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Liver microRNAs: potential mediators and biomarkers for metabolic and cardiovascular disease?

Peter Willeit^{1,2,3}, Philipp Skroblin¹, Stefan Kiechl², Carlos Fernández-Hernando⁴, and Manuel Mayr^{1*}

¹King's British Heart Foundation Centre, King's College London, London, UK; ²Department of Neurology, Medical University of Innsbruck, Innsbruck, Austria; ³Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK; and ⁴Vascular Biology and Therapeutics Program, Yale University School of Medicine, New Haven, CT, USA

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Recent discoveries have revealed that microRNAs (miRNAs) play a key role in the regulation of gene expression. In this review, we summarize the rapidly evolving knowledge about liver miRNAs (including miR-33, -33*, miR-223, -30c, -144, -148a, -24, -29, and -122) and their link to hepatic lipid metabolism, atherosclerosis and cardiovascular disease, non-alcoholic fatty liver disease, metabolic syndrome, and type-2 diabetes. With regards to its biomarker potential, the main focus is on miR-122 as the most abundant liver miRNA with exquisite tissue specificity. MiR-122 has been proposed to play a central role in the maintenance of lipid and glucose homeostasis and is consistently detectable in serum and plasma. This miRNA may therefore constitute a novel biomarker for cardiovascular and metabolic diseases.

Keywords MicroRNAs • Biomarkers • Lipid metabolism • Cardiovascular disease

Introduction

MicroRNAs (miRNAs, miRs) are small ~22 nucleotides long non-coding regulatory molecules.¹ They are generated from primary transcripts (pri-miR), which are processed by endonuclease complexes into miRNA precursors (pre-miR) and further into a duplex of two miRNA strands (5p and 3p strand).² In most cases, only one of the two strands (termed the guide or mature strand) is stable and biologically active. In some cases, the other strand (termed passenger or star [*] strand) also regulates distinct targets. MiRNAs are able to modify gene expression at the post-transcriptional level by binding to the 3'-untranslated regions of target messenger RNAs and thereby inducing their degradation or repressing their translation.¹ They often have multiple targets within the same biological pathway, closely interact with each other, and target both activators and inhibitors of a functional regulator.³ MiRNAs therefore constitute a layer of epigenetic regulation that provides additional control of intricate processes such as metabolism, cell growth, differentiation, stress response, and tissue remodelling, and safeguards the

stability of biological systems. In 2015, the European Society of Cardiology Working Group Atherosclerosis and Vascular Biology has identified miRNAs as an area of major interest in the search for novel biomarkers.⁴

Over the past years, evidence has emerged that miRNAs involved in the regulation of cholesterol and lipid metabolism in the liver may contribute to the development of metabolic disturbances and cardiovascular disease. The present review (i) describes the mechanistic role of liver miRNAs in hepatic lipid metabolism; (ii) assesses the detectability of liver miRNAs in the circulation, a prerequisite for their exploitation as soluble biomarkers; (iii) summarizes the evidence from epidemiological studies on associations of liver miRNAs in circulation (i.e. those measured in acellular samples of serum or plasma) with metabolic and cardiovascular outcomes; and (iv) discusses further steps required for their use in clinical practise. The role of miRNAs in the regulation of lipid metabolism in non-hepatic tissues such as adipose tissue or in macrophages⁵ and their respective implications for obesity and atherosclerosis⁶ have been reviewed elsewhere.

* Corresponding author. Tel: +44 20 7848 5446, Fax: +44 20 7848 5298, Email: manuel.mayr@kcl.ac.uk

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Liver miRNAs as regulators of hepatic lipid metabolism

Most miRNAs involved in lipid homeostasis have been reported to modulate lipid transport processes and assembly rather than degradation and clearance. A key protein mediating the efflux of cholesterol to high-density lipoprotein (HDL)-forming apolipoprotein A I is the ATP-binding cassette transporter A1 (ABCA1). ABCA1 appears to be regulated by several miRNAs. This is due to the fact that ABCA1 has an exceptionally long 3' UTR (3.3 kb, the average length of human 3' UTRs is 0.8 kb), allowing the binding of multiple different miRNAs. MiRNAs directly targeting ABCA1 include miR-10b, miR-17, miR-19b, miR-26, miR-27a/b, miR-33a/b, miR-33a*/b*, miR-93, miR-101, miR-106b, miR-128, miR-144, miR-145, miR-148a, miR-302a, and miR-758,^{7–16} whereas miR-223 has been shown to regulate ABCA1 indirectly.¹⁷ This regulation of ABCA1 is a prime example of a miRNA regulatory network converging on one target, yet the physiological and pathological relevance of some of these miRNAs as regulators of cholesterol transport remains to be validated. In the following sections, we summarize the role of several miRNAs in hepatic lipid metabolism.

MiR-33a/b

In humans, *miR-33a* and *miR-33b* are encoded in introns of the genes coding sterol regulatory element-binding proteins (*SREBP-2* and *SREBP-1*), while mice express only one miR-33 isoform, located in an intron of *Srebp-2*. MiR-33 is a key regulator of lipid metabolism and transport.^{18,19} MiR-33 targets ABCA1 and thus suppresses HDL synthesis by attenuation of cholesterol efflux to apolipoprotein A1 and nascent HDL.²⁰ Loss of miR-33 in low-density lipoprotein receptor null (*LDLR*^{-/-}) mice raises plasma HDL-cholesterol levels and promotes reverse cholesterol transport. As expected by these findings, anti-miR-33 therapy attenuates the progression and enhances the regression of atherosclerosis in *LDLR*^{-/-} mice.^{21,22} Similarly, genetic ablation of miR-33 in *ApoE*^{-/-} mice markedly reduces the progression of atherosclerosis.²³ In contrast, a study has recently shown that anti-miR-33 therapy using different antisense oligonucleotides (ASOs) fails to prevent atherogenesis,²⁴ suggesting that the chemical modification of the ASOs might influence their therapeutic efficacy. In addition to its prominent role in regulating lipid metabolism, a recent report revealed that miR-33 antagonism increased mitochondrial function and de-repressed ABCA1 in macrophages, which, in combination, resulted in an increased cholesterol efflux from macrophages.²⁵ In the same study, anti-miR-33 treatment in atherosclerotic *ApoE*^{-/-} mice reduced lesion size even though circulating lipid levels were unaffected. Moreover, elevated miR-33 and reduced expression of the mitochondria regulatory genes *PGC-1 α* , *SLC25A25*, *NRF1*, and *TFAM* were observed in human atherosclerotic plaques.²⁵ Another biological effect of miR-33 is the attenuation of hepatic secretion of very-low-density lipoprotein (VLDL)-triglycerides by targeting N-ethylmaleimide-sensitive factor, an essential component of the exocytic pathway.²⁶

In addition to the guide strand miR-33 (miR-33-5p), the passenger strand miR-33* (miR-33-3p) has been implicated in the regulation of cholesterol and lipid metabolism.⁹ MiR-33* represses several genes

encoding key enzymes and transcription factors involved in cholesterol efflux (including ABCA1), fatty acid (FA) metabolism, and insulin signalling.⁹ Overexpression of miR-33* in hepatic cells reduces FA oxidation. Thus, two primary transcripts (*SREBP-1* and *SREBP-2*) give rise to six important regulators of lipid and cholesterol metabolism: *SREBP-1*, *SREBP-2*, miR-33a, miR-33a*, miR-33b, and miR-33b*. MiR-33, in turn, represses *SREBP-1* thus fine-tuning cholesterol homeostasis by an auto-feedback loop.²⁷ Because of its essential role in lipid metabolism, miR-33 has been considered and tested as a therapeutic target for metabolic disorders. Inhibition of miR-33 in non-human primates resulted in elevated plasma HDL-C and decreased VLDL-C levels.²⁸ However, there are conflicting reports on the long-term inhibition of miR-33 in mice, which may or may not have detrimental effects such as elevated plasma triglyceride (TG) levels and moderate hepatic steatosis.^{29,30} Notably, the adverse effects were observed only upon feeding mice a high-fat diet.²⁹ Nonetheless, the observation has clinical relevance given the potential target population for miR-33 therapy. Therefore, further studies are required to determine long-term effects and safety of miR-33 inhibition.

MiR-223

MiR-223 was initially reported to be myeloid cell-specific, but it has recently been shown to be also expressed in hepatocytes where it regulates cholesterol homeostasis.¹⁷ Hepatic miR-223 reduces cholesterol biosynthesis by repressing 3-hydroxy-3-methylglutaryl-CoA synthase 1 and sterol-C4-methyloxidase-like protein, and inhibits HDL-C uptake by targeting the scavenger receptor class B member 1. Furthermore, miR-223 promotes cholesterol efflux by positively regulating ABCA1 expression via its direct target Sp3. Importantly, miR-223 levels are regulated by the cholesterol level; in a low-cholesterol state, miR-223 is suppressed. As a consequence, cholesterol synthesis and uptake are increased, whereas cholesterol efflux to HDL is attenuated in order to raise cellular cholesterol levels.¹⁷

MiR-30c

MiR-30c exerts lipid-lowering effects in the liver: It represses the microsomal triglyceride transfer protein (MTP), which is essential for lipoprotein assembly. Hepatic miR-30c reduces lipid synthesis and lipoprotein secretion and decreases atherosclerosis in *ApoE*^{-/-} mice.³¹ In addition to its role in regulating lipoprotein metabolism and the progression of atherosclerosis, miR-30c overexpression in cardiomyocytes results in dilated cardiomyopathy.³²

MiR-144

MiR-144 is another regulator of cholesterol metabolism and transport in the liver³³ and in macrophages.³⁴ In the liver, the nuclear bile acid receptor farnesoid X receptor up-regulates miR-144, which, in turn, targets ABCA1. This results in lower plasma HDL-cholesterol levels.³³ Similarly, in macrophages, activation of a related receptor, liver X receptor, leads to an up-regulation of miR-144 and a down-regulation of ABCA1. As a consequence, cholesterol efflux from macrophages is attenuated.³⁴ Interestingly, overexpression of miR-144 increases atherosclerosis in *ApoE*^{-/-} mice.³⁵

MiR-148a

Two independent studies have identified miR-148a-3p as a major regulator of lipoprotein metabolism using different experimental approaches.^{8,10} Hepatic miR-148a-3p negatively regulates LDL receptor (LDLR) and ABCA1 levels.^{8,10} *In vivo* inhibition of miR-148a-3p increases hepatic LDLR and ABCA1 expression lowering LDL-cholesterol and increasing HDL-cholesterol in plasma.^{8,10} MiR-148a-3p is also highly expressed in macrophages where it controls ABCA1 expression and cholesterol efflux.¹⁰ Importantly, the locus encoding miR-148a-3p is located in a genomic region enriched for single nucleotide polymorphisms associated with abnormal circulating total cholesterol, TG, or LDL-cholesterol levels.^{10,36,37} Thus, genetic determinants of lipoprotein metabolism in humans are also likely to affect the expression of miR-148a-3p in the liver and/or other tissues.

MiR-122

MiR-122 is the predominant miRNA in the liver and completely conserved in vertebrates. It accounts for >70% of the total hepatic miRNA expression³⁸ and regulates a number of genes associated to cholesterol and FA metabolism (Figure 1).³⁹ In mice, inhibition of miR-122 using ASO lowered circulating cholesterol by ~25–35%, reduced hepatic lipid synthesis, and enhanced hepatic FA oxidation.^{40,41} Similarly, antagonism of miR-122 in non-human primates markedly lowers plasma cholesterol levels.^{42,43} Antisense targeting of miR-122 results in the down-regulation of a broad array of genes involved in lipid synthesis and lipoprotein assembly including 3-hydroxy-3-methylglutaryl-coenzyme A reductase, the rate-limiting enzyme in the cholesterol biosynthesis pathway,

3-hydroxy-3-methylglutaryl-CoA synthase 1, and MTP.⁴⁴ All these proteins are no direct targets of miR-122, and the mechanisms by which miR-122 regulates their expression are still unknown.

MiR-122 has also been implicated in systemic iron homeostasis by directly targeting mRNAs that encode activators of hepcidin expression (*Hfe* and *Hjv*),⁴⁵ and in glucose homeostasis by indirect effects on AMP-activated kinase and glucose 6-phosphatase, a key regulatory enzyme of hepatic gluconeogenesis.^{40,44} Preliminary data from miR-122 null mice kept on high-fat diet for 3 months suggest improved insulin sensitivity compared with wild-type mice along with lower fasting glucose levels, enhanced thermogenesis, and lower body weight.⁴⁶ However, genetic ablation of *miR-122* in mice also causes a marked reduction of hepatic MTP expression and VLDL secretion leading to hepatosteatosis.⁴⁷ Similar observations were found in a separate study, in which *miR-122* deletion resulted in hepatic lipid accumulation, hepatitis, and the development of hepatocellular carcinoma-like tumours.⁴⁸ There is accumulating evidence that miR-122 exerts tumour-suppressive effects⁴⁹ but also inhibits collagen maturation in hepatic stellate cells.⁵⁰

Detection of liver miRNAs in circulation

In circulation, miRNAs are packaged in membranous microvesicles and protein complexes,⁵¹ protecting them from enzymatic degradation.⁵² While their levels in serum and plasma are remarkably stable,³⁸ miRNAs may be released into circulation by different tissues and upon different triggers (see Figure 2). For instance, we have previously shown that activation of platelets triggers the

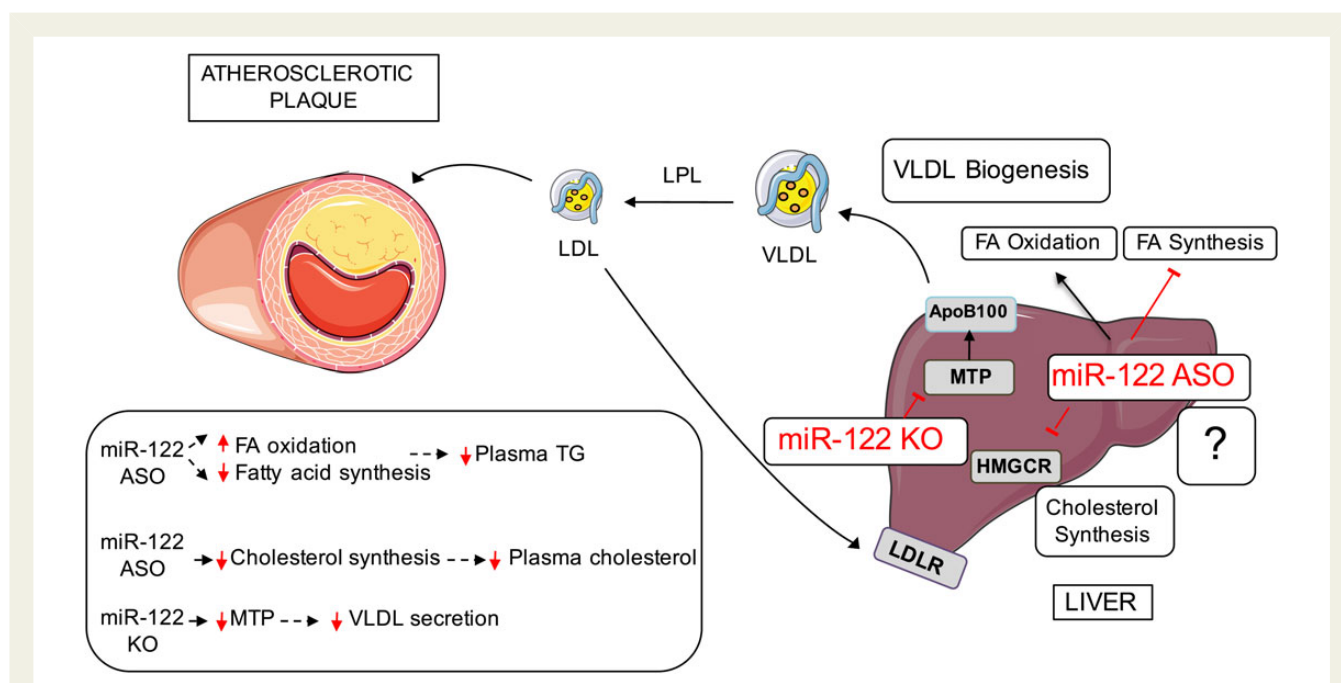


Figure 1 Role of miR-122 in lipid homeostasis. ASO, antisense oligonucleotide; FA, fatty acid; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; KO, knock-out; LDL, low-density lipoprotein; LPL, lipoprotein lipase; LDLR, low-density lipoprotein receptor; MTP, microsomal triglyceride transfer protein; TG, triglycerides; VLDL, very-low-density lipoprotein.

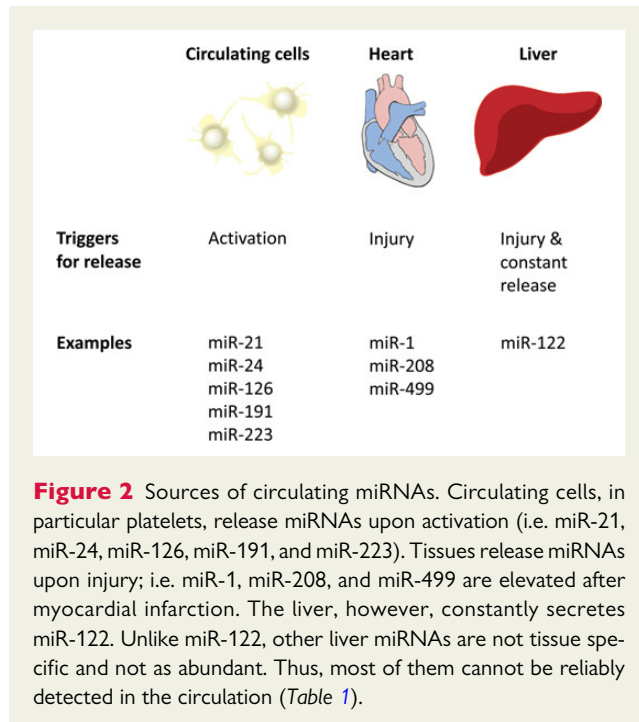


Table 1 Detectability of liver miRNAs implicated in lipid homeostasis in plasma samples taken from healthy individuals

MiRNA	Average Ct	Average rpm	First author, reference
Detected reliably			
223	18.4	275.7	Vickers ¹⁷
320	23.8	214.5	Chen ⁹¹
122	25.3	60.8	Esau⁴⁰
27b	25.4	938.0	Vickers, ⁹² Goedeke ⁹³
30c	26.8	447.5	Soh ³¹
27a	27.9	103.5	Alvarez ⁹⁴
378	28.6	42.6	Carrer ⁹⁵
335	28.9	36.5	Nakanishi ⁹⁶
148a	29.8	616.9	Wagschal ¹⁰
Not detected reliably			
370	33	6.9	Iliopoulos ⁹⁷
613	36.3	0.0	Ou ⁹⁸
33a*	37.5	0.1	Goedeke ⁹
378*	38.6	0.1	Carrer ⁹⁵
33a	39.7	1.1	Rayner ²⁸
33b	40	1.2	Rayner ²⁸
33b*	n.d.	0.0	Goedeke ⁹
144	n.d.	28.6	de Aguiar Vallim ³³

Shown is the average Ct value for individual miRNAs assessed by qPCR using Taqman assays in 12 random plasma samples from the Bruneck cohort and the average rpm (reads per million) from small RNA sequencing in 4 plasma samples. MiRNAs are categorised by the ability to reliably detect them in plasma samples using both methods (qPCR and sequencing). MiR-122 (in **bold**) is the only liver-specific miRNA reliably detectable in circulation. n.d., not determined.

release of miRNAs, including miR-21, miR-24, miR-126, miR-191, and miR-223, into circulation, whereas therapeutic platelet inhibition reduces their levels. Measurement of circulating platelet miRNA levels could therefore be useful as an *in vivo* test for platelet activation.^{53–56} Another important trigger is tissue damage. MiRNAs released from the heart upon myocardial injury have been suggested as markers for diagnosis and prognosis of acute coronary syndrome but without clear evidence for superiority over troponins.⁵⁷ Liver miRNAs, especially circulating miR-122, are sensitive markers of acute liver failure and acetaminophen toxicity⁵⁸ and explored for *in vitro* assessment of drug-induced hepatocyte toxicity.⁵⁹ Remarkably, even under normal conditions, miR-122 is released from the liver, mainly via hepatic exosomes,^{60,61} a mechanism that is modulated by statins.⁶² MiR-122 is, to our knowledge, the only tissue-specific miRNA that is released into the circulation in a constant manner. Due to its abundance in the liver, miR-122 is readily detectable in the circulation. Also, it deserves comment that circulating miR-122 level and hepatic miR-122 expression were highly correlated ($r = 0.46$) in a recent study of 67 patients with non-alcoholic fatty liver disease (NAFLD).⁶³

In comparison, we have assessed the ability to detect other liver miRNAs related to lipid homeostasis in plasma samples taken from healthy individuals (Table 1). Using quantitative real-time polymerase chain reactions (qPCR) and next generation sequencing, we found that apart from miR-122 only 8 out of 16 miRNAs could be reliably detected. However, many of these miRNAs are expressed in various tissues (Table 1) and thus lack specificity for the liver. Importantly, the high platelet contribution to circulating levels of miR-223, miR-320, miR-27b, miR-335, miR-148a, and miR-30c^{53–56} hampers their use as putative biomarkers for metabolic processes in the liver. As anticipated, the only miRNA with known impact on lipid metabolism that is liver-specific and reliably detectable in circulation was miR-122, making it a promising candidate biomarker.

Liver miRNAs as biomarkers in epidemiological studies

We performed a literature search for case-control and prospective studies that reported on the potential association of miR-122 levels with metabolic and cardiovascular outcomes. The identified studies are summarized in Table 2.

Metabolic outcomes

Non-alcoholic fatty liver disease occurs when the accumulation of liver fat exceeds 5% and encompasses a broad spectrum of pathological conditions ranging from steatosis to non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis. NAFLD represents the most common liver disease in western countries with a common link to metabolic syndrome and type 2 diabetes. An invasive procedure (liver biopsy) is still required to confirm the diagnosis and assess the degree of fibrosis. Recently, liver miRNAs in the context of NAFLD have been implicated both as causal mediators and promising non-invasive biomarkers. Several studies strived for the identification of a serum miRNA signature of NAFLD,^{64–68} and one performed external validation in an independent cohort of patient

Table 2 Overview of published epidemiological studies on miR-122 and metabolic and cardiovascular outcomes

First author	Study design	Sample type	Cases	Controls	No. of participants or cases/controls	Reported fold differences (FD) or odds ratios (OR) for circulating miR-122 with outcome
Metabolic outcomes						
Becker ⁶⁷	Case-control	Serum	NASH	Healthy controls	87/61	OR per 1 dCt decrease 1.69 ($P < 0.001$) ^a
			NAFLD	Healthy controls	50/61	Higher miR-122 ($P < 0.001$)
Celikbilek ⁶⁸	Case-control	Serum	NASH	Healthy controls	20/20	n.s. ^b
Cermelli ⁶⁴	Case-control	Serum	NAFLD-SS	Healthy controls	34/19	FD 5.7 ($P < 0.001$)
			NASH	Healthy controls		FD 11.4 ($P < 0.001$)
Pirola ⁶⁶	Case-control	Serum	NAFLD-SS	Healthy controls	16/16	FD 2.3 ($P < 0.05$) ^b
			NASH	Healthy controls	16/16	FD 7.2 ($P < 0.05$) ^b
			NAFLD-SS	Healthy controls	30/19	FD 1.7 (n.s.) ^b
			NASH	Healthy controls	47/19	FD 3.3 ($P = 0.006$) ^b
Tan ⁶⁹	Case-control	Serum	NAFLD	Healthy controls	20/20	FD 9.3 ($P < 0.01$) ^c
			NAFLD	Healthy controls	152/90	FD 8.7 ($P < 0.001$) ^c
Yamada ⁶⁵	Case-control	Serum	NAFLD	Healthy controls	48/90	Men: FD 3.1 ($P < 0.001$)
			NAFLD	Healthy controls	44/221	Women: FD 2.6 ($P < 0.001$)
Gao ⁷⁶	Case-control	Plasma	Hyperlipidaemia	Controls	155/100	FD 2.0 ($P = 0.008$)
Wang ⁷⁷	Case-control	Serum	Insulin resistance	Controls	123/107	OR 3.4 ($P = 0.028$) ^d
			Obese	Normal weight	123/107	FD 3.1 ($P < 0.001$)
			Obese	Normal weight	56/56	FD 3.2 ($P < 0.001$)
Ortega ⁷⁸	Intervention	Plasma	Surgery-induced weight loss		22	FD 0.05 ($P < 0.001$)
			Diet-induced weight loss		9	FD 0.6 (n.s.)
Janssen ⁷⁹	Phase-2 RCT	Not applicable	Miravirsin vs. placebo in HCV patients		27/9	Total cholesterol ↓
Willeit ⁸⁰	Prospective	Serum and plasma	Incident metabolic syndrome in general population		810	OR per SD 1.60 ($P < 0.001$) ^e
			Incident type-2 diabetes in general population		810	OR per SD 1.37 ($P = 0.021$) ^e
Cardiovascular outcomes						
Li ⁸¹	Case-control	Plasma	AMI	Healthy controls	115/16	FD 4.0 ($P = 0.036$)
			AMI	Non-CHD	30/21	FD 17.8 ($P = 0.023$)
			AMI	Non-CHD	77/51	n.s.
D'Alessandra ⁸⁴	Case-control	Serum	AMI	Healthy controls	33/17	FD 0.13 ($P < 0.01$)
Corsten ⁸³	Case-control	Plasma	AMI	Normal angiogram	32/36	FD 0.83 ($P = 0.35$)
Gao ⁷⁶	Case-control	Plasma	CAD	No CAD	155/100	OR 1.08 ($P = 0.034$) ^f
Corsten ⁸³	Case-control	Plasma	Acute heart failure	Healthy controls	33/34	FD 2.5 ($P < 0.05$)
D'Alessandra ⁸²	Case-control	Plasma	Unstable angina	Healthy controls	19/20	FD 4.0 ($P = 0.011$)
Jickling ⁸⁵	Case-control	PBCs	Ischaemic stroke	Healthy controls	24/24	FD 0.44 ($P = 0.047$)
Willeit ⁸⁰	Prospective	Serum and plasma	Incident CVD in general population		810	OR per SD 1.10 ($P = 0.330$) ^e

AMI, acute myocardial infarction; CAD, coronary artery disease; CHD, coronary heart disease; CVD, cardiovascular disease; FD, fold difference; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; n.s., not significant; OR, odds ratio; PBCs, peripheral blood cells; RCT, randomized controlled trial.

^aAdjusted for age, sex, and body mass index.

^bAge- and sex-matched.

^cAge-, sex-, and ethnicity-matched.

^dAdjusted for age, sex, body mass index, waist-hip ratio, fat percentage, systolic blood pressure, diastolic blood pressure, lipid profile and liver function.

^eAdjusted for age, sex, socio-economic status, smoking, physical activity, and alcohol consumption.

^fAdjusted for age, sex, BMI, smoking, hypertension, diabetes, and lipids.

($n = 152$) and control ($n = 90$) samples with the final selection of miRNAs comprising miR-122, -1290, -27b, and -192 and the diagnostic yield surpassing that of standard transaminase levels

(Table 2).⁶⁹ Unsurprisingly, the common readout of the various studies was miR-122, the most abundant liver miRNA with exquisite tissue specificity, which was consistently elevated in all series of

NAFLD patients and increased with disease severity.^{64,66,67} Large-scale epidemiological studies are now required to clarify whether high circulating miR-122 levels, alone or in concert with other miRNAs, offer sufficient diagnostic performance to limit future necessity of liver biopsies for NAFLD.

From a pathophysiological perspective, NAFLD, featured by excess accumulation of TGs, reflects an unbalance between *de novo* lipogenesis in the liver, FA and lipoprotein uptake, FA oxidation, and lipoprotein assembly and release (TG export). MiRNAs including miR-122 are crucially involved in all these processes as epigenetic regulators on a post-transcriptional level.⁷⁰ MiR-122 is a key positive regulator of *de novo* lipogenesis, which accounts for roughly one-quarter of hepatic fat deposits in NAFLD patients and produces lipid species that confer a high cardiovascular disease risk.⁷¹ In line, silencing of miR-122 (by ASO) prevents hepatosteatosis in response to high-fat diet.⁴⁰ Of note, some residual miR-122 activity is required for achieving favourable effects on liver fat content. MiR-122 null mice spontaneously develop severe hepatosteatosis,⁴⁷ probably due to diminished expression of MTP and subsequent impairment of VLDL assembly and secretion.⁷⁰ Remarkably, in NASH as well as in alcoholic hepatitis, packaging of miR-122 in exosomes is up-regulated leading to increased levels of circulating miR-122, increased miR-122 delivery to macrophages eliciting an inflammatory response,⁷² and decreased liver miR-122 levels potentially promoting fibrosis.⁷³ Other miRNAs particularly relevant to NAFLD include (i) miR-33, down-regulation of which leads to severe liver steatosis²⁹; (ii) miR-24, which targets insulin-induced gene 1 and inhibition of which blocks both hepatic steatosis and hyperlipidaemia⁷⁴; and (iii) miR-29, which prevents lipoprotein lipase from being expressed in liver and inhibition of which promotes hepatic lipid accumulation in mice.⁷⁵

The effects of miR-122 on *de novo* lipogenesis—and lipid metabolism in general—are also reflected in the cross-sectional correlations with major lipid species. Previous studies generally reported positive correlations of circulating miR-122 with total cholesterol, TG, LDL-cholesterol, and liver function enzymes, and inverse correlations with HDL-cholesterol. Participants with hyperlipidaemia and insulin resistance have markedly higher circulating miR-122 levels than healthy controls (Table 2).^{76,77}

Circulating miR-122 levels are higher in obese people compared with people with normal weight (Table 2).⁷⁷ In a study of morbidly obese patients, surgery-induced weight loss led to a major reduction of miR-122 levels.⁷⁸ A Phase-2 clinical trial that aimed to assess the efficacy of the miR-122 inhibitor Miravirsin in reducing viral load in patients with hepatitis C reported a concurrent reduction in total cholesterol over 14 weeks of treatment, but no shift in the ratio of LDL-cholesterol to HDL-cholesterol.⁷⁹ As the first prospective population-based study investigating the biomarker potential of miR-122 in the general population, the Bruneck study reported that miR-122 is significantly associated with incident metabolic syndrome and type 2 diabetes, even after adjustment for age, sex, socio-economic status, smoking, physical activity, and alcohol consumption.⁸⁰

Cardiovascular outcomes

In contrast to metabolic outcomes, evidence available for cardiovascular outcomes is sparse (Table 2), with some studies reporting

positive^{76,81,82} and some studies reporting inverse^{83–85} associations. The interpretation of these studies is further complicated by the reporting of crude fold differences (i.e. those unadjusted for potential confounding variables) and the notion that statins may influence the release of miR-122 from the liver into circulation.⁶² In the Bruneck study, circulating miR-122 was not significantly associated with the development of incident cardiovascular disease over a 15-year time frame.⁸⁰ However, there is evidence that liver miRNAs may adversely influence plaque composition through their aforementioned effects on lipid metabolism or effects on other cell types.⁸⁶ Antagonism of miR-33 in atherosclerosis-susceptible mice promoted accumulation of anti-inflammatory M₂ macrophages and regulatory T-cells (FOXP3 + T_{regs})⁸⁷ and enhanced cholesterol efflux from lesional macrophages.²¹ Both effects are assumed to stabilize advanced plaques. MiR-24, in turn, targets pro-inflammatory chitinase-3-like protein 1, macrophage apoptosis, and matrix metalloproteinase-14 expression and activation in macrophages.⁸⁸ Inhibition of miR-24 resulted in a vulnerable plaque phenotype with decreased collagen content and increased macrophage infiltration.⁸⁹

Steps required for clinical translation

Epidemiological research into the role of miR-122 in metabolic and cardiovascular diseases is still in its infancy. Published studies on the cross-sectional correlates of miR-122 are limited to the major lipid classes (e.g. total cholesterol, HDL-cholesterol, and LDL-cholesterol), whereas a further breakdown of lipid classes would provide a better mechanistic understanding of the regulation of lipid homeostasis by miR-122. Furthermore, only one prospective study has investigated the association of baseline miR-122 levels with subsequent development of metabolic and cardiovascular diseases. Because prospective studies measure miR-122 well before the outcome of interest, they can establish a temporal relationship, are less prone to biases, and therefore are superior to cross-sectional and case-control studies.

Detection of miRNAs in plasma and serum by qPCR offers an opportunity for large-scale epidemiological studies into the role of miRNAs in human diseases. However, to prove clinical utility as a biomarker, more studies are needed that establish and define clinical cut-offs, quantify within-person variability over time and potential circadian fluctuations,⁹⁰ provide assay standardization, and give more insights into the dependence of results on specimen type and storage requirements. Our in-house data show that, among a range of miRNAs, miR-122 showed the highest stability for repeat measurements taken 5 years apart. The pre-analytic requirements with regards to sample preparation that have become crucial in the study of circulating miRNAs, given the recent evidence for a predominant platelet origin,⁵⁶ do not equally apply to miR-122, as this miRNA is liver specific and shows reproducible levels across different types of samples (e.g. serum, platelet-poor and platelet-rich plasma). Statins reduce the levels of miRNA-122 in circulation, whereas other types of medications such as platelet inhibitors do not show an effect on miR-122 but alter the levels of platelet-derived miRNAs in the circulation.^{54,56} Hence, further studies are

needed to obtain a better understanding of the different factors that can affect the hepatic release of miR-122 into circulation.

As a drug target, it will be challenging to advance miR-122 to a licenced therapy given that duration, method, and degree of miR-122 lowering as well as the stage of liver pathology may all modify potential treatment effects. Also, the usefulness of miR-122 as a biomarker over and beyond the information provided by existing liver markers such as liver function enzymes (alanine aminotransferase, aspartate aminotransferase, or gamma-glutamyl transpeptidase) remains to be determined in detail.

Concluding remarks

Experimental studies and preliminary clinical data suggest that miR-122 may be relevant to common metabolic and cardiovascular diseases. Prospective epidemiological studies with long-term follow-up are required to confirm and further characterize this association in humans and to elaborate their predictive performance. If secreted liver miRNAs indeed emerge as useful biomarkers, this would offer the prospect of developing new diagnostics to complement existing markers in the early identification of people at risk of metabolic syndrome, type 2 diabetes, and associated complications.

Authors' contributions

P.W. and P.S. performed statistical analyses. M.M. handled funding and supervision. P.W., P.S., S.K., and M.M. acquired the data. P.W. and M.M. conceived and designed the research. P.W., P.S., and M.M. drafted the manuscript. S.K. and C.F.-H. made critical revision of the manuscript for key intellectual content.

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Conflict of interest: King's College London and Medical University of Innsbruck have filed patent applications on miRNAs as biomarkers.

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