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Manuel Mayr, MD, PhD

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2. *Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, Lewis GD, Fox CS, Jacques PF, Fernandez C, O'Donnell CJ, Carr SA, Mootha VK, Florez JC, Souza A, Melander O, Clish CB, Gerszten RE. Metabolite profiles and the risk of developing diabetes.* Nat Med. *2011;17:448–453.*

Study Hypothesis

Initial studies using nuclear magnetic resonance spectroscopy for plasma metabolomics claimed to predict angiographically defined advanced coronary artery disease with a high degree of accuracy and specificity. Later studies demonstrated that these initial findings were due to plasma lipids, and the accuracy and specificity of prediction was greatly reduced in patients treated with statins. Two recent studies described here used mass spectrometry–based technologies to test whether advanced metabolomic profiling in large, well-defined cohorts can identify new biomarkers for cardiovascular disease and diabetes.[1,2]

How Was the Hypothesis Tested?

Gerszten (Wang and colleagues)[2] performed targeted metabolic profiling of >60 water-soluble metabolites, including amines, amino acids, and other polar metabolites in 2 longitudinal studies and assessed their prognostic value for type 2 diabetes. Hazen (Wang and colleagues)[1] performed untargeted metabolic profiling of an initial 2000+ analytes in patients with cardiovascular disease. Using plasma metabolomics in combination with animal models and integrative mouse genetics, they provided mechanistic insights into the role of novel proatherogenic metabolites in cardiovascular disease.

Principal Findings

Cholesterol and triglycerides are thought to be the key culprits in atherosclerosis. Results reported by Hazen and colleagues point toward an important role for a third class of lipids: phospholipids. Phospholipids are a major component of all cell membranes and form the lipid bilayers. Most phospholipids contain a diglyceride, a phosphate group, and a simple organic molecule such as choline. The novel finding in this study is that plasma levels of choline, betaine, and trimethylamine N-oxide (TMAO) were strongly correlated with cardiovascular disease in subjects (n=1876) undergoing elective cardiac evaluations. The initial breakdown of dietary phospholipids gives rise to choline and other trimethylamine (TMA)-containing species such as betaine. TMA is released by the gut flora and converted to TMAO by at least 1 member of the hepatic flavine monooxygenase (FMO) family of enzymes, FMO3. Patients with trimethylaminuria, an autosomal recessive disorder caused by deficiency of FMO3, develop a fish odor of their sweat, urine, and breath after the consumption of choline-rich foods, such as red meat, milk products, and eggs because they lack the ability to convert TMA, with its characteristic smell of rotten fish, to the odorless TMAO. Notably, dietary supplementation of choline and TMAO was sufficient to aggravate the development of atherosclerotic lesions in apolipoprotein E null (apoE−/−) mice without significant alterations in plasma cholesterol, triglycerides, lipoproteins, glucose levels, and hepatic triglyceride content. Moreover, apoE−/− mice from an F2 intercross between atherosclerosis-prone (C57BL/6J) and atherosclerosis-resistant (C3H/HeJ) strains differed in their hepatic expression levels of FMO3. Significant positive associations were readily found with quantitative measures of atherosclerosis.

Gerszten and colleagues identified a plasma amino acid profile that can serve as a predictor of future diabetes. Using a nested case-control design, metabolic profiling was performed on 189 individuals from the Framingham Offspring Study who developed new-onset diabetes during a 12-year follow-up period. Control subjects were closely matched with respect to age, sex, body mass index, and fasting glucose. In paired analysis, the branched-chain amino acids leucine, isoleucine, and valine as well as the aromatic amino acids phenylalanine and tyrosine showed higher fasting concentrations in cases than in control subjects (with probability values of ≤0.001), confirming previous reports from cross-sectional studies that amino acids may constitute a metabolic signature of insulin resistance. For the 5 amino acids of interest, each SD increment in log marker was associated with a 1.57 to 2.02 increase in the odds ratios for diabetes. These findings were replicated in the Malmö Diet and Cancer study, comprising 167 cases and control subjects. Even in a random cohort (n=400) of the Framingham Offspring

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Study, the amino acid profile was associated with risk of future diabetes, but the adjusted odds ratio was reduced to 1.36 (1.08 to 1.70) per SD increment in the combined amino acid score ($P=0.008$).

Implications

There are 2 main implications: (1) Are these plasma metabolites better biomarkers? The reported associations are impressive but await confirmation in additional cohorts. Plasma levels of choline and TMAO will be dependent on dietary habits, in particular the consumption of a proatherosclerotic phospholipid–rich diet, and are likely to be a risk factor rather than a direct marker of cardiovascular disease. Similarly, amino acid profiles correlated with standard biochemical measures of insulin resistance and β-cell function. Their predictive value was better in high-risk individuals with elevated fasting insulin and glucose concentrations than in the normal population. (2) Are these plasma metabolites contributing to disease? Choline and TMAO feeding accelerated atherosclerosis in apoE−/− mice, a widely used mouse model of atherosclerosis. Reverse cholesterol transport differs between mice and humans. A negative correlation was reported between hepatic FMO3 expression and plasma high-density lipoprotein concentrations in apoE−/− mice, but no correlation was observed between plasma levels of TMAO and high-density lipoproteins in subjects. Also, previous metabolomics analysis of atherosclerotic aortas from apoE−/− mice demonstrated a 2-fold rise in choline without significant changes in tissue concentrations of TMAO, suggesting that plasma TMAO may act systemically rather than locally in the atherosclerotic plaque. This would be consistent with the observation that TMAO feeding induced foam cell formation in peritoneal macrophages. The precise molecular mechanisms in which TMAO mediates its proatherosclerotic effect are currently unknown. Interestingly, TMAO was also measured in the study by Gerszten et al: According to their correlation matrix for plasma metabolite levels (see their Figure 1), TMAO is positively correlated with N-monomethyl-arginine, a competitive inhibitor of nitric oxide synthase, providing an alternative explanation for the association of TMAO with cardiovascular disease. Similarly, the relationship of hyperaminoacidemia and type 2 diabetes requires further investigation. If hyperaminoacidemia is an early manifestation of insulin resistance, does it directly affect glucose metabolism and insulin secretion, or act through independent pathways?

In summary, both studies illustrate how metabolomics can drive biomarker discovery and generate new hypotheses, which open exciting avenues for future research.

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Disclosures

None.