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META-ANALYSIS

Identification of microRNA biomarkers in type 2 diabetes: a meta-analysis of controlled profiling studies

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Abstract
Aims/hypothesis The aim was to identify potential microRNA (miRNA) biomarkers of type 2 diabetes.
Methods Controlled studies were retrieved from PubMed to compare miRNA expression profiles of type 2 diabetes and nondiabetic control samples. Meta-analysis under a random effects model was conducted. Subgroup analyses examined tissue specificity and species specificity. Sensitivity analyses were also performed to explain the heterogeneity among studies. Results were represented as log odds ratios (logOR), 95% confidence intervals (CI) and *p* values after Bonferroni correction.
Results Among 343 differentially expressed miRNAs in 38 miRNA expression profiling studies published between 1993 and March 2014, only 151 miRNAs were tested by multiple studies, out of which 102 miRNAs were reported to be upregulated or downregulated. Meta-analysis identified 51 significantly dysregulated miRNAs. The top upregulated miRNA was miR-142-3p (logOR 6.4721; 95% CI 4.9537, 7.9904; adjusted $p=4.60\times10^{-16}$). The top downregulated miRNA was miR-126a (logOR 7.5237; 95% CI 4.7159, 10.3316; adjusted $p=3.01\times10^{-07}$). The dysregulation of two miRNAs (miR-199a-3p and miR-223) was highly pancreas-specific and liver-specific. miR-30e was downregulated in patients with type 2 diabetes, while miR-92a was downregulated in animal models of diabetes. In sensitivity analysis, 40 out of 47 miRNAs (85%) were robustly and consistently dysregulated.
Conclusions/interpretation This meta-analysis confirms that 40 miRNAs are significantly dysregulated in type 2 diabetes. miR-29a, miR-34a, miR-375, miR-103, miR-107, miR-132, miR-142-3p and miR-144 are potential circulating biomarkers of type 2 diabetes. In addition, miR-199a-3p and miR-223 are potential tissue biomarkers of type 2 diabetes.

Keywords Meta-analysis · MicroRNA · Type 2 diabetes

Abbreviations

LogOR	Log odds ratio
MIAME	Minimum information about a microarray experiment
MIQE	Minimum information for publication of quantitative real-time PCR experiments
miRNA	MicroRNA
qPCR	Quantitative PCR

Introduction

Type 2 diabetes is a complex metabolic disorder characterised by insulin resistance [1] that is often undetected until hyperglycaemia is observed [2]. Over time, multiple organ damage can occur, especially to the heart, blood vessels, eyes, kidneys and nerves [3]; thus, exploring novel early biomarkers and therapeutics for diabetes is of great importance. MicroRNAs (miRNAs) are likely to represent early biomarkers of type 2 diabetes that can be used to detect and monitor progression of the disease [4].

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miRNAs are a class of small (approximately 22 nucleotides), endogenous, noncoding, highly stable RNAs that regulate gene and protein expression. miRNAs are involved in many biological processes, including cellular differentiation, metabolism and cancer development [5–7], and their modes of dysregulation are linked to many diseases [8]. In studies attempting to identify novel early biomarkers of type 2 diabetes, miRNA expression profiling was often performed in cultured cells, blood or solid tissue samples [9–12]. Large numbers of miRNAs were identified to be differentially expressed, either overexpressed or underexpressed, while only a small number may actually be important signatures or therapeutic targets. Different profiling studies have employed different profiling platforms and different methods. It is, therefore, challenging to determine which miRNAs are potential biomarkers, which miRNAs are tissue-specific, whether circulating miRNAs make the best biomarkers and whether animal models are sufficient for pilot studies. The results of these studies are, however, subject to evaluation by meta-analysis. Recent studies have indicated that the seminal findings from academic laboratories could only be reproduced 11–50% of the time [13, 14]. A survey on data reproducibility of biomedical science also found the problem of data irreproducibility [15]. As literature reviews on miRNAs in type 2 diabetes were purely narrative without any meta-analysis, it came as no surprise that the overall findings were inconsistent (Table 1). Therefore, no consistent and definitive picture is available. For example, a review [16] reported that the regulation of miR-103 in adipose tissue was different according to different experiments, while a second review [18] deemed miR-103 to be downregulated in adipose tissue and a third review [19] found it to be upregulated in adipose tissue. This study aims to fill this gap by using a meta-analysis to identify consistently dysregulated miRNAs that have been shown in reproducible profiling results to be potential biomarkers for type 2 diabetes.

Methods

Search strategies PubMed was searched for type 2 diabetes miRNA expression profiling studies published between 1993 and 11 March 2014 using the following terms: ('miRNA', 'diabetes' and 'expression' in the title/abstract) or ('miRNA', 'diabetes' and 'profile' in the title/abstract) or ('miRNA', 'diabetes' and 'profiling' in the title/abstract) or ('microRNA', 'diabetes' and 'expression' in the title/abstract) or ('microRNA', 'diabetes' and 'profile' in the title/abstract) or ('microRNA', 'diabetes' and 'profiling' in the title/abstract).

Study selection Eligible studies had to meet the inclusion criteria: (1) they were miRNA expression profiling studies on patients with type 2 diabetes or on animal models of diabetes; (2) they used diabetic and nondiabetic control samples for comparison; (3) they used miRNA expression arrays; (4) they reported cut-off criteria of differentially expressed miRNAs; and (5) they reported sample sizes. miRNA profiling studies using saliva or urine of type 2 diabetes patients were excluded because we focused on miRNAs in blood, and miRNAs studies in saliva and urine were mostly related to oral cancer [21] and urinary tract cancer [22–24], respectively.

Data extraction and quality assessment From the full text and supplementary information of each expression profiling study, the following eligibility items were collected and recorded: first author, year of publication, location of study, selection and characteristics of recruited type 2 diabetes patients or animal models of diabetes, miRNA expression profiling

Table 1 Inconsistent findings of literature reviews on possible associations of miRNA dysregulation

miRNA	Literature review							Meta-analysis
	Guay 2011 [16]	Guay 2012 [17]	Hamar 2012 [18]	Karolina 2012 [2]	McClelland 2014 [19]	Natarajan 2012 [20]	Shantikumar 2012 [4]	The present study
miR-103 (adipose)	N	–	D	–	U	–	N	N
miR-107 (adipose)	D	–	–	–	U	–	–	N
miR-132 (adipose)	–	–	U	–	–	–	D	N
miR-143 (adipose)	N	–	–	D	–	–	N	N
miR-144 (liver)	D	–	–	U	–	–	–	N
miR-192 (kidney)	–	–	U	N	N	N	–	N
miR-192 (liver)	D	–	–	–	–	–	–	U
miR-21 (kidney)	–	–	–	D	U	N	–	N
miR-29c (liver)	N	–	–	–	–	–	–	U
miR-375 (islets)	D	U	–	U	U	–	U	U

D, downregulated; U, upregulated; N, inconsistent findings identified; –, not reported

platform, sample sizes, tissue types, cut-off criteria of upregulated and downregulated miRNAs and the list of differentially expressed miRNAs and their corresponding fold changes (if available). Quality assessment of microarrays was performed according to the Minimum Information About a Microarray Experiment (MIAME) guideline version 2.0 [25]. Studies involving quantitative PCR (qPCR)-based miRNA arrays were assessed according to the Minimum Information for Publication of Quantitative Real-time PCR Experiments (MIQE) guideline [26], which is equivalent to the MIAME guideline.

Meta-analysis Extracted data were transferred to the statistical software R (Revolution R Enterprise, version 6.1.0) with the Metafor package (http://cran.r-project.org/web/packages/metafor/index.html, version1.9-5) [27] for meta-analysis under a random effects model. The outcomes are presented as $\log_{10}$ odds ratios (logORs), based on the numbers of dysregulation events in both type 2 diabetes and nondiabetic control samples, with their 95% confidence intervals. Bonferroni corrections were performed on p values. Adjusted p values less than 0.05 were considered significant. When compared with nondiabetic control groups, a significant logOR higher than 1 indicated miRNA upregulation. When compared with the diabetic group, a significant logOR higher than 1 indicated miRNA downregulation. Potential circulating biomarkers should be significantly upregulated or downregulated and detectable in both human and animal blood or in both blood and tissues. Potential tissue biomarkers should be significantly upregulated or downregulated and highly tissue-specific. Differentially expressed miRNAs in type 2 diabetes and nondiabetic control samples were ranked according to the following order of importance: (1) p values; (2) the number of consistent reports; and (3) logOR values.

Subgroup analysis miRNAs are differentially expressed among tissue types and species, with corresponding overall effects and heterogeneities. Subgroup analyses split the extracted data according to tissue types and species in order to compare miRNA expression profiles among tissue types (blood, muscle, pancreas, liver, etc.) and species (i.e. tissue specificity and species specificity). Studies using serum, plasma or peripheral blood mononuclear cells were classified as blood as they were from blood and aimed to investigate circulating miRNAs. Studies that did not report the pancreatic tissue was whole pancreas or pancreatic islets were pooled with the other studies on pancreatic islets.

Sensitivity analysis Sensitivity analysis was performed on the sample size to test the robustness of findings. Sample size is a dominant factor that affects precision in determining the overall effects. Thus, the meta-analysis was repeated after excluding the studies for which sample sizes were ten or less.

Results

Included studies and their characteristics Figure 1 shows the selection process of the studies. A total of 253 potentially relevant studies were identified in PubMed. After removal of duplicated publications and reviews, 38 articles met the

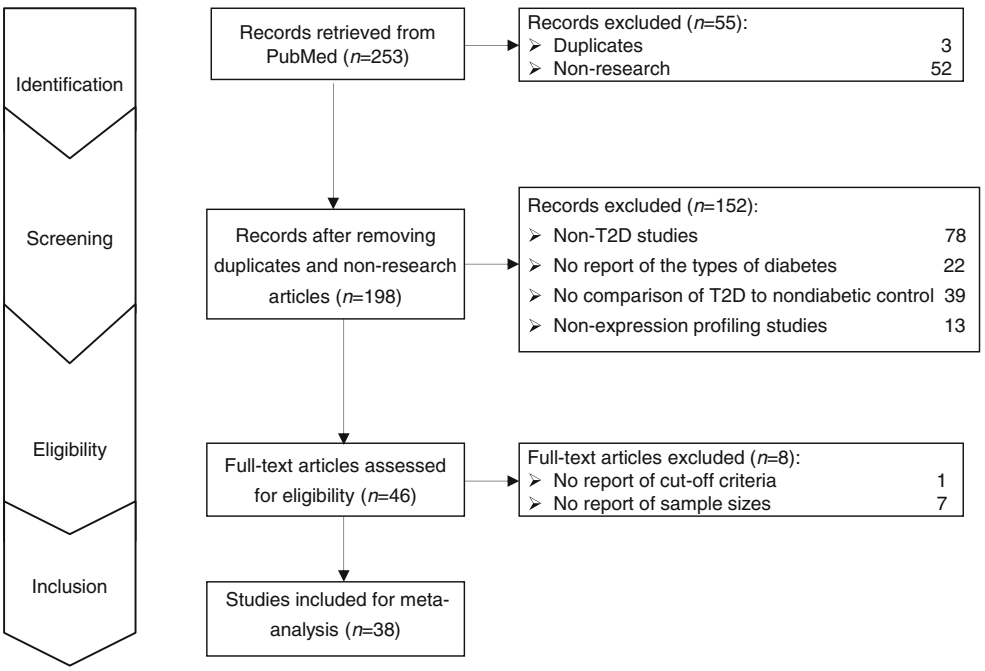

Fig. 1 Flow diagram of study selection. The process of study selection including identification, screening, eligibility extraction and inclusion steps is depicted in the flow diagram. Out of the 253 records identified from PubMed, 38 studies met the selection criteria. T2D, type 2 diabetes

eligibility criteria. Details of study characteristics are shown in Tables 2 and 3. Among the 38 included studies, 22 reported only human miRNA expression profiles (Table 2), 15 focused on miRNA expression profiles in animal models (Table 3), and only one was based on both humans and animals. The numbers of differentially expressed miRNAs ranged from 1 to 130, with a median of 10. Fold change information of miRNAs was available in 17 of the 38 studies.

Quality assessment of studies MIAME guideline 2.0 [25] and MIQE guideline [26] were used to assess study quality. Figure 2 and electronic supplementary material (ESM) Table 1 summarise the quality assessment process. Of the included studies, 74% did not report raw data of hybridisation and 82% of studies did not give sufficient information about experimental design and sample data relationships. For more than 30% of the studies, other key aspects (such as final processed data and array design) were not always fully reported.

Differentially expressed miRNAs Out of 343 differentially expressed miRNAs reported in the 38 studies that compared type 2 diabetes samples with nondiabetic control samples, 151 miRNAs (44.0%) were reported in at least two substudies. Among the 151 differentially expressed miRNAs, 51 (33.8%) were consistent in their direction of dysregulation (30 were reported to be upregulated and 21 downregulated). In the meta-analysis of dysregulated miRNAs, the dysregulation of these 51 miRNAs was significant, as shown in ESM Tables 2 and 3 (30 upregulated and 21 downregulated). The most reported upregulated miRNA was miR-29a, which was reported in ten substudies with an adjusted $p=3.72\times10^{-14}$. miR-487b (adjusted $p=6.24\times10^{-06}$) was the most reported downregulated miRNA, which was reported in four substudies.

Subgroup analysis Eighteen of the 38 studies investigated circulating miRNAs, which used plasma, serum, peripheral blood mononuclear cells and whole blood as profiling samples. Six investigated muscle tissue, eight investigated pancreas tissue, three investigated glomeruli tissue, six investigated liver tissue and six investigated adipose tissue. Among the eight pancreas tissue profiling studies, five studies reported using pancreatic islets and the others reported using whole pancreas. Details are shown in Tables 2 and 3. Significant dysregulation of miRNAs in different tissue types is shown in ESM Tables 4–9. Among the dysregulated miRNAs, ten upregulated miRNAs (miR-103, miR-107, miR-132, miR-143, miR-144, miR-21, miR-29a, miR-29b, miR-34a and miR-375) were consistently reported in at least two tissue types, while 36 miRNAs (26 upregulated and ten downregulated) were reported in only one tissue. The other two dysregulated miRNAs (miR-199a-3p and miR-223) were upregulated in one tissue type and downregulated in another (ESM Table 10), which suggests that their regulation is highly tissue-specific.

In subgroup analysis of species, 190 miRNAs were reported in 23 human profiling studies with 54 reported in at least two substudies. Among these 54 miRNAs, 18 were upregulated and seven were downregulated (ESM Table 11), while differential expression of the other 29 was not significant (adjusted $p>0.05$).

A total of 241 miRNAs were reported in 16 animal profiling studies with 119 reported in at least two substudies. Among the 119 differentially expressed miRNAs, 35 miRNAs had adjusted p values <0.05 (27 were upregulated and eight were downregulated). Details are shown in ESM Table 12. Comparison of upregulated miRNAs ($n=30$) in all profiling studies showed that miR-185 (adjusted $p=6.29\times10^{-05}$), miR-187 (adjusted $p=5.27\times10^{-05}$) and miR-103 (adjusted $p=1.40\times10^{-03}$) were upregulated in human profiling studies, while miR-19a (adjusted $p=9.47\times10^{-04}$), let-7d (adjusted $p=5.53\times10^{-06}$), miR-191 (adjusted $p=1.14\times10^{-04}$), miR-320 (adjusted $p=2.08\times10^{-06}$), miR-27a (adjusted $p=5.36\times10^{-04}$) and miR-29b (adjusted $p=1.57\times10^{-07}$), amongst other miRNAs, were upregulated in animal profiling studies. Comparison of significantly downregulated miRNAs ($n=21$) in all profiling studies showed that miR-652 (adjusted $p=8.64\times10^{-05}$) and miR-30e (adjusted $p=5.56\times10^{-08}$) were downregulated in human profiling studies, while miR-92a (adjusted $p=3.15\times10^{-04}$) was downregulated in animal profiling studies. These results indicate that miRNAs might be expressed differently in various species. Human tissue-specific and animal tissue-specific miRNAs had already been analysed. There were no sufficient samples for analysis; thus, the results are not included in this manuscript.

Sensitivity analysis Sensitivity analysis was conducted to examine the robustness of the findings and to determine what effect sample size had on the overall analysis. Thirty of the 38 included reports had sample sizes greater than ten. We excluded studies where sample size was no more than ten. Analysing the thirty studies identified 47 miRNAs that were significantly differentially expressed, with 29 upregulated and 18 downregulated (ESM Table 13). Forty of the 47 miRNAs (85%) were significantly differentially expressed both in the sensitivity analysis and the overall analysis, while the other seven were not significantly differentially expressed in the overall analysis. This result indicated that the small sample sizes used in miRNA profiling studies may explain some differences in the results of miRNA profiling studies.

Discussion

This study is the first meta-analysis of type 2 diabetes miRNA expression profiling studies and identified specific miRNAs

Table 2 Characteristics of human miRNA expression profiling studies (T2D vs nondiabetic controls)

Study	Year	T2D					Differentially expressed miRNAs				
		Country	Period	Tissue	Clinical status	No. of samples (T2D/nondiabetic)	Platform	Cut-off criteria	Total	Upregulated	Downregulated
Balasubramanyam [28]	2011	Chennai India	NR	PBMCs	Diagnosed	40 (20/20)	miRCURY LNA miRNA PCR system (Exiqon)	$p<0.05$	1	0	1
Corral-Fernández [29]	2013	Mexico	NR	PBMCs	Diagnosed	40 (20/20)	TaqMan miRNA Assays (Applied Biosystems)	$p<0.05$	2	0	2
Gallagher [30]	2010	Scotland, UK	NR	Skeletal muscle tissue	Diagnosed	92 (45/47)	miRCURY LNA miRNA array (Exiqon) and TaqMan Assays (Applied Biosystems)	SAM; FDR <0.1 (FC >1.3)	62	29	33
Karolina [31]	2011	Singapore	Jul 2008 to Apr 2009	Whole blood	Diagnosed	15 (8/7)	miRCURY LNA miRNA array (Exiqon) and TaqMan Assays (Applied Biosystems)	$p<0.05$	81	48	34
Karolina [11]	2012	Singapore	Jul 2008 to Jul 2012	Whole blood	Diagnosed	96 (50/46)	miRCURY LNA miRNA array (Exiqon) and TaqMan Assays (Applied Biosystems)	$p<0.05$	11	5	6
Klöting [32]	2009	Germany	6 months	Subcutaneous (SC)/omental adipose	Newly diagnosed	15 (6/9)	TaqMan miRNA Assays (Applied Biosystems)	$p<0.05$	11	3	8
Kong [33]	2010	Shandong, China	Feb 2009 to Jun 2009	Serum	Newly diagnosed	44 (18/26)	TaqMan miRNA Assays (ABI company)	$C_t=40$; $p<0.01$	1	1	0
Kong [34]	2010	Shandong, China	NR	Serum	Newly diagnosed	37 (18/19)	TaqMan miRNA Assays (Applied BioSystems)	$p<0.05$	7	7	0
Locke [35]	2014	USA and UK	NR	Pancreatic islets	Diagnosed	20 (11/9) 20 (10/10)	TaqMan miRNA Assays (Life Technologies)	$p<0.05$	2	2	0
Lu [36]	2010	UK	NR	Left ventricular biopsies	Diagnosed	12 (6/6)	TaqMan miRNA Assay (Applied Biosystems)	FC <−2 or >2, $p\leq0.05$	18	10	8
Meng [37]	2012	Shanghai, China	NR	PBMCs	Diagnosed	30 (15/15)	miRNA array probes (LC Sciences) and TaqMan miRNA Assays (Applied BioSystems)	$p<0.01$	5	0	5
Ortega [38]	2010	Girona, Spain	NR	Adipose	Diagnosed	T2D/nondiabetic: 9/6 T2D + obese/obese: 9/13	miRNA microarrays (Agilent Technologies) TaqMan miRNA Assays (Applied BioSystems)	$p<0.05$	8	4	4
Ortega [39]	2014	Girona, Spain	NR	Plasma	Diagnosed	T2D/nondiabetic: 30/35 T2D + obese/obese: 18/10	TaqMan Low-Density Arrays (Applied Biosystems)	$p<0.05$	6	3	3
Pescador [40]	2013	Madrid, Spain	NR	Serum	Diagnosed	T2D: 13 nondiabetic: 20 obese: 20 T2D + obese: 16	MiRCURY LNA microRNA PCR system	FC >5	4	2	2
Rong [41]	2013	Hubei, China	Mar 2011 to Jun 2011	Plasma	Newly diagnosed	180 (90/90)	TaqMan miRNA Assays (Applied Biosystems)	$p<0.05$	1	1	0

Table 2 (continued)

Study	Year	T2D					Differentially expressed miRNAs				
		Country	Period	Tissue	Clinical status	No. of samples (T2D/nondiabetic)	Platform	Cut-off criteria	Total	Upregulated	Downregulated
Spinetti [42]	2013	NR	NR	Serum and PACs from PBMCs	Diagnosed	T2D + CLI/nondiabetic: 99 (101)/18 T2D/nondiabetic: 6/5 T2D + CLI/CLI: 6/6 T2D/nondiabetic: 52/17	TaqMan-validated RT-PCR (Applied Biosystems)	$p<0.05$	19	17	2
Sun [43]	2014	Xinjiang, China	2010 to 2011	Plasma	Diagnosed	200 (100/100)	TaqMan miRNA Assays (Applied Biosystems)	$p<0.05$	1	1	0
Wang [44]	2012	Guangxi, China	Mar 2010 to Mar 2011	PBMCs	Diagnosed	116 (30/86)	All-in-One miRNA Q-PCR (GeneCopoeia)	$p<0.05$	1	1	0
Wang [45]	2014	Sweden and Iraq	Feb 2010 to Mar 2010	Plasma	Diagnosed	152 (33/119)	miRCURY LNA miRNA PCR system (Exiqon)	FC >1.5	9	9	0
Zampetaki [46]	2010	Bolzano, Italy	1995 to 2005	Plasma	Diagnosed	160 (80/80)	TaqMan miRNA Assays (Applied Biosystems)	$p<0.05$	13	1	12
Zhang [47]	2013	Shenzhen, China	NR	Plasma	Diagnosed	60 (30/30)	Bio-Rad CFX-96 qPCR system (Bio-Rad)	$p<0.05$	1	0	1
Zhao [48]	2010	Hong Kong, China	NR	Pancreatic tissue	Diagnosed	55 (40/15)	TaqMan miRNA Assays (Applied BioSystems)	$p<0.05$	1	1	0
Zhou [49]	2013	Chongqing, China	2010 to 2012	Whole blood	Diagnosed	16 (7/9)	LC sciences (Houston) TaqMan miRNA Assays (Applied Biosystems)	$p<0.05$	1	1	0

CLI, critical limb ischaemia; FC, fold change; FDR, false discovery rate; NR, not reported; PACs, proangiogenic cells; PBMCs, peripheral blood mononuclear cells; SAM, significance of microarray analysis; T2D, type 2 diabetes

Table 3 Characteristics of animal miRNA expression profiling studies (T2D vs nondiabetic control)

First author (reference)	Year	Country	T2D				Differentially expressed miRNAs				
			Traceable animal source	Tissue	Animal model	No. of samples (T2D/nondiabetic)	Platform	Cut-off criteria	Total	Upregulated	Downregulated
Chettimada [50]	2014	USA	NR	Aorta	GK rats	14 (7/7)	TaqMan miRNA Assays (Applied Biosystems)	$p<0.05$	4	4	0
Esguerra [51]	2011	Malmo, Sweden	NR	Pancreatic islets	GK rats	12 (6/6)	miRCURY LNA array (Exiqon)	SAM; median FDR=0%	30	24	6
He [52]	2007	Beijing, China	NR	Skeletal muscle tissue	GK rats	4 (2/2)	Microarray analysis (the microRNA Registry, Release 8.0)	FC ≥1.5	15	4	11
Herrera [53]	2009	Oxford, UK	Oxford	Liver, adipose tissue	GK rats	8 (liver: 4/4) 8 (fat: 4/4)	Ambion mirVana miRNA arrays	$p<0.05$	17	14	3
Herrera [54]	2010	Oxford, UK	Oxford	Liver, white adipose tissue, skeletal muscle	GK rats	9 (liver: 4/5) 10 (muscle: 5/5) 9 (fat: 4/5)	miRCURY LNA miRNA array (Exiqon)	$p<0.05$ or FDR <5%	29	17	12
Jordan [55]	2011	Cologne, Germany	NR	Liver, heart, skeletal muscle, adipose and pancreatic tissue	*db*/*db* mice	40 (liver: 20/20); 29 (heart: 13/16); 30 (muscle: 15/15); 62 (fat: 30/32); 22 (pancreas: 12/10)	TaqMan miRNA Assays (Applied Biosystems)	$p\leq0.05$	2	2	0
Karolina [31]	2011	Singapore	NR	Whole blood; adipose tissues, pancreas, skeletal muscle and liver	HFD and STZ induced T2D	*n*=12 (6/6) for each tissue	miRCURY LNA miRNA array (Exiqon) and TaqMan Assays (Applied Biosystems)	FC ≥1.5 and $p<0.05$	130	74	43
Kato [56]	2009	California, USA	Jackson Laboratory	Glomeruli	*db*/*db* mice	8 (4/4)	SYBR Green PCR Master Mix and 7300 Real-time PCR System (Applied Biosystems)	$p<0.05$	2	2	0
Kato [57]	2010	California, USA	Jackson Laboratory	Glomeruli	*db*/*db* mice	6 (3/3)	SYBR Green PCR Master Mix and 7300 Real-time PCR System (Applied Biosystems)	$p<0.05$	1	1	0
Kaur [58]	2011	Delhi, India	CSIR, Lucknow, India	Liver	*db*/*db* mice	8 (4/4)	miRNA microarray (Agilent Technologies)	$p<0.05$ and FDR <5%	13	11	2
Lovis [59]	2008	Lausanne, Switzerland	NR	Pancreatic islets	*db*/*db* mice	9 (5/4)	LC Science (Houston) mirVana qRT-PCR miRNA (Ambion)	$p<0.05$	2	2	0
Nesca [60]	2013	Lausanne, Switzerland	Sydney, Australia	Pancreatic islets	*db*/*db* mice	14 (7/7)	miRNA microarrays (Agilent Technologies)miRCURY LNA miRNA PCR system (Exiqon)	$p\leq0.05$; FC >1.5	65	35	30
Poy [61]	2009	Berlin, Germany	NR	Pancreatic islets	*ob*/*ob*	12 (6/6)	qPCR	$p<0.05$	1	1	0
Trajkovski [62]	2011	Zurich, Switzerland	NR	Liver	*ob*/*ob*	6 (3/3)	miRCURY LNA miRNA Labelling (Exiqon) miRNA arrays (miRXplore)	FC >1.2 and $p<0.05$	60	27	33
Suvh [63]	2010	California, USA	Jackson Laboratory	Mouse vascular smooth muscle cells	*db*/*db* mice	14 (7/7)	RT-qPCR	$p<0.01$	1	1	0
Zhang [64]	2009	Chongqing, China	Shanghai, China	Glomeruli	*db*/*db* mice	18 (9/9)	LC Sciences (Houston)	$p<0.05$	66	35	31

FC, fold change; FDR, false discovery rate; HFD, high fat diet; NR, not reported; SAM, significance of microarray analysis; STZ, streptozotocin; T2D, type 2 diabetes

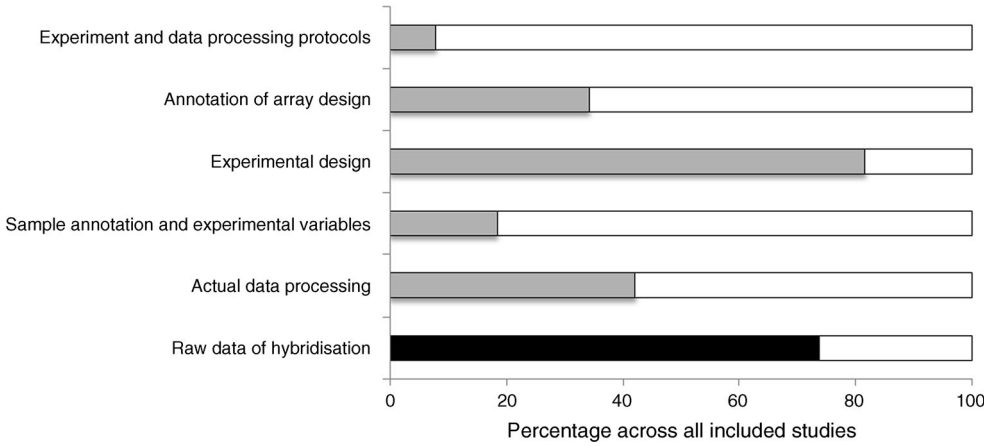

Fig. 2 Quality assessment according to the MIAME guidelines. White bars, grey bars and black bars, respectively, indicate the items that were sufficient in annotation, not sufficient in annotation and not reported

as potential biomarkers of type 2 diabetes. A Venn diagram of miRNA categories and a flow chart of the meta-analysis is shown in Fig. 3.

A total of 151 differentially expressed miRNAs were reported in at least two independent substudies. Among these 151 miRNAs, only 51 (34%) were significantly differentially expressed, while 100 (66%) were not. This result is not a surprise as initially we expected that the number of significantly differentially expressed miRNAs might be the same as or less than that of nonsignificantly differentially expressed

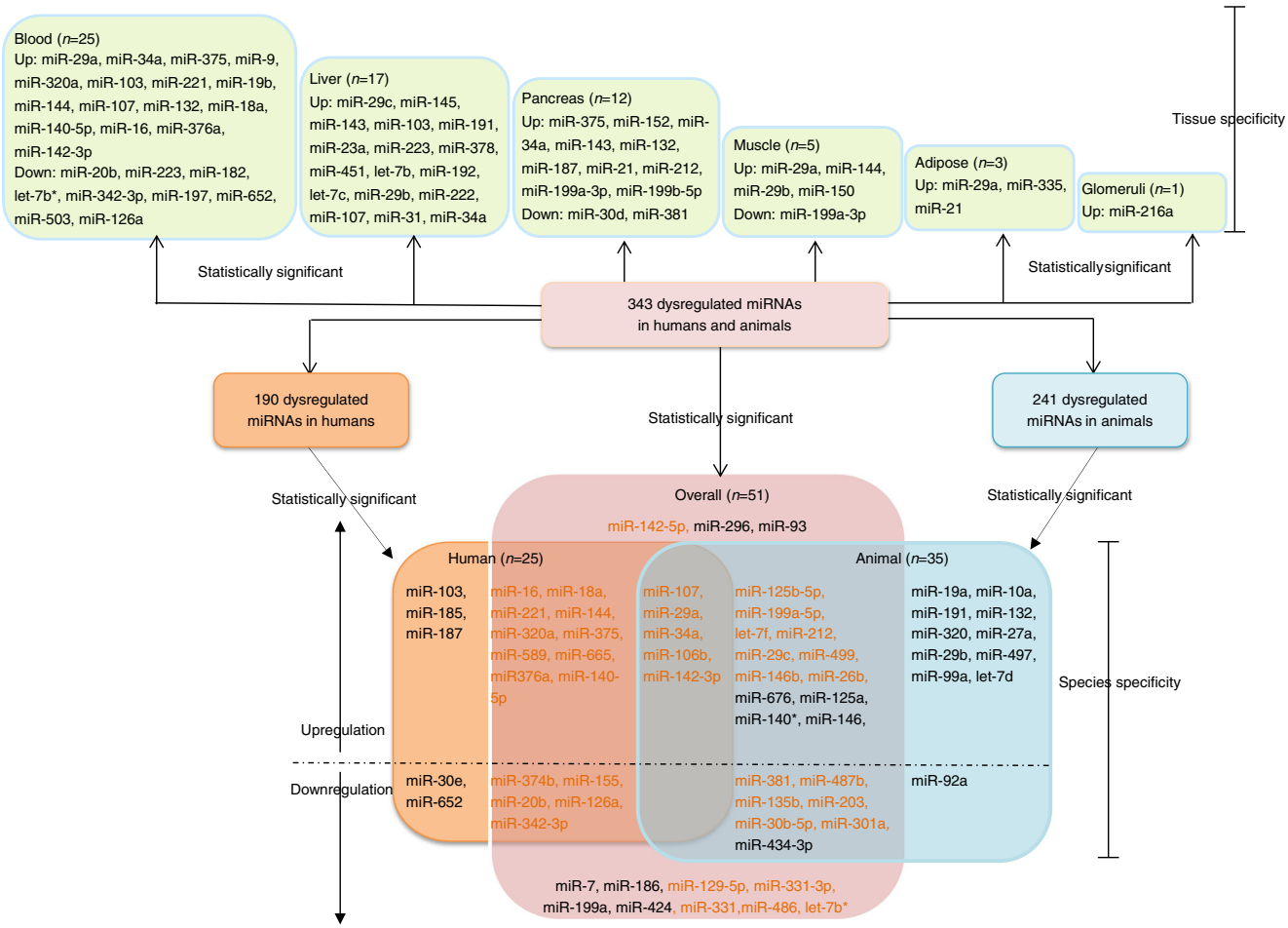

Fig. 3 Flow diagram showing miRNA categories analysed in this meta-analysis. The blocks in the upper part of the diagram show the tissue specificity. The Venn diagram in the lower part summarises the results of the overall analysis, species specificity and sensitivity analyses. miRNAs in orange (n=40) were found to be robustly and consistently dysregulated in sensitivity analysis. n, number of statistically significant miRNAs

miRNAs. Several factors may explain the meta-analysis results of the differentially expressed miRNAs, such as publication bias, biological complexity (for example, variations in environmental backgrounds and gene susceptibility), insufficient information about the pattern of miRNA expression in different tissue types (the heterogeneity in different kinds of specimens) and heterogeneous conditions.

The miRNAs exert actions in different tissues, while circulating miRNAs hold much promise as biomarkers of type 2 diabetes. In the tissue subgroup study, 36 miRNAs were only expressed in one tissue, while two (miR-199a-3p and miR-223) were upregulated in one tissue and downregulated in another. Ten miRNAs (miR-103, miR-107, miR-132, miR-143, miR-144, miR-21, miR-29a, miR-29b, miR-34a and miR-375) were consistently reported in multiple tissue types, for example muscle, adipose tissue, liver and pancreas, and seven of these (miR-103, miR-107, miR-132, miR-144, miR-29a, miR-34a and miR-375) were identified in blood. This finding indicates that circulating miRNAs hold much potential as biomarkers of type 2 diabetes [65]. Laterza et al [66] and Kosaka et al [67] have demonstrated how circulating miRNAs may indicate the physiological state at the tissue level. The miRNAs that circulate in the blood are in a stable form and remain stable even after multiple freeze–thaw cycles. They can be detected by minimally invasive techniques [68] and are specific to tissue and disease states [69]. If these miRNAs in circulating blood can serve as biomarkers, they would provide a minimally invasive biomarker approach that would be extremely useful in diagnosing and monitoring type 2 diabetes. Studies on multiple tissues were also analysed (ESM Table 14). Ten miRNAs with significant differential expression were consistently found in liver and adipose tissue among all the studies. The high agreement between the liver and adipose tissue analyses indicate the reliability of liver-specific and adipose-tissue-specific miRNAs. However, more studies are needed to identify miRNAs that are tissue-specific in other tissue types.

miRNAs can be differentially expressed between animals and humans. Although animal studies might be informative about type 2 diabetes indicators in humans, animals are still different from humans. In the species subgroup analysis, the differential expression of some miRNAs was significant in both humans and animals, but other miRNAs, such as let-7d (adjusted $p=5.53\times10^{-06}$) and miR-29b (adjusted $p=1.57\times10^{-07}$), were significantly differentially expressed in animals but not in humans. These results indicate that miRNAs can be differentially expressed in various species and animal models are insufficient to determine indicators of type 2 diabetes in humans. Thus, miRNAs identified as candidates in animal models require verification in humans.

This study identified ten miRNAs (miR-103, miR-107, miR-132, miR-142-3p, miR-144, miR-199a-3p, miR-223, miR-29a, miR-34a and miR-375) as potential biomarkers of type 2 diabetes, including circulating and tissue biomarkers. Circulating biomarkers are preferred to tissue biomarkers due to easier sampling and testing. For extensive validation of tissue biomarkers, animal experiments would be less costly than clinical studies. In cases in which resources for clinical studies are constrained, we would suggest validating the tissue miRNA biomarkers commonly found in both animals and humans before those found only in humans. The potential circulating biomarkers detected in both human and animal blood or in both blood and tissues are shown in Fig. 4. The potential tissue biomarkers were miR-199a-3p and miR-223. Among all the profiling studies, the most frequently reported (ten studies) and upregulated miRNA was miR-29a (adjusted $p=3.72\times10^{-14}$), which was overexpressed together with miR-29c (adjusted $p=5.13\times10^{-10}$) during hyperglycaemia and hyperinsulinaemia. Overexpression of the miR-29 family impairs insulin-stimulated glucose uptake by inhibiting insulin signalling via the Akt signalling pathway [52]. miR-34a (adjusted $p=3.69\times10^{-14}$) ranked second among the upregulated miRNAs in all profiling studies. miR-34a in beta cells increased in response to palmitate, impairing nutrient-induced insulin secretion by repressing vesicle-associated membrane protein 2 (VAMP2) expression [59] and making the beta cells more susceptible to apoptosis [70]. miR-103 and miR-107, which play a central role in regulating insulin sensitivity by targeting caveolin-1 (a regulator of the insulin receptor) [62], were upregulated in the blood of both humans and animals in our analysis. The most upregulated miRNA in human profiling studies was miR-375 (adjusted $p=3.60\times10^{-13}$), which inhibits insulin secretion by repressing its targets myotrophin [71] and phosphoinositide-dependent protein kinase-1 (PDK1) [72]. In both overall analysis and subgroup analysis of species, miR-144 (adjusted $p=4.81\times10^{-10}$) and miR-142-3p (adjusted $p=9.71\times10^{-13}$) were upregulated in human type 2 diabetes blood samples. miR-144 impairs insulin signalling by inhibiting the expression of insulin receptor substrate 1 (IRS1) [31] and miR-142-3p regulates Akt1 (also known as protein kinase B) in adipogenesis regulation [73, 74]. In the subgroup analysis of tissue types, miR-223 was downregulated in one tissue and upregulated in another. Upregulated miR-223 in cardiomyocytes induces glucose transporter 4 (GLUT4) protein expression to restore normal glucose uptake [36]. miR-132 was upregulated in both blood and liver. miR-132 targets insulin-mediated regulation of CYP2E1 (cytochrome P450, family 2, subfamily E, polypeptide 1), which is involved in hepatic metabolism [75].

miR-132 inhibits the expression of LRRFIP1 (leucine-rich repeat (in Flightless 1) interacting protein-1) to block vascular smooth muscle cell proliferation in atherosclerosis and restenosis [76], and miR-144 is reported to regulate haematopoiesis and vascular development by repressing the expression of meis homeobox 1 [77]. Likewise, the miR-29 family, which directly targets *Mmp2* (the matrix

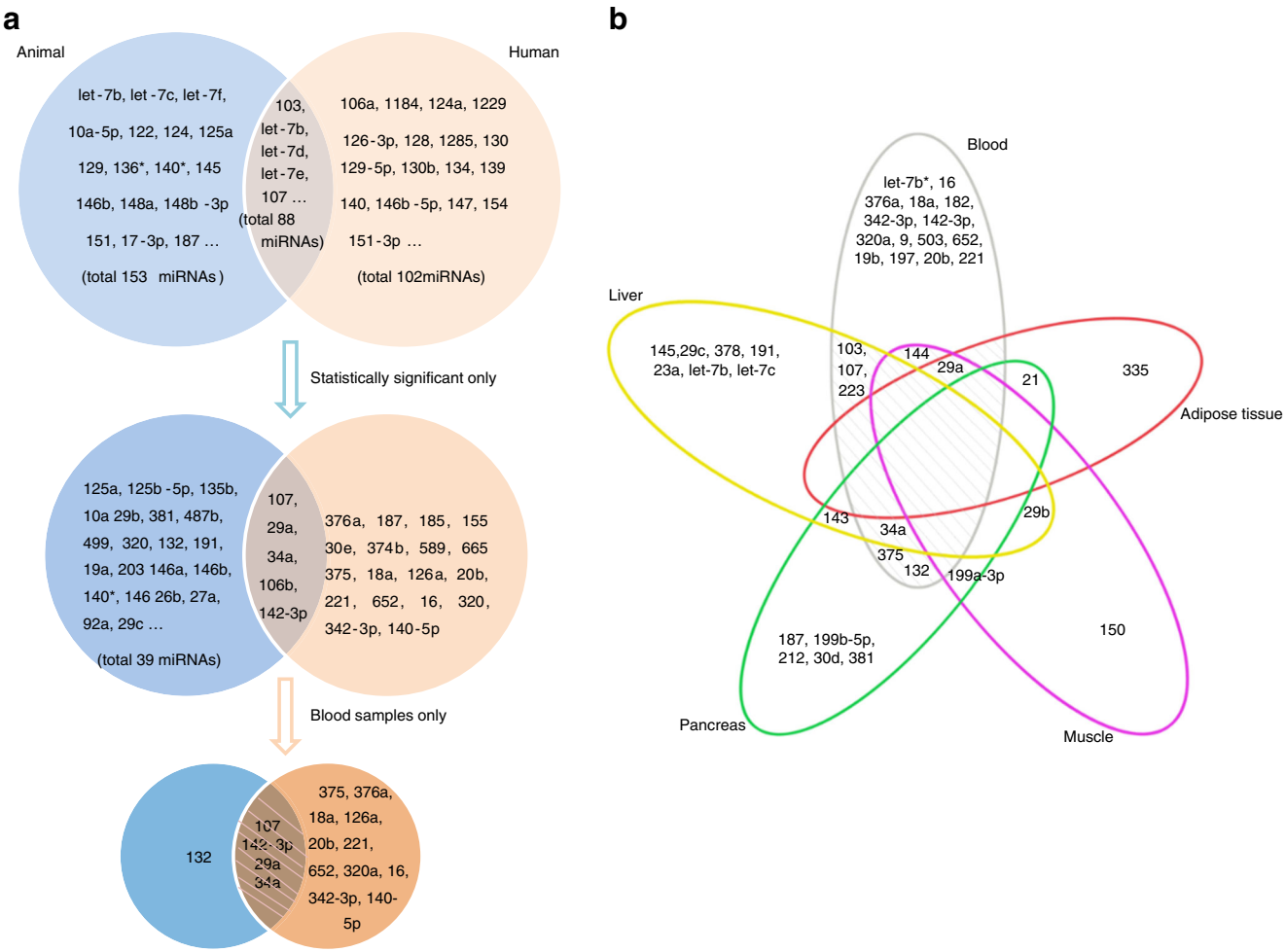

Fig. 4 Venn diagrams showing some potential miRNA biomarkers of type 2 diabetes. (**a**) Stepwise selection of miRNAs; (**b**) commonality of miRNAs among tissues including blood. The prefix 'miR-' of miRNA identifiers is omitted. The potential miRNA biomarkers are in the shaded area. The shaded area of Fig. 4b also contains identified potential miRNA biomarkers in both blood and tissues

metalloproteinase 2 gene), *Col1a1* (the collagen, type I, alpha 1 gene) and *Col3a1* (the collagen, type III, alpha 1 gene), is involved in renal and cardiovascular injury [78]. miR-34a modulates p53 that is related to human atherosclerosis [79]. It also inhibits sirtuin-1 and HMGCR (3-hydroxy-3-methyl-glutaryl-CoA reductase) in nonalcoholic fatty liver disease [80]. miR-375 targets caveolin1, janus kinase 2 (JAK2) and yes-associated protein 1 (YAP1) that are related to cancer [81, 82]. These plausible relationships indicate that miRNAs are associated with not only type 2 diabetes but also the complications of type 2 diabetes. Since specific miRNAs were consistently detected as being dysregulated in multiple studies of (1) both human and animal blood or (2) both blood and tissues, they represent potential biomarkers of type 2 diabetes for extensive validation.

Conclusion This meta-analysis of type 2 diabetes miRNA expression profiling studies identified 40 significantly dysregulated miRNAs. Eight miRNAs (miR-103, miR-107, miR-132, miR-144, miR-142-3p, miR-29a, miR-34a and miR-375) are potential blood biomarkers, while two miRNAs (miR-199a-3p and miR-223) have high tissue-specific regulation and are potential tissue biomarkers.

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Contribution statement SL is the guarantor of this work and conceived the meta-analysis. SL and HZ designed the protocol. HZ searched the databases, assessed and selected studies, and extracted and analysed the data from the selected studies according to the eligibility criteria. SL and HZ interpreted the data and drafted a report on the findings. SL revised the manuscript for submission. All authors read and approved the final version of the manuscript.

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