Hypertension Research (2016) 39, 576–577

www.nature.com/hr

COMMENTARY

Metabolomic signatures in atherosclerotic disease: what is the potential use?

Peter M Nilsson and Martin Magnusson

Hypertension Research (2016) **39,** 576–577; doi:10.1038/hr.2016.44; published online 12 May 2016

In recent years, the development of human genetics and the new 'omics' techniques have shed new light on the patterns of risk markers of cardiometabolic disease and its associated phenotypes. If the discovery of new biomarkers leads to the elucidation of causal mechanisms of pathophysiological importance, the results may enable the identification of potential new drug targets that may be used, for example, in the prevention of atherosclerosis and its clinical consequences.[1] Among the new 'omics', one of the most innovative methods of identifying novel causes of disease is metabolomics, for example, liquid chromatography/mass spectrometry. Metabolomics allows the acquisition of high-throughput profiles of the metabolic status of entire organisms, allowing for comprehensive assessment of molecules that are substrates and/or products of metabolic pathways.

For example, in a recent clinical case-control study from Estonia, the metabolomic signature of arterial stiffness has been investigated in a group of 42 men with peripheral artery disease (PAD) compared with 46 healthy male controls.[2] The measurements and metabolomic analyses seem to have been properly performed, and the statistical analysis appears to have been adequate. The importance of the results of this study should be put in perspective.

The general findings of this observational study[2] are that aortic pulse wave velocity (aPWV), a marker of arterial stiffness, as well as the serum levels of lactate, free carnitine and 11 different amino acids, including tyrosine, were all higher among patients with PAD. In contrast, the serum levels of pyruvate, citrate, alpha-ketoglutarate, aconitate and cysteine were higher in control subjects. In multiple regression analysis models, aPWV was independently determined by log-tyrosine and log-oxidized low density lipoprotein (log-oxLDL) in the PAD patients, and by age, log-pyruvate and log-oxLDL in the controls. Thus, the study has described significant differences in the metabolomic signatures of patients with advanced atherosclerosis compared with clinically healthy male controls. The authors have concluded that measurement of low-molecular-weight metabolites, which are related to changes in vascular phenotypes, may lead to the identification of novel vascular risk markers.

Despite these new data from Estonia, a major limitation of this study is its cross-sectional nature because it suggests differences related only to the phenotype analyzed and does not allow disease prediction because no time axis of observations is available, which is particularly obvious in the commenting of other studies using metabolomics with a longitudinal approach for event risk prediction. Thus, another limitation results from differences in study design, which may preclude studies from replicating the findings of similar works. For example, this study was not performed to identify predictive risk biomarkers, as has been done in a study by Tang *et al.*,[3] in which the phenotype analysis was much more informative regarding the status of cardiovascular disease (CVD) with respect to what can be inferred from arterial stiffness. Furthermore, the larger number of patients provided a stronger statistical power for the analysis.

Carnitine has been linked to CVD via the biomarker trimethylamine N-oxide in another recent study,[4] thus suggesting that differences in dietary intake may exist in PAD subjects compared with controls. This finding may explain some of the findings of Zagura M *et al.*[2] and also represent another of its limitations.

The study by Zagura M *et al.*[2] describes differences in the metabolomic signatures of patients with advanced atherosclerosis compared with clinically healthy controls. The main finding of this study is that aPWV is independently associated with serum levels of tyrosine and oxLDL in patients with PAD and is related to pyruvate and oxLDL levels in control subjects. These findings are interesting because earlier studies have also reported that increased levels of branched, chained and aromatic amino acids (for example, tyrosine, phenylalanine and isoleucine) are significantly associated with an increased risk of future diabetes.[5] However, the authors have failed to report that the same metabolic signature (a score of tyrosine, phenylalanine and isoleucine) has been shown to be significantly associated with an increased risk of atherosclerosis, as well as incident cardiovascular disease, in Sweden.[6] In addition, Shah *et al.*[7] have published prospective data accounting for a principal component analysis-derived pattern of several branched-chain amino acids (valine, leucine, methionine, tyrosine, isoleucine and phenylalanine) that are significantly associated with a lower hazard ratio for mortality. Because these authors have previously shown that oxLDL adds power to discriminate survival time in PAD patients,[8] it may be interesting to examine the results of the inclusion of

PM Nilsson is at Department of Internal Medicine Skåne University Hospital, Malmö, Sweden and The Lund University, Department of Clinical Sciences, Malmö, Sweden, M Magnusson is at Lund University Department of Clinical Sciences, Malmö, Sweden, and The Department of Heart Failure and Valvular Disease, Skåne University Hospital, Malmö, Sweden
E-mail: Peter.Nilsson@med.lu.se

oxLDL in a multivariate analysis (in addition to body mass index (BMI), ankle brachial pulse index (ABPI), cholesterol, triglycerides, high sensitive C-reactive protein (hs-CRP), estimated glomerular filtration rate (eGFR), glucose and heart rate) examining the relationship between atherosclerosis and low-molecular-weight metabolites (for example, tyrosine).

In summary, the new fields of omics and metabolomics can increase knowledge through the discovery of new pathways associated with hypertension[9,10] and cardiometabolic disease.[11] Because differences may exist among study populations regarding age, sex and comorbidities, as well as geographical distribution, ethnicity and lifestyle (diet), more comparative studies are needed. Ideally, such studies should not only have case-control design but also incorporate cohort follow-up design to predict real clinical events. In addition, intervention studies are needed, for example, to evaluate the effects of smoking cessation on oxidative stress and improvements in endothelial function.[12] When metabolic patterns are found to be associated with disease phenotypes, the next step should be to use modern genetics to dissect causality on the basis of Mendelian randomization methodology. Ultimately, new drug targets based on causal pathways of disease may be identified and further developed in clinical intervention studies. This is a good example of translational research ultimately aimed at clinical applications.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Swedish Research Council (to PMN), the Medical Faculty of Lund University, Skåne University Hospital, the Crafoord Foundation, the Ernhold Lundström Research Foundation, and the Hulda and Conrad Mossfelt Foundation (to MM).

1 Hoefer IE, Steffens S, Ala-Korpela M, Bäck M, Badimon L, Bochaton-Piallat ML, Boulanger CM, Caligiuri G, Dimmeler S, Egido J, Evans PC, Guzik T, Kwak BR, Landmesser U, Mayr M, Monaco C, Pasterkamp G, Tuñón J, Weber CESC Working Group. Atherosclerosis and vascular biology. Novel methodologies for biomarker discovery in atherosclerosis. *Eur Heart J* 2015; **36**: 2635–2642.
2 Zagura M, Kals J, Kilk K, Serg M, Kampus P, Eha J, Soomets U, Zilmer M. Metabolomic signature of arterial stiffness in patients with peripheral arterial disease. *Hypertens Res* 2015; **38**: 840–846.
3 Tang WH, Wang Z, Cho L, Brennan DM, Hazen SL. Diminished global arginine bioavailability and increased arginine catabolism as metabolic profile of increased cardiovascular risk. *J Am Coll Cardiol* 2009; **53**: 2061–2067.
4 Tang WH, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, Wu Y, Hazen SL. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N Engl J Med* 2013; **368**: 1575–1584.
5 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.
6 Magnusson M, Lewis GD, Ericson U, Orho-Melander M, Hedblad B, Engström G, Östling G, Clish C, Wang TJ, Gerszten RE, Melander O. A diabetes-predictive amino acid score and future cardiovascular disease. *Eur Heart J* 2013; **34**: 1982–1989.
7 Shah SH, Sun JL, Stevens RD, Bain JR, Muehlbauer MJ, Pieper KS, Haynes C, Hauser ER, Kraus WE, Granger CB, Newgard CB, Califf RM, Newby LK. Baseline metabolomic profiles predict cardiovascular events in patients at risk for coronary artery disease. *Am Heart J* 2012; **163**: 844–850.
8 Kals J, Lieberg J, Kampus P, Zagura M, Eha J, Zilmer M. Prognostic impact of arterial stiffness in patients with symptomatic peripheral arterial disease. *Eur J Vasc Surg* 2014; **48**: 308–315.
9 Nikolic SB, Sharman JE, Adams MJ, Edwards LM. Metabolomics in hypertension. *J Hypertens* 2014; **32**: 1159–1169.
10 Takiwaki M, Tomoda F, Koike T, Taki T, Inoue H, Kigawa M, Kitajima I, Uji Y. Increased levels of small dense low-density lipoprotein cholesterol associated with hemorheological abnormalities in untreated, early-stage essential hypertensives. *Hypertens Res* 2014; **37**: 1008–1013.
11 Magnusson M, Wang TJ, Clish C, Engström G, Nilsson P, Gerszten RE, Melander O. Dimethylglycine deficiency and the development of diabetes mellitus. *Diabetes* 2015; **64**: 3010–3016.
12 Kato T, Umeda A, Miyagawa K, Takeda H, Adachi T, Toyoda S, Taguchi I, Inoue T, Node K. Varenicline-assisted smoking cessation decreases oxidative stress and restores endothelial function. *Hypertens Res* 2014; **37**: 655–658.