dom.15786.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are not publicly available due to restrictions in the informed consent. Access can be requested via the corresponding author, but access to data must be granted by the respective steering committee who will check for compliance with the informed consent.

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REFERENCES

1. Nair ATN, Wesolowska-Andersen A, Brorsson C, et al. Heterogeneity in phenotype, disease progression and drug response in type 2 diabetes. *Nat Med*. 2022;28(5):982-988. doi:10.1038/s41591-022-01790-7
2. Ahlqvist E, Storm P, Karajamaki A, et al. Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. *Lancet Diabetes Endocrinol*. 2018;6(5):361-369. doi:10.1016/S2213-8587(18)30051-2
3. Slieker RC, Donnelly LA, Fitipaldi H, et al. Replication and cross-validation of type 2 diabetes subtypes based on clinical variables: an IMI-RHAPSODY study. *Diabetologia*. 2021;64(9):1982-1989. doi:10.1007/s00125-021-05490-8
4. Slieker RC, Donnelly LA, Fitipaldi H, et al. Distinct molecular signatures of clinical clusters in people with type 2 diabetes: An IMI-RHAPSODY study. *Diabetes*. 2021;70(11):2683-2693. doi:10.2337/db20-1281
5. de Klerk JA, Beulens JWJ, Mei H, et al. Altered blood gene expression in the obesity-related type 2 diabetes cluster may be causally involved in lipid metabolism: a Mendelian randomisation study. *Diabetologia*. 2023;66(6):1057-1070. doi:10.1007/s00125-023-05886-8
6. Pordzik J, Jakubik D, Jarosz-Popek J, et al. Significance of circulating microRNAs in diabetes mellitus type 2 and platelet reactivity: bioinformatic analysis and review. *Cardiovasc Diabetol*. 2019;18(1):113. doi:10.1186/s12933-019-0918-x
7. van der Heijden AA, Rauh SP, Dekker JM, et al. The Hoorn diabetes care system (DCS) cohort. A prospective cohort of persons with type 2 diabetes treated in primary care in The Netherlands. *BMJ Open*. 2017;7(5):e015599. doi:10.1136/bmjopen-2016-015599
8. t Hart LM, de Klerk JA, Bouland GA, et al. Small RNA sequencing reveals snoRNAs and piRNA-019825 as novel players in diabetic kidney disease. *Endocrine*. 2024. doi:10.1007/s12020-024-03884-3
9. Rozowsky J, Kitchen RR, Park JJ, et al. exceRpt: a comprehensive analytic platform for extracellular RNA profiling. *Cell Syst*. 2019;8(4):352-357 e353. doi:10.1016/j.cels.2019.03.004
10. Griffiths-Jones S. miRBase: the microRNA sequence database. *Methods Mol Biol*. 2006;342:129-138. doi:10.1385/1-59745-123-1:129
11. Sai Lakshmi S, Agrawal S. piRNABank: a web resource on classified and clustered Piwi-interacting RNAs. *Nucleic Acids Res*. 2008;36-(Database issue):D173-D177. doi:10.1093/nar/gkm696
12. Harrow J, Frankish A, Gonzalez JM, et al. GENCODE: the reference human genome annotation for the ENCODE project. *Genome Res*. 2012;22(9):1760-1774. doi:10.1101/gr.135350.111
13. Glazar P, Papavasileiou P, Rajewsky N. circBase: a database for circular RNAs. *RNA*. 2014;20(11):1666-1670. doi:10.1261/rna.043687.113
14. Chan PP, Lowe TM. GtRNAdb: a database of transfer RNA genes detected in genomic sequence. *Nucleic Acids Res*. 2009;37(Database issue):D93-D97. doi:10.1093/nar/gkn787
15. Consortium GT. The genotype-tissue expression (GTEx) project. *Nat Genet*. 2013;45(6):580-585. doi:10.1038/ng.2653
16. Ma Q, Jiang H, Ma L, et al. The moonlighting function of glycolytic enzyme enolase-1 promotes choline phospholipid metabolism and tumor cell proliferation. *Proc Natl Acad Sci U S A*. 2023;120(15):e2209435120. doi:10.1073/pnas.2209435120
17. Li N, Liu X. Phosphoglycerate mutase 1: its glycolytic and non-glycolytic roles in tumor malignant behaviors and potential therapeutic significance. *Onco Targets Ther*. 2020;13:1787-1795. doi:10.2147/OTT.S238920
18. Huang B, Ma Z, Xie T, et al. Glucose-6-phosphate isomerase deficiency hemolysis. *Quant Imaging Med Surg*. 2023;13(12):8869-8872. doi:10.21037/qims-22-1154
19. Cosentino C, Toivonen S, Diaz Villamil E, et al. Pancreatic beta-cell tRNA hypomethylation and fragmentation link TRMT10A deficiency with diabetes. *Nucleic Acids Res*. 2018;46(19):10302-10318. doi:10.1093/nar/gky839
20. Ma XX, Xiao L, Wen SJ, et al. Small noncoding vault RNA2-1 disrupts gut epithelial barrier function via interaction with HuR. *EMBO Rep*. 2023;24(2):e54925. doi:10.15252/embr.202254925
21. Rahimi N. Defenders and challengers of endothelial barrier function. *Front Immunol*. 2017;8:1847. doi:10.3389/fimmu.2017.01847
22. Song X, Lv Y, Huang N, et al. Clinical characteristics of inpatients with new-onset diabetes mellitus in eastern China: based on novel clustering analysis. *Front Endocrinol (Lausanne)*. 2022;13:927661. doi:10.3389/fendo.2022.927661
23. Ngo D, Benson MD, Long JZ, et al. Proteomic profiling reveals biomarkers and pathways in type 2 diabetes risk. JCI. *Insight*. 2021;6(5). doi:10.1172/jci.insight.144392
24. Luo X, Luan C, Zhou J, et al. Glycolytic enzyme Enolase-1 regulates insulin gene expression in pancreatic beta-cell. *Biochem Biophys Res Commun*. 2024;706:149735. doi:10.1016/j.bbrc.2024.149735
25. Chandel NS. Glycolysis. *Cold Spring Harb Perspect Biol*. 2021;13(5). doi:10.1101/cshperspect.a040535
26. Feve B, Bastard JP. The role of interleukins in insulin resistance and type 2 diabetes mellitus. *Nat Rev Endocrinol*. 2009;5(6):305-311. doi:10.1038/nrendo.2009.62
27. Akbari M, Hassan-Zadeh V. IL-6 signalling pathways and the development of type 2 diabetes. *Inflammopharmacology*. 2018;26(3):685-698. doi:10.1007/s10787-018-0458-0
28. Kochumon S, Madhoun AA, Al-Rashed F, et al. Adipose tissue gene expression of CXCL10 and CXCL11 modulates inflammatory markers in obesity: implications for metabolic inflammation and insulin resistance. *Ther Adv Endocrinol Metab*. 2020;11:2042018820930902. doi:10.1177/2042018820930902

29. Puschel GP, Klauder J, Henkel J. Macrophages, low-grade inflammation, insulin resistance and hyperinsulinemia: a mutual ambiguous relationship in the development of metabolic diseases. *J Clin Med*. 2022;11(15). doi:10.3390/jcm11154358
30. Kadatane SP, Satariano M, Massey M, Mongan K, Raina R. The role of inflammation in CKD. *Cells*. 2023;12(12):1581. doi:10.3390/cells12121581
31. Tanabe H, Saito H, Kudo A, et al. Factors associated with risk of diabetic complications in novel cluster-based diabetes subgroups: a Japanese retrospective cohort study. *J Clin Med*. 2020;9(7). doi:10.3390/jcm9072083
32. Schrader S, Perfilyev A, Ahlqvist E, et al. Novel subgroups of type 2 diabetes display different epigenetic patterns that associate with future diabetic complications. *Diabetes Care*. 2022;45(7):1621-1630. doi:10.2337/dc21-2489
33. Moore KJ, Rayner KJ, Suarez Y, Fernandez-Hernando C. The role of microRNAs in cholesterol efflux and hepatic lipid metabolism. *Annu Rev Nutr*. 2011;31:49-63. doi:10.1146/annurev-nutr-081810-160756
34. Jopling C. Liver-specific microRNA-122: biogenesis and function. *RNA Biol*. 2012;9(2):137-142. doi:10.4161/rna.18827
35. Esau C, Davis S, Murray SF, et al. miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. *Cell Metab*. 2006;3(2):87-98. doi:10.1016/j.cmet.2006.01.005
36. Elmen J, Lindow M, Silahtaroglu A, et al. Antagonism of microRNA-122 in mice by systemically administered LNA-antimiR leads to up-regulation of a large set of predicted target mRNAs in the liver. *Nucleic Acids Res*. 2008;36(4):1153-1162. doi:10.1093/nar/gkm1113
37. Zhang H, Guan M, Townsend KL, et al. MicroRNA-455 regulates brown adipogenesis via a novel HIF1an-AMPK-PGC1alpha signaling network. *EMBO Rep*. 2015;16(10):1378-1393. doi:10.15252/embr.201540837
38. Lei Sun HX, Mori MA, Alexander R, et al. Mir193b–365 is essential for brown fat differentiation. *Nat Cell Biol*. 2011;13:958-965.
39. Pafili K, Roden M. Nonalcoholic fatty liver disease (NAFLD) from pathogenesis to treatment concepts in humans. *Mol Metab*. 2021;50:101122. doi:10.1016/j.molmet.2020.101122
40. Schroder B, Kahl S, Roden M. Non-alcoholic fatty liver disease in type 2 diabetes - a specific entity? *Liver Int*. 2021;41(Suppl 1):105-111. doi:10.1111/liv.14846
41. Kan Changez MI, Mubeen M, Zehra M, et al. Role of microRNA in non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH): a comprehensive review. *J Int Med Res*. 2023;51(9):3000605231197058. doi:10.1177/03000605231197058
42. Giraudi PJ, Salvoza N, Bonazza D, et al. Ficolin-2 plasma level assesses liver fibrosis in non-alcoholic fatty liver disease. *Int J Mol Sci*. 2022;23(5). doi:10.3390/ijms23052813
43. Liu X, Tan S, Liu H, et al. Hepatocyte-derived MASP1-enriched small extracellular vesicles activate HSCs to promote liver fibrosis. *Hepatology*. 2023;77(4):1181-1197. doi:10.1002/hep.32662
44. Wu Y, Cao Y, Xu K, et al. Dynamically remodeled hepatic extracellular matrix predicts prognosis of early-stage cirrhosis. *Cell Death Dis*. 2021;12(2):163. doi:10.1038/s41419-021-03443-y
45. Lung T, Sakem B, Risch L, et al. The complement system in liver diseases: evidence-based approach and therapeutic options. *J Transl Autoimmun*. 2019;2:100017. doi:10.1016/j.jtauto.2019.100017

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How to cite this article: de Klerk JA, Beulens JWJ, Bijkerk R, et al. Circulating small non-coding RNAs are associated with the insulin-resistant and obesity-related type 2 diabetes clusters. *Diabetes Obes Metab*. 2024;26(10):4375-4385. doi:10.1111/dom.15786