Use of Multiple Metabolic and Genetic Markers to Improve the Prediction of Type 2 Diabetes: the EPIC-Potsdam Study

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OBJECTIVE — We investigated whether metabolic biomarkers and single nucleotide polymorphisms (SNPs) improve diabetes prediction beyond age, anthropometry, and lifestyle factors.

RESEARCH DESIGN AND METHODS — A case-cohort study within a prospective study was designed. We randomly selected a subcohort (n = 2,500) from 26,444 participants, of whom 1,962 were diabetes free at baseline. Of the 801 incident type 2 diabetes cases identified in the cohort during 7 years of follow-up, 579 remained for analyses after exclusions. Prediction models were compared by receiver operating characteristic (ROC) curve and integrated discrimination improvement.

RESULTS — Case-control discrimination by the lifestyle characteristics (ROC-AUC: 0.8465) improved with plasma glucose (ROC-AUC: 0.8672, P < 0.001) and A1C (ROC-AUC: 0.8859, P < 0.001). ROC-AUC further improved with HDL cholesterol, triglycerides, γ-glutamyltransferase, and alanine aminotransferase (0.9000, P = 0.002). Twenty SNPs did not improve discrimination beyond these characteristics (P = 0.69).

CONCLUSIONS — Metabolic markers, but not genotyping for 20 diabetogenic SNPs, improve discrimination of incident type 2 diabetes beyond lifestyle risk factors.

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A ccurate identification of individuals who are at increased risk for type 2 diabetes is a requirement for a targeted prevention. We therefore tested whether metabolic and genetic markers add substantial prognostic information to age, anthropometry, and lifestyle characteristics.

RESEARCH DESIGN AND METHODS — The European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study involves 27,548 participants (16,644 women, mainly aged 35–65 years, and 10,904 men, mainly aged 40–65 years) recruited from the general population in Potsdam, Germany, between 1994 and 1998. Follow-up questionnaires were sent out every 2–3 years to identify incident cases of type 2 diabetes (response rates 93–97%), and self-reports were verified by questionnaires mailed to physicians. Informed consent was obtained from participants; approval was given by the ethics committee of the State of Brandenburg, Germany.

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A prospective case-cohort study was designed (1) (supplemental Fig. 1, which can be found in the online appendix [available at http://care.diabetesjournals.org/cgi/content/full/dc09-0197/DC1]). Of 2,500 individuals randomly selected from 26,444 participants with collected blood, 1,962 remained after exclusion of prevalent diabetes, self-reported but unverified diabetes during follow-up, missing biomarker data, abnormal plasma glucose, or more than four missing genotypes. Of 801 incident cases identified in the full cohort with blood samples (mean follow-up 7.1 years), 579 remained for analyses after exclusions.

We used baseline information on age, waist circumference, height, history of hypertension, physical activity, smoking, and consumption of red meat, whole-grain bread, coffee, and alcohol to compute the German Diabetes Risk Score (DRS), a prediction model previously described (2). Measurement of glucose, HDL cholesterol, triglycerides, γ-glutamyltransferase, alanine aminotransferase, high-sensitivity C-reactive protein (hs-CRP), and A1C followed standard procedures (1). Total adiponectin was measured with an ELISA (LINCO Research, St. Charles, MO). Genotyping of 20 single nucleotide polymorphisms (SNPs) related to diabetes risk (3–6) (supplemental Tables 1–2) was performed with TaqMan technology (Applied Biosystems, Foster City, CA). The genotyping error was ≤0.5%, and genotype distributions were in Hardy-Weinberg equilibrium (P > 0.05). We computed an unweighted count genetic score assuming an additive genetic model for each SNP, applying a linear weighting of 0, 1, and 2 to genotypes containing 0, 1, or 2 risk alleles, respectively. Scores for individuals with missing genotypes were standardized to those for individuals with complete data (7).

We evaluated different prediction models through receiver operating characteristics (ROCs) based on logistic regression models comparing the area under the curve (AUC) of the fuller model with that of the sparser model (8). Model calibration was tested by Hosmer-
Relatives of the German DRS and biochemical and genetic markers to prediction of type 2 diabetes risk

| ROC* | IDI† | Relative IDI (%) |
|------|------|-----------------|
| C statistic (95% CI) | P | Absolute IDI (95% CI) | |
| DRS only‡ | 0.8465 (0.8299–0.8630) | Ref. | Ref. | |
| DRS and A1C | 0.8859 (0.8716–0.9003) | <0.0001 | 0.0974 (0.0792–0.1155) | 34.11 |
| DRS and glucose | 0.8672 (0.8515–0.8830) | <0.0001 | 0.0553 (0.0407–0.0699) | 19.37 |
| DRS, A1C, and glucose | 0.8926 (0.8785–0.9067) | 0.0040 | 0.0230 (0.0135–0.0325) | 6.01 |
| DRS, glucose, and A1C | 0.8926 (0.8785–0.9067) | <0.0001 | 0.0651 (0.0506–0.0797) | 19.11 |
| DRS, glucose, A1C, triglycerides, HDL cholesterol, γ-glutamyltransferase, and alanine aminotransferase | 0.9000 (0.8862–0.9137) | 0.0022 | 0.0223 (0.0142–0.0304) | 5.50 |
| DRS, glucose, A1C, and genetic markers§ | 0.8928 (0.8787–0.9070) | 0.7361 | 0.0014 (−0.0010–0.0039) | 0.36 |
| DRS, glucose, A1C, triglycerides, HDL cholesterol, γ-glutamyltransferase, alanine aminotransferase, and adiponectin | 0.9023 (0.8887–0.9158) | 0.0471 | 0.0064 (0.0022–0.0107) | 1.50 |
| DRS, glucose, A1C, triglycerides, HDL cholesterol, γ-glutamyltransferase, alanine aminotransferase, and hs-CRP | 0.9016 (0.8880–0.9151) | 0.1523 | 0.0029 (−0.0007–0.0066) | 0.69 |
| DRS, glucose, A1C, triglycerides, HDL cholesterol, γ-glutamyltransferase, alanine aminotransferase, and genetic markers | 0.9002 (0.8865–0.9140) | 0.6868 | 0.0015 (−0.0010–0.0039) | 0.34 |

*The ROC curve is a plot of sensitivity versus false-positive rate across all possible cut points for a continuous predictor or prediction model. The area under the ROC curve (C statistic) is a measure of discrimination between case patients and control participants based on ranks and reflects the probability that the predicted risk is higher for a case subject than for a control subject. It ranges from 0.5 (no predictive ability) to a theoretical maximum of 1 (perfect discrimination)—the latter achieved if the scores or predicted risks for all case subjects are higher than those for all control subjects. †IDI is the difference between two models in discrimination slopes, which reflect the mean difference in predicted risk between case and control subjects. Instead of the difference, relative IDI expresses the discrimination slope achieved if the scores or predicted risks for all case subjects are higher than those for all control subjects. ‡IDI is the difference between two models in discrimination.

Results — Baseline characteristics of the random subcohort and incident cases are presented in supplemental Table 3. ROC-AUC increased significantly when A1C or glucose was incorporated into a model with the German DRS (Table 1), most notably for A1C (from 0.8464 to 0.8862). P values for Hosmer-Lemeshow tests indicated better model calibration when A1C (P = 0.1181) or glucose (P = 0.3823) was included compared with a model with the German DRS alone (P = 0.0157). Measuring both glucose and A1C improved case-control discrimination. Also, blood lipids, γ-glutamyltransferase, and alanine aminotransferase significantly improved discrimination beyond the German DRS, A1C, and glucose (ROC-AUC: 0.9000). Moreover, IDI, as a marker of improved risk classification, was significantly different from zero (Table 1). In contrast to hs-CRP, additional information on adiponectin improved ROC-AUC. However, relative IDI was rather small (1.5%).

Diabetes risk increased with increasing number of prevalent risk alleles (supplemental Fig. 2). When genetic information was included along with the German DRS and metabolic markers, improvements of ROC-AUC and IDI were small and nonsignificant (Table 1).

Conclusions — Numerous diabetes prediction models have been developed, but few studies have investigated whether metabolic markers improve prediction beyond conventional risk factors. In the Atherosclerosis Risk in Communities study, clinical variables combined with fasting plasma glucose discriminated...
better compared with clinical variables only (11). Further improvement in discrimination was observed with HDL cholesterol and triglycerides. In the Framingham Offspring Study, a model involving additional information on hypertension, fasting plasma glucose, HDL cholesterol, and triglycerides performed substantially better than a model including age, sex, BMI, and parental history (12). Our results support and extend these findings by indicating that a comprehensive basic model including important lifestyle risk factors such as physical activity, smoking, alcohol consumption, and diet is significantly improved by glucose, A1C, HDL cholesterol, triglycerides, and liver enzymes but not hs-CRP, adiponectin, or genetic markers.

Our observation that multiple SNPs do not substantially improve discrimination beyond age, sex, and clinical markers confirms previous studies (7,13–15). We extended their results by using a comprehensive set of anthropometric, nutritional, and lifestyle variables in the basic model; by including additional biomarkers (adiponectin, hs-CRP, and A1C); and by using the full set of currently confirmed diabetogenic SNPs. It should be noted that the prospective design rendered it necessary to exclude prevalent diabetes cases at baseline. Thus, results reflect genetic prediction in middle-aged individuals but not prediction at birth. Furthermore, we did not consider gene–gene interactions.

In conclusion, our study indicates that both plasma glucose and A1C considerably improve discrimination of incident type 2 diabetes by age, anthropometry, and lifestyle characteristics (DRS). HDL cholesterol, triglycerides, γ-glutamyltransferase, and alanine aminotransferase, but not 20 diabetogenic SNPs, further improve discrimination.

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