ESM Methods:

Modeling the OGTT Curve

Glucose tolerance status was defined as a binary trait: 0 for NGT subjects, and 1 for IFG/IGT subjects. Peak-wise classifications were based on the number of incline and decline phases in glucose as previously described [1]. Frequencies of all peak-wised classified curves in Botnia and MPP studies are reported in Table 1. Given that glucose levels were measured at 0, 20, 40, 60, and 120 minutes in MPP, a small number of biphasic (n = 13), and triphasic (n = 20) curves were observed compared to 2046 monophasic curves. As a result, peak-wise classification analyses were not investigated in MPP. Unclassified curves (n = 163) were excluded from peak-wise analyses in Botnia along with monotonous ones (n = 29) due to their small sample size.

FPG-wise classifications were based on the time at which glucose level drops below FPG level as previously described [2]. Frequencies of all classified FPG-wised curves in both Botnia and MPP studies are reported in the Table 1. Both groups I (n = 17) and II (n = 6) were excluded from analyses in MPP due to their small numbers.

OGTT curve shapes embody dynamical glucose homeostasis mechanisms that are represented by changes in PG across time. Classification approaches do not account for within group variations. Two monophasic curves may vary by magnitude or relative magnitudes of rise and fall in glucose. We therefore developed quantitative shape indices for in-depth analyses of OGTT curves. Two well-established OGTT curve indices are total and incremental area under the OGTT curve of glucose (total $AUC_{glucose}$ and incremental $AUC_{glucose}$ in mmol/l × min). Both indices contain curve shape information, but two distinctly different OGTT curve shapes could have similar total $AUC_{glucose}$, incremental $AUC_{glucose}$ and mean glucose (Supplementary Figure 1). We therefore developed a new approach based on continuous changes in glucose across time.
to investigate curve shapes more accurately. We focused our attention on the total and relative changes in glucose. The strategy was developed by holding either one of the two variables constant and accounting for the other. Let \( G_0, G_1, \ldots, G_n \) and \( t_0, t_1, \ldots, t_n \) be \( n+1 \) OGTT glucose measurements and their respective times measured at fasting and post glucose ingestion. We defined curve size in mmol/l as the absolute sum of changes in glucose given by

\[
|\Delta G_1| + |\Delta G_2| + \cdots + |\Delta G_n|,
\]

where \( \Delta G_i = G_{i+1} - G_i \) is the amount of change in glucose following the \( i^{th} \) measurement.

We further defined peak sharpness of OGTT curves as a trait with both magnitudes and relative changes in glucose information as mmol l\(^{-1} \) min\(^{-2} \), where a complete peak (equal incline and decline in glucose) with large changes in glucose is considered sharp.

\[
\text{Peak sharpness} = 1000 \times \sum_{i=1}^{n-1} \frac{2 \times \min(|\Delta G_{i-1}|, |\Delta G_i|) + \Delta G_{i-1} + \Delta G_i \times |\Delta^2 G_i|}{2 \times \max(|\Delta G_{i-1}|, |\Delta G_i|) + \Delta G_{i-1} + \Delta G_i \times \Delta t_i^2},
\]

where

\[
\frac{|\Delta^2 G_i|}{\Delta t_i^2} = \frac{2}{\Delta t_i - \Delta t_{i-1}} \times \left( \frac{\Delta G_i}{\Delta t_i} - \frac{\Delta G_{i-1}}{\Delta t_{i-1}} \right)
\]

The relationship between curve size (total changes in glucose) and peak sharpness is that small curves with little changes in glucose cannot form sharper peaks than significantly larger curves. For peak sharpness of small and large curves to be comparable, there is a need to normalize peak sharpness by the potential peak sharpness of each curve. We defined potential peak sharpness as the maximum peak sharpness a curve could achieve given total changes in glucose.

\[
\text{Potential peak sharpness} = 1000 \times \sum_{i=1}^{n-1} \frac{2}{\Delta t_i - \Delta t_{i-1}} \left[ \frac{|\Delta G_i|}{\Delta t_i} + \frac{|\Delta G_{i-1}|}{\Delta t_{i-1}} \right].
\]

Potential peak sharpness was calculated assuming changes in glucose formed a complete peak (equal incline and decline in glucose). We normalized the effect of curve size on peak sharpness and introduced the index:
Peak completeness is the percent component of peak sharpness occurring as a result of equal adjacent inclines and declines in glucose. Two OGTT curves with the same relative changes in glucose, but different sizes will have the same peak completeness. It contains information on relative changes in glucose independent of curve size.

Lastly, we investigated unidirectional changes in glucose using the deviation of curve peak sharpness from its potential. We referred this information in mmol l\(^{-1}\) min\(^{-2}\) as peak swing (i.e. peak swing = potential peak sharpness – peak sharpness). Large and small curves with complete peaks have zero deviation from their potential peak sharpness (i.e. peak swing = 0), whereas incomplete peaks show a deviation that increases as more changes in glucose occur without contributing to peak completeness. Two incomplete peak curves with different sizes but the same relative changes in glucose (i.e. the same peak completeness) have different peak swings. The larger curve will have a larger peak swing compared to the other curve. Additional information on shape indices are reported in the Supplementary Table 1 and Supplementary Figure 2. All four shape indices (Curve size, Peak Sharpness, Peak Completeness and Peak Swing) were studied alongside PG concentrations, and both total and incremental AUC\(_{\text{glucose}}\) to optimize extracted information from OGTTs in assessing risk of incident T2D. For accuracy purposes, we calculated the exact total AUC\(_{\text{glucose}}\) as the sum of decomposed rectangular areas under OGTT line segments. Total AUC\(_{\text{glucose}}\) is given as

\[
AUC_{\text{glucose}} = \sum_{i=1}^{n-1} \frac{1}{2} \times (t_{i+1} - t_i) \times (G_{i+1} - G_i) ,
\]

which was further used to calculate incremental AUC\(_{\text{glucose}}\) as

\[
AUC_{\text{incr}} = AUC_{\text{glucose}} - 120 \times G_0 .
\]
Statistics

All statistical analyses were performed with R version 2.13.1. We assessed the association between continuous OGTT traits using Pearson correlation. The predictive ability for incident T2D of OGTT traits alone or in combination with baseline clinical risk factors (age, sex, BMI, family history of T2D) as an extended baseline model was assessed using logistic regression analyses. The outcome was incident T2D and the predictors were baseline clinical risk factors and / or OGTT traits. All predictors were continuous variables except for the discrete sex, family history of T2D, glucose tolerance status, peak-wise and FPG-wise classifications. Generalized estimating equations (GEE) models with a binomial family via logit link function were used to adjust for family clusters in the family-based Botnia study [3]. GEE models were fitted assuming an exchangeable correlation structure using the geepack package in R [4].

Receiver-operator-characteristics (ROC) curve analyses were used to assess the discriminatory ability of OGTT glucose traits between incident and non-incident T2D events, and whether they improve discriminatory ability of the clinical baseline model that includes age, sex, BMI, and family history of T2D. ROC curve analyses were performed using pROC package in R [5] with P values calculated using 5000 bootstrap permutations with stratified sampling of equal T2D events and non-events to the original sample. We included continuous net-reclassification-improvement (NRI) analysis to confirm ROC results and assess event-NRI and non-event-NRI proportions that make up total NRI value [6]. Continuous NRI analyses were done using Hmisc package in R [7].

We assessed the cumulative ability of two or more OGTT traits to predict future T2D using logistic regression or GEE models. We ran a combination of OGTT traits as extensions to our baseline model with age, sex, BMI, and family history of T2D to predict incident T2D. We
excluded models with independent variables having a Variance Inflation Factor (VIF) > 10 to avoid multicollinearity issues. The best model was compared with the best extended baseline model using a single OGTT trait. Model comparisons were based on their discriminatory AUC$_{ROC}$ values. External validations for OGTT traits in predicting T2D were carried out using Botnia as the model development set, and MPP as the model validation set.

We defined a 1h-PG threshold that maximizes sum of sensitivity and specificity for incident T2D prediction from a 5% percentile increment. The clinical utility of 1h-PG cut-off values was assessed by i) the odd ratios (± 95 CI) for incident T2D ii) the proportion of total incident T2D cases captured within the high-risk group iii) the positive and negative predictive values.

References: