EDITORIAL (SEE KOLBERG ET AL., P. 1207)

Multiple Biomarker Prediction of Type 2 Diabetes

Type 2 diabetes is predictable and preventable. Obesity, familial diabetes, and higher-than-normal blood glucose levels are well-known risk factors for development of type 2 diabetes by middle age; recent prediction models have incorporated these with other readily measurable features of metabolic syndrome (elevated blood pressure, low HDL cholesterol, and elevated triglycerides) to generate validated prediction rules (1–3). In clinical care, a patient of European descent with a family history of diabetes, obesity, and features of metabolic syndrome is at ~25-fold increased risk to develop type 2 diabetes in the next few years relative to a patient without these characteristics (4). With regard to the public health of the population, these characteristics very reliably discriminate groups at relatively high risk from those at low risk (3). Once high-risk patients are identified, those adherent to a program of ~30 min of moderate physical activity per day and weight loss of 5–10% of initial body weight can expect that risk for type 2 diabetes will be reduced by at least 50% relative to patients not following a therapeutic lifestyle program (5).

The relatively simple recognition of pre-diabetes and prevention of its transformation to diabetes may be well-known to the readers of *Diabetes Care*. However, some will raise concerns regarding practical problems obtaining fasting or oral glucose tolerance blood tests, the high intraindividual variability in blood glucose levels (6), the fact that fewer than 20% of white people with obesity will progress to diabetes over subsequent years (7), and the fact that the genetics that presumably underlie familial type 2 diabetes do not seem to add much to its predictability (8). Perhaps less well-known is the expanding understanding of pathophysiological axes beyond the classic triumvirate of β-cell, skeletal muscle, and liver (9) that contribute to the pre-diabetic state. Abnormal adipocyte signaling (10), subclinical inflammation (11), endothelial dysfunction (12), iron overload (13), incretin system abnormalities (14), and variation in the Circadian system (15,16) all appear to add substantial complexity to type 2 diabetes–risk physiology, offering potential targets for alternate or improved type 2 diabetes screening approaches.

In this issue of *Diabetes Care*, Kolberg et al. (17) used the experience of the Inter99 cohort to develop a model for assessing the 5-year risk of incident type 2 diabetes based on a panel of 64 circulating candidate biomarkers. In a nested case-control design, they selected from a population-based sample of ~6,600 Danes 160 individuals who developed type 2 diabetes and 472 who did not. They measured several clinical variables, collected fasting serum, and measured many candidate biomarkers from multiple diabetes-associated pathways. Their assay system employed an ultrasensitive immunoassay microsample molecular counting technology. The biomarkers were initially selected based on a thorough search of the current diabetes biomarker literature, narrowed based on the availability of assay reagents that could be incorporated with high quality into the assay system, and then further selected using statistical modeling.

Their biomarker selection and modeling method was thorough and exhaustive. They used a wide variety of approaches: biomarker selection based on biologically sensible hand-fit models or highly data-driven trial-and-error models. The modeling led ultimately to six biomarkers that gave a Diabetes Risk Score. The approach involved a high degree of multiple hypothesis testing. They appropriately controlled the type 1 error rate through permutation estimations of minimum *P* values observed for a randomly distributed outcome given the number of tests performed. Although they did not test the Diabetes Risk Score in an entirely independent sample, they provided good estimates of both the error around the model fit and discrimination using split-sample and bootstrap techniques. Given the thoroughness of the approach, there is no doubt that Kolberg et al. present the most robust multimarker prediction model possible given the biomarkers initially measured and the source population. The authors appropriately note that the approach may only apply to white Northern Europeans enrolled in a lifestyle intervention trial. Whether the model would produce the same biomarkers or discriminate well in race/ethnicity populations that are differentially affected by diabetes was not addressed.

The best predicting model included adiponectin, C-reactive protein (CRP), ferritin, interleukin-2 receptor A (IL2RA), glucose, and insulin, with area under the a receiver operator characteristic curve (AROC) of 0.76–0.78, indicating that the model would correctly pick the higher-risk subject from a pair of at-risk subjects 76–78% of the time. This model had better discrimination than models including the single variables A1C, fasting plasma glucose (FPG), fasting serum insulin, BMI, or sex-adjusted waist circumference; a model using FPG and insulin (that is, adding adiponectin, CRP, ferritin, and IL2RA to glucose and insulin; AROC = ~75%) that increased the AROC by about 1–3%; or a model including age, BMI, waist circumference, and family history of type 2 diabetes in first-degree relatives (AROC = 70%). Combining age, BMI, waist circumference, and family history of diabetes with the Diabetes Risk Score (that is, adding novel biomarkers to obesity, family history, FPG, and insulin) resulted in an AROC of 79%, which increased the AROC by 1%. Subjects in the highest 10% of the Diabetes Risk Score distribution had a 5-year risk of diabetes ~3.5 times higher than the population's average risk, which was higher than the relative risk associated with impaired fasting glucose (IFG) (FPG >100 mg/dl, risk relative to normal glucose tolerance = 1.4). IFG represented 56% of the sample; not reported is the risk associated with the top 10% of the FPG distribution relative to the population's average risk, which would be a fairer way to make this comparison.

There a couple of ways to view the meaning of the data in this article, including the ability of biomarkers to discriminate future disease and the ability of biomarkers to reveal pathophysiology. The authors focus on the former, but the

latter may be where the true interest of the approach is found. Here, they show that in addition to levels of glucose and insulin, markers of inflammation (CRP and IL2RA) and adipocyte signaling (adiponectin) are also independently associated with type 2 diabetes risk. Whether levels of ferritin reflect inflammation or iron storage physiology cannot be resolved by the data. By and large, diabetes biomarker literature has focused on single markers of physiological axes, although some studies have attempted multimarker analyses of adipose tissue signaling, inflammation, or endothelial dysfunction (12,18,19). Kolberg et al. exhaustively examined biomarkers from many different axes to arrive at the final set. In the online appendix, they point out that although the biomarker selection process may not have identified the best possible model, it identified a good model, and that there may have been other combinations of markers with equal performance. They do not present the results of every model; this may explain why levels of plasminogen activator inhibitor 1, von Willebrand factor, interleukin-6, or sex hormone–binding globulin, shown in other datasets to be independently associated with type 2 diabetes risk (18,20,21), were measured but not found to be in the final Diabetes Risk Score. Multimarker approaches have likewise been revealing with regard to the complex pathophysiology underlying risk of other cardiometabolic disorders, including hypertension, metabolic syndrome, and cardiovascular disease (22–25). The present report is the first such effort to create a multimarker "map" for pre-diabetes physiology. The results are consistent with the notion that the physiology underlying development of type 2 diabetes involves multiple systems beyond the classic triumvirate of liver, muscle, and pancreas.

Kolberg et al., however, focus primarily on the ability of their Diabetes Risk Score to discriminate future diabetes. The value of the score for this use is not clear. First, the marginal discriminatory value of the novel biomarkers seems quite small after considering the classic diabetes risk factors. Next, the proposed Diabetes Risk Score is now being marketed by Tethys Bioscience as the "PreDx Diabetes Risk Test," which is fine but warrants extra scrutiny with respect to the new technology's real practical clinical value. The test requires a 10-h fasting blood sample. One imagines that in most routine clinical practice where fasting blood samples are taken, a lipid panel is also measured as part of recommended adult risk factor screening. One also imagines that if patients present for fasting blood testing, they also will be weighed and have their blood pressure measured. Family history of diabetes may or may not be assessed, but if the focus of the exam includes diabetes risk screening, then familial diabetes is important to ascertain.

Thus, the key question that is not addressed by Kolberg et al. is what the marginal discriminatory capacity of the Diabetes Risk Score is after considering fasting glucose and triglycerides, HDL cholesterol, BMI, blood pressure, and family history of diabetes. Or, put another way, it is hard to imagine a situation where one would measure the "PreDx Diabetes Risk Test" but not fasting lipids and anthropometrics. That the Diabetes Risk Score can be used to generate a continuous estimate of risk is not a unique advantage because other metabolic syndrome characteristics can be handled in the same manner (3). Further, whether generation of a Diabetes Risk Score would be useful clinically is uncertain. As the authors point out, it is unclear that these have ever been widely adopted by physicians in practice. A distinct advantage of the metabolic syndrome concept, as opposed to sophisticated biomarker measurement or modeling, is that metabolic syndrome offers instant pattern recognition of patients likely to be at elevated risk for future type 2 diabetes. It is not clear that we need more than a few simple routine clinical measures to identify diabetes risk. The real challenge is not the need for new ways to identify pre-diabetes. The challenge is to find better approaches to help at-risk patients to change their lifestyle and lose weight because we know for certain that these changes are powerful means to prevent the development of type 2 diabetes.

JAMES B. MEIGS, MD, MPH

From the General Medicine Division, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts.

Corresponding author: James B. Meigs, jmeigs@partners.org.

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