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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, dyslipidaemia**,** and hypertension. Around 20-25% of the world's adult population is estimated to have the MetS (1). MetS has been estimate to confer a 5-fold increase in the risk of type 2 diabetes mellitus (T2DM) over the next 5-10 years (2). Both MetS and T2DM increase the risk of cardiovascular diseases (CVD) (3). MetS diagnosis is cumbersome. A simple non-invasive and inexpensive test allowing to risk stratify the general population before MetS onset would allow primary prevention strategies, especially aggressive lifestyle modifications, and to monitor more stringently those individuals positive to the predictor. This may have an enormous impact on our national health systems, ultimately enabling to slow down the diabetic epidemics.

MicroRNAs (miRs) are small non-coding RNA regulatory molecules that inhibit the expression of a plethora of messenger RNAs (mRNAs), which they target within their parent cells, but also in other cells that they reach *via* different shuttling mechanisms (4). Such shuttles, include lipoproteins and extracellular vesicles, protect their cargos from degradation and deliver active miRs from cells to cells contributing in cell-to-cell communication. Moreover, by conferring resilience to miRs, the shuttles incidentally increase our possibilities to develop miRs in extracellular biomarkers. Amongst many different actions, miRs are now recognized as regulator of lipid and glucose metabolism and 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 miR 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 this issue, Willeit *et al* report that circulating miR-122 is associated with variations in the human plasma lipidome and apolipoproteome as well as with insulin resistance, obesity, MetS, T2DM, and an overall adverse lipid profile. Authors propose that miR-122 could be developed into a predictive biomarker for new onset of MetS and T2DM (11). First, working on their Bruneck general population biobank, Authors found circulating miR-122 to be positively associated with MetS and T2DM, 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 to detect 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 including 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 response 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 complex regulatory interaction be the 2 miRs. Anti-miR-122 treatment additionally affected the murine hepatic levels of 11 proteins linked to lipid metabolism and downregulated the GTPase Rab27a, which is important for the release of exosomes (the smallest endogenous extracellular vesicles described so far) from cells (13).

With this study, Willeit *et al* advance our understanding of miR-122 biology through the combination of various omics data - gene expression, proteomics and lipidomics - in humans, 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 focus on clinically relevant endpoints – 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 hypertensive patients (randomised from the ASCOT trial, whose second primary hypothesis was that adding statin to anti-hypertensive treatment would further protect against coronary heart disease endpoints in hypertensive subjects with a total cholesterol < or = 6.5 mmol/l)(14).

One person's risk of developing MetS and/or T2DM results from the interaction between genetic and environmental factors. In this context, miRs 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, miR 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 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 problem with liver metabolisms and require further laboratory and clinical analyses going beyond the normal routine.

Some questions remain open: 1) Given the strong association between MetS and T2DM 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 working on larger populations. 2) Willeit *et al* speculate that circulating miR-122 level depends upon 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 intra-hepatic and circulating miR-122. Calculating the miR-122 concentration ratio between exosomes and whole plasma/serum would have helped to understand if the variations in circulating miR-122 in the Brunek and ASCOT samples were mainly attributable to exosomes transport. Noteworthy, miR-122 can also be transported *via* LDL

particles (17), thus making its circulating level sensible to lipid lowering drugs. 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 if genetic variants dictate miR-122 level and function.

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 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 during the 24 hours.

In conclusion, this article opens new perspectives for the study of the regulation and sensing of metabolic and lipid profiles by miRs and should pave the way for further studies. Another key perspective introduced by this study is the empirical need to pay more attention to cell-to-cell communication and crosstalk between distant organs – which will require 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 – whilst remaining directly relevant to the clinic. Beyond mechanisms, the next translational challenge is to prove that miRs are useful markers and understand if they are suitable to implement precision medicine strategies.

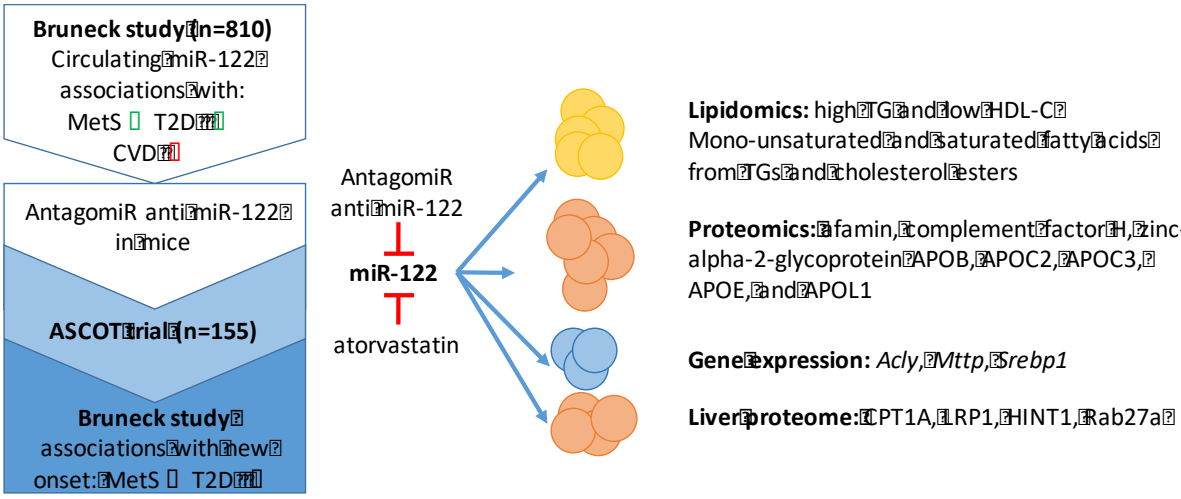

Figure 1—Summary of the study design and findings reported in Willeit et al. (11). Acly, ATP citrate lyase; APO, apolipoprotein; CPT1, carnitine palmitoyltransferase I; HINT1, histidine triad nucleotide binding protein 1; LRP1, low-density lipoprotein receptor-related protein 1; Mttp, microsomal triglyceride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

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