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Diabetes Subphenotypes and Metabolomics: The Key to Discovering Laboratory Markers for Personalized Medicine?

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For decades, glucose, hemoglobin A_{1c}, insulin, and C peptide have been the laboratory tests of choice to detect and monitor diabetes *(1)*. However, these tests do not identify individuals at risk for developing type 2 diabetes (T2Dm)[4] (so-called prediabetic individuals and the subphenotypes therein), which would be a prerequisite for individualized prevention. Nor are these parameters suitable to identify T2Dm subphenotypes, a prerequisite for individualized therapeutic interventions. The oral glucose tolerance test (oGTT) is still the only means for the early and reliable identification of people in the prediabetic phase with impaired glucose tolerance (IGT). This procedure, however, is very time-consuming and expensive and is unsuitable as a screening method in a doctor′s office. Hence, there is an urgent need for innovative laboratory tests to simplify the early detection of alterations in glucose metabolism.

The search for diabetic risk genes was the first and most intensively pursued approach for individualized diabetes prevention and treatment. Over the last 20 years cohorts of tens of thousands of people have been analyzed, and more than 70 susceptibility loci associated with T2Dm and related metabolic traits have been identified *(2)*. But despite extensive replication, no susceptibility loci or combinations of loci have proven suitable for diagnostic purposes.

Why did the genomic studies fail? One reason might be that T2Dm is a polygenetic disease, but there is another more important reason. The large diabetes cohorts investigated in these studies were very heterogeneous, consisting of poorly characterized individuals who were usually selected because they had an increase in blood glucose. Subsequently it has become clear that many different subphenotypes already exist in the prediabetic phase *(3, 4)*.

Metabolomics represents a new potential approach to move the diagnosis of diabetes beyond the application of the classical diabetic laboratory tests. This strategy means the profiling of hundreds (targeted metabolomics) or thousands (nontargeted metabolomics) of metabolites *(5)*.

In the current issue of *Clinical Chemistry*, Liu and coworkers *(6)* present data on the nontargeted metabolomic investigation of fasting serum samples of a T2Dm subtype with IGT as demonstrated by an oGTT [2-h glucose concentration ≥200 mg/dL (11.1 mmol/L)] but fasting glucose concentrations within reference intervals. Following the nomenclature of Liu and coworkers, this T2Dm subtype will henceforth be referred to as isolated postchallenge diabetes (IPD). Of note, IPD remains undetected when only fasting glucose is measured. Fifteen IPD-specific metabolites were identified. Concentrations of these metabolites were significantly different not only between healthy controls and patients with IPD, but also between patients with IPD and individuals with newly diagnosed T2Dm and impaired fasting glucose. Linoleic acid, oleic acid, and dehydroepiandrosterone sulfate were the most powerful markers. In a replication study (n = 400), the area under the ROC curve when applying a combination of these 3 metabolites to discriminate between IPD and non-IPD individuals was 0.849. The identification by these investigators of a metabolic pattern in fasting plasma that is capable of identifying IPD, a diagnosis which currently is only possible after an oGTT, demonstrates the strengths of metabolomics in diabetes research. Their findings together with the results of other metabolomics studies could represent the first step toward replacing the oGTT. However, there are pitfalls in current metabolomics approaches to search for diagnostic biomarkers, such as failure to validate the diagnostic power of a given approach in a sample set that includes sera from patients with other diseases.

The identification of specific (pre)diabetic metabolite patterns is an essential prerequisite for this diagnostic application. However, the metabolic profiles obtained are often very complex, and bioinformatics tools such as feature subset selection approaches are needed

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[4] Nonstandard abbreviations: T2Dm, type 2 diabetes; oGTT, oral glucose tolerance test; IGT, impaired glucose tolerance; IPD, isolated postchallenge diabetes.

to find the relevant pattern in the hundreds or thousands of detected metabolite signals *(7)*. It is worth noting that bioinformatically elucidated patterns found suitable to separate subphenotypes may include a mixture of metabolites, some of which, when considered univariately, are not significantly different between phenotypes, and others which are significantly different *(7)*. The advantage of metabolite patterns over single parameters can be seen in the ability of a multivariate pattern to predict future development of T2Dm by use of fasting plasma concentrations of 5 amino acids *(8)* and by the differentiation of prediabetic subphenotypes (metabolically benign vs malignant fatty liver) by a pattern of 7 plasma metabolites *(7)*.

Several metabolite classes or candidate biomarkers have emerged from metabolomic (pre)diabetes studies. The metabolites most consistently found are amino acids, in particular, branched-chain amino acids *(8, 9)*, lyso-phosphatidylcholines *(6, 7, 10, 11)*, fatty acids, and acyl carnitines *(6, 7, 9)*. In this context, it is important to note that these diabetes-associated markers do not preclude the utility of other metabolic biomarkers. The current dominance of these biomarkers is partially attributable to the application of targeted metabolomics as the most frequently used strategy, which covers a limited number of selected 100–200 metabolites. Other relevant aspects include the metabolite plasma concentration and the performance and selectivity of the analytical platform. For example, in liquid chromatography–electrospray ionization–mass spectrometry approaches, most of the metabolites mentioned above are among the favored ions dominating the metabolite ion pattern. All these aspects lead to the selection of distinct groups of metabolites and, consequently, certain markers dominate the current reports in the literature.

An important issue to be considered when selecting a targeted approach or interpreting the data is whether the study has been designed to focus on pathways relevant in the (pre)diabetic context. The use of such a strategy can lead to the detection of an impressive number of significantly altered metabolite concentrations, as recently shown by the group of Robert E. Gerszten, who found the concentrations of 91 of 110 analyzed metabolites to be significantly increased or decreased following an oGTT *(12)*. The often-used alternative is that the targeted metabolites are not directly related to the studied context, but are based rather on already existing analytical platforms with different foci. Consequently, missing data, from the tricarboxylic acid cycle for example, may lead to difficulty in interpreting the results.

Furthermore, the quality of the samples used is an underestimated pitfall. The success of metabolomic investigations depends highly on the integrity of the clinical samples *(13)*. Important preanalytical requirements *(13)* may not always be fulfilled by samples obtained from biobanks, particularly when samples are collected in large multicenter diabetes cohort studies.

With respect to the validation of new laboratory diagnostics, all (pre)diabetes metabolomics markers or patterns detected to date have been carefully evaluated. They have been replicated in independent and large studies. It should be stressed, however, that these validations always have been performed in the "diabetes environment," i.e., by comparing selected healthy controls with (pre)diabetic individuals. To my knowledge, no validations of these diabetes markers have been performed by inclusion of samples from patients with other diseases.

It is important to note that even when metabolite patterns fail in these diagnostic tests, the detected metabolites are still valuable from the mechanistic point of view. They should be specified as functional biomarkers. Metabolomics can essentially contribute to a better understanding of the pathophysiology of this complex disease. However, many of the metabolomics reports in the diabetes field have been disappointing, because they are only descriptive. They have failed for the most part to provide experimentally proven functional insights into the pathogenesis of T2Dm. Although such knowledge is not essential for the discovery of diagnostics, it can be invaluable for identifying new targets for individual interventions; functional metabolomics provides optimal possibilities, particularly when stable-isotope-assisted metabolomics or lipidomics approaches are applied *(14)*. Sophisticated functional metabolomics studies may give important impetus to translational diabetes research, particularly when they are combined with data from proteomics and/or transcriptomics investigations *(14, 15)*.

Metabolomics research is full of promise and pitfalls. Diabetes research groups are exploring its utility. The fear is that if an increasing number of scientists fail to reach their intended goals owing to poor sample quality or inappropriate selected cohorts, they may abandon this approach. To prevent such a scenario, lessons should be learned from the disappointing results of the genomics studies in diabetes. One option would be to start the metabolomic project by complementary targeted and nontargeted investigation of a small group of deeply phenotyped individuals in a very carefully selected subphenotype ($n \leq 50$), e.g., prediabetic individuals who do not benefit from lifestyle intervention. Individuals in the latter phenotype need to be identified as early as possible for the initiation of alternative therapeutic interventions. Such individuals could benefit from the availability of a well-developed metabolomic profile for their early identification.

In conclusion, applying metabolomics means picking up the biochemical trails of our scientific forefathers from the pregenomic era and discovering new causal metabolic relations. It can be foreseen that the application of sophisticated metabolomics and bioinformatics approaches for the investigation of (pre)diabetes subphenotypes may open new perspectives by the discovery of unexpected targets for interventions (functional biomarkers) and novel laboratory diagnostics (diagnostic biomarkers). Without question, the use of metabolomics for identification and differentiation of (pre)diabetic subphenotypes would facilitate personalized medicine in the form of individualized prevention and treatment for one of the most burdensome diseases of the 21st century.

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References

1. WHO. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of WHO/IDF consultation. Geneva: WHO; 2006. http://whqlibdoc.who.int/publications/2006/9241594934_eng.pdf (Accessed June 2013).
2. Morris AP, Voight BF, Teslovich TM, Ferreira T, Segre AV, Steinthorsdottir V, et al. Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. Nat Genet 2012;44:981–90.
3. Stefan N, Kantartzis K, Machann J, Schick F, Thamer C, Rittig K, et al. Identification and characterization of metabolically benign obesity in humans. Arch Intern Med 2008;168:1609–16.
4. Stefan N, Haring HU. The metabolically benign and malignant fatty liver. Diabetes 2011;60:2011–7.
5. Rhee EP, Gerszten RE. Metabolomics and cardiovascular biomarker discovery. Clin Chem 2012;58:139–47.
6. Liu L, Wang M, Yang X, Bi M, Na L, Niu Y, et al. Fasting serum lipid and dehydroepiandrosterone sulfate as important metabolites for detecting isolated postchallenge diabetes: serum metabolomics via ultra-high-performance LC-MS. Clin Chem 2013;59:1338–48.
7. Lehmann R, Franken H, Dammeier S, Rosenbaum L, Kantartzis K, Peter A, et al. Circulating lysophosphatidylcholines are markers of a metabolically benign nonalcoholic fatty liver. Diabetes Care 2013;36:2331–8.
8. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, et al. Metabolite profiles and the risk of developing diabetes. Nat Med 2011;17:448–53.
9. Newgard CB. Interplay between lipids and branched-chain amino acids in development of insulin resistance. Cell Metab 2012;15:606–14.
10. Floegel A, Stefan N, Yu Z, Muhlenbruch K, Drogan D, Joost, HG, et al. Identification of serum metabolites associated with risk of type 2 diabetes using a targeted metabolomic approach. Diabetes 2012;62:639–48.
11. Wang-Sattler R, Yu Z, Herder C, Messias AC, Floegel A, He Y, et al. Novel biomarkers for pre-diabetes identified by metabolomics. Mol Syst Biol 2012;8:615.
12. Ho JE, Larson MG, Vasan RS, Ghorbani A, Cheng S, Rhee EP, et al. Metabolite profiles during oral glucose challenge. Diabetes 2013;62:2689–98.
13. Yin P, Peter A, Franken H, Zhao X, Neukamm SS, Rosenbaum L, et al. Preanalytical aspects and sample quality assessment in metabolomics studies of human blood. Clin Chem 2013;59:833–45.
14. Wolf M, Chen S, Zhao X, Scheler M, Irmler M, Staiger H, et al. Production and release of acylcarnitines by primary myotubes reflect the differences in fasting fat oxidation of the donors. J Clin Endocrinol Metab 2013;98:E1137–42.
15. Dutta T, Chai HS, Ward LE, Ghosh A, Persson XM, Ford GC, et al. Concordance of changes in metabolic pathways based on plasma metabolomics and skeletal muscle transcriptomics in type 1 diabetes. Diabetes 2012;61:1004–16.