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Circulating MicroRNAs to Predict the Risk for Metabolic Diseases in the General Population?

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Metabolic syndrome (MetS) results from the cluster of several risk factors, including central obesity, insulin resistance, dyslipidemia, and hypertension. Around 20%–25% of the world's adult population is estimated to have MetS (1). MetS has been estimated to confer a fivefold increase in the risk of type 2 diabetes (T2D) over the next 5–10 years (2). Both MetS and T2D increase the risk of cardiovascular disease (CVD) (3). MetS diagnosis is cumbersome. A simple noninvasive and inexpensive test that risk stratifies the general population before MetS onset would allow for primary prevention strategies, especially aggressive lifestyle modifications, and the more stringent monitoring of those individuals positive to the predictor. This may have an enormous impact on our national health systems, ultimately slowing down the diabetes epidemics.

MicroRNAs (miRNAs, or miRs) are small noncoding RNA regulatory molecules that inhibit the expression of a plethora of mRNAs, which they target within their parent cells but also in other cells that they reach via different shuttling mechanisms (4). Such shuttles, including lipoproteins and extracellular vesicles, protect their cargos from degradation and deliver active miRNAs from cells to cells contributing to cell-to-cell communication. Moreover, by conferring resilience to miRNAs, the shuttles incidentally increase our possibilities to develop miRNAs in extracellular biomarkers. Among many different actions, miRNAs are now recognized as regulators of lipid and glucose metabolism and as involved in the development of metabolic and cardiovascular diseases (5,6). The liver-enriched miR-122 (now identified as miR-122-5p) was the first miRNA to be recognized functionally associated with a metabolic phenotype, and in particularly to regulate cholesterol and lipid metabolism (7–10). miR-33a/b is also important for cholesterol regulation (6).

In the February issue of *Diabetes*, Willeit et al. (11) reported that circulating miR-122 is associated with variations in the human plasma lipidome and apolipoproteome, as well as with insulin resistance, obesity, MetS, T2D, and an overall adverse lipid profile. The authors propose that miR-122 could be developed into a predictive biomarker for new-onset MetS and T2D. First, working from their Bruneck Study general population biobank, the authors found circulating miR-122 to be positively associated with MetS and T2D but not with CVD. Next, they associated circulating miR-122 with targeted lipids, typically monounsaturated and saturated fatty acyls from triacylglycerols and cholesterol esters and proteins, mostly apolipoproteins, in blood profiles using shotgun lipidomics and targeted proteomics, which allowed for the detection of additional correlations. Of interest, miR-122 correlated positively with afamin, a secreted vitamin E–binding glycoprotein primarily transcribed in the liver, already associated with MetS in a previous population-based study that included the Bruneck biobank (12). Next, the team found that miR-122 inhibition decreased total circulating cholesterol in healthy mice, presumably driven by a downregulation of the sterol regulatory element–binding protein 1 (Srebp1), which regulates hepatic cholesterol metabolism. Interestingly, *Srebp1* hosts *miR-33a* gene, and miR-122 inhibition reduced the liver level of miR-33, suggesting a regulatory interaction in the two miRNAs (11). Anti–miR-122 treatment additionally affected the murine hepatic levels of 11 proteins linked to lipid metabolism and downregulated GTPase Rab27a, which is important for the release of exosomes (the smallest endogenous extracellular vesicles described so far) from cells (13) (Fig. 1).

With this study, Willeit et al. (11) advance our understanding of miR-122 biology through the combination of

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COMMENTARY

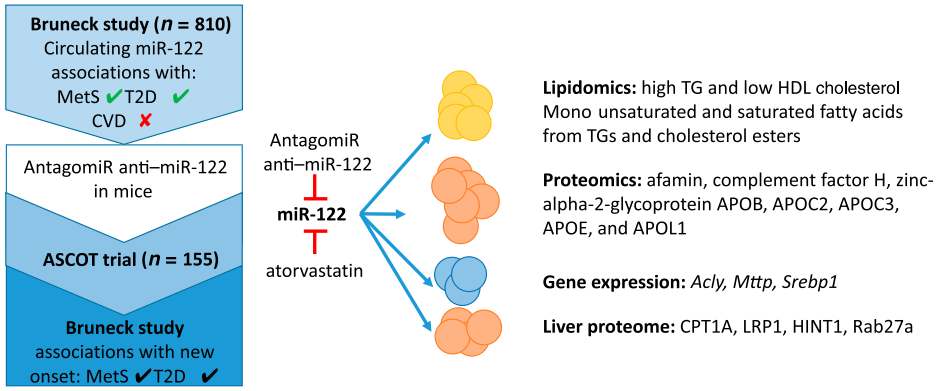

Figure 1—Summary of the study design and findings reported in Willeit et al. (11). *Acly*, ATP citrate lyase; APO, apolipoprotein; CPT1, carnitine palmitoyltransferase I; HINT1, histidine triad nucleotide binding protein 1; LRP1, low-density lipoprotein receptor-related protein 1; *Mttp*, microsomal triglyceride transfer protein; TG, triglyceride.

various "omics" data—RNA expression, proteomics, and lipidomics—in humans and cell and murine models. The use of targeted lipidomics and proteomics for human blood, though limited in scope, reduces the burden of multiple statistical testing and focuses on clinically relevant end points: apolipoproteins and fatty acids. The team provides additional evidence that the relationship between miR-122 and lipid metabolism is not unidirectional. In fact, they show reduced extracellular miR-122 levels after statin treatment of cultured hepatocytes, healthy mice, and patients with hypertension (randomized from the Anglo-Scandinavian Cardiac Outcomes Trial [ASCOT] trial, which had a second primary hypothesis that adding statin to antihypertension treatment would further protect against coronary heart disease end points in subjects with hypertension and a total cholesterol ≤6.5 mmol/L) (14).

One person's risk of developing MetS and/or T2D results from the interaction between genetic and environmental factors. In this context, miRNAs can provide an insight on the complex endogenous gene expression regulation mechanisms at work before, during, and after the onset of the disease. As such, miRNA variation can be seen as the systems integrative response to host genetic susceptibility and environmental effects and can be harnessed for predictive, diagnostic, and prognostic purposes. In particular, once validated, circulating miR-122 appears to be a promising functional biomarker, able to sense changes in hepatic metabolism as well as to induce them. In support of this hypothesis, an association of miR-122-5p with fatty liver and related lipoprotein metabolism was recently described in a Finnish general population cohort (15). This reinforces the concept that increased circulating miR-122 might flag a problem with liver metabolisms and require further laboratory and clinical analyses going beyond the normal routine.

Some open questions will need to be addressed in future studies. First, given the strong association between MetS and T2D with CVD, it is puzzling that miR-122 could not be associated with cardiovascular events, which were otherwise registered in the Bruneck Study participants (16). It is possible that these associations will show up in work with larger populations. Second, Willeit et al. (11) speculate that the circulating miR-122 level depends on exosome-mediated hepatic secretion rather than reflecting the miR-122 hepatic expression. Indeed, in vitro statin treatment reduced miR-122 in the culture medium of hepatocytes but not intracellularly. Nonetheless, in mice, statin reduced both intrahepatic and circulating miR-122. Calculating the miR-122 concentration ratio between exosomes and whole plasma/serum would have helped elucidate whether the variations in circulating miR-122 in the Bruneck and ASCOT samples were mainly attributable to exosomes transport. It is noteworthy that miR-122 can also be transported via LDL particles (17), thus making its circulating level sensible to lipid-lowering drugs. Third, to better appreciate the cause and significance of changes in circulating miR-122, it is now essential to gain understanding of the mechanisms regulating its transcription and maturation and understand if genetic variants dictate miR-122 level and function. Fourth, from a biomarker prospective, defining the normality range of circulating miR-122 for the average population is essential. In this context, in addition to possible ethnicity-associated differences, we should take into account that even modest alcohol consumption increases the miR-122 level (18) and that *miR-122* transcription in the mouse liver follows a circadian rhythm (19), suggesting the necessity to control for fluctuation of miR-122 over 24 h.

In conclusion, the article by Willeit et al. (11) opens new perspectives for the study of the regulation and sensing of metabolic and lipid profiles by miRNAs. Another key perspective introduced by the study is the empirical need to pay more attention to cell-to-cell communication and cross talk between distant organs, which will require the implementation of specific experimental designs and improved "omics" profiling strategies and translational systems medicine strategies able to cope with the complexity of the profiles and their regulation while remaining

directly relevant to the clinic. Beyond mechanisms, the next translational challenge is to prove that miRNAs are useful markers and understand if they are suitable to implement precision medicine strategies.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

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