Machine Learning in Multi-Omics Data to Assess Longitudinal Predictors of Glycaemic Health

Laurie Prélot[1], Harmen Draisma[1], Mila D. Anasanti[1], Zhanna Balkhiyarova[1], Matthias Wielscher[2], Loic Yengo[3], Beverley Balkau[4,5], Ronan Roussel[6,7], Sylvain Sebert[2,8,9], Mika Ala-Korpela[10-15], Philippe Froguel[1,16], Marjo-Riitta Jarvelin[2,8,9,17,18], Marika Kaakinen[1,19,20#], Inga Prokopenko[1,20#*]

[1]Section of Genomics of Common Disease, Department of Medicine, Imperial College London, London W12 0NN, United Kingdom;

[2]Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London W2 1NY, United Kingdom;

[3]Institute for Molecular Bioscience, The University of Queensland, 306 Carmody Road, St Lucia Qld 4072, Australia;

[4]Inserm, CESP Center for Research in Epidemiology and Public Health, U1018, Villejuif, France;

[5]Univ Paris-Saclay, Univ Paris Sud, UVSQ, UMRS 1018, UMRS 1018, Villejuif, France;

[6]Inserm U1138, Centre de Recherche des Cordeliers, Paris, France;

[7]University Paris Diderot, Sorbonne Paris Cite, Paris, France; Diabetology, Endocrinology and Nutrition Department, DHU FIRE, Hopital Bichat, AP-HP, Paris, France;

[8]Center for Life Course Health Research, University of Oulu, FI-90014 University of Oulu, Finland;

[9]Biocenter Oulu, University of Oulu, FI-90014 University of Oulu, Finland;

[10]Systems Epidemiology, Baker Heart and Diabetes Institute, Melbourne VIC 3004, Australia;

[11]Population Health Science, Bristol Medical School, University of Bristol, Bristol, UK;

[12]Medical Research Council Integrative Epidemiology Unit at the University of Bristol, Bristol BS8 1QU, United Kingdom;

[13]Computational Medicine, Faculty of Medicine, University of Oulu and Biocenter Oulu, FI-90014 University of Oulu, Finland;

[14]*NMR Metabolomics Laboratory, School of Pharmacy, University of Eastern Finland, FI-70210 Kuopio, Finland;*

[15]*Department of Epidemiology and Preventive Medicine, School of Public Health and Preventive Medicine, Faculty of Medicine, Nursing and Health Sciences, The Alfred Hospital, Monash University, Melbourne VIC 3004, Australia;*

[16]*CNRS, Pasteur Institute of Lille, University of Lille, 59000 Lille, France;*

[17]*Oulu University Hospital, 90220 Oulu, Finland;*

[18]*Department of Life Sciences, College of Health and Life Sciences, Brunel University London, Uxbridge UB8 3PH, United Kingdom;*

[19]*Centre for Pharmacology and Therapeutics, Department of Medicine, Imperial College London, London W12 0NN, United Kingdom;*

[20]*School of Biosciences and Medicine, Department of Clinical and Experimental Medicine, University of Surrey, Guildford, United Kingdom.*

#These authors contributed equally.

*Corresponding author:

Inga Prokopenko, i.prokopenko@imperial.ac.uk

Abstract

Type 2 diabetes (T2D) is a global health burden that will benefit from personalised risk prediction and targeted prevention programmes. Omics data have enabled more detailed risk prediction; however, most studies have focussed on directly on the ability of DNA variants predicting T2D onset with less attention given to epigenetic regulation and glycaemic trait variability. By applying machine learning to the longitudinal Northern Finland Birth Cohort 1966 (NFBC 1966) at 31 (T1) and 46 (T2) years old, we predicted fasting glucose (FG) and insulin (FI), glycated haemoglobin (HbA1c) and 2-hour glucose and insulin from oral glucose tolerance test (2hGlu, 2hIns) at T2 in 513 individuals from 1,001 variables at T1 and T2, including anthropometric, metabolic, metabolomic and epigenetic variables. We further tested whether the information obtained by the machine learning models in NFBC could be used to predict glycaemic traits in the independent French study with 48 matching predictors (DESIR, N=769, age range 30-65 years at recruitment, interval between data collections: 9 years). In this study, FG and FI were best predicted, with average R^2 values of 0.38 and 0.53. Sex, branched-chain and aromatic amino acids, HDL-cholesterol, glycerol, ketone bodies, blood pressure at T2 and measurements of adiposity at T1, as well as multiple methylation marks at both time points were amongst the top predictors. In the validation analysis, we reached R^2 values of 0.41/0.55 for FG/FI when trained and tested in NFBC1966 and 0.17/0.30 when trained in NFBC1966 and tested in DESIR. We identified clinically relevant sets of predictors from a large multi-omics dataset and highlighted the potential of methylation markers and longitudinal changes in prediction.

Key Words

Glycaemic traits, longitudinal, machine learning, metabolomics, methylation, prediction, type 2 diabetes

Background

Diabetes accounts for the yearly deaths of about four million people between 20 and 79 years old (2017) world-wide and the prevalence of diabetes is expected to increase from 8.8% to 9.9% by 2045.[1] Moreover, glucose tolerance impairment is progressing in young individuals, leading to high risk of developing type 2 diabetes (T2D) later in life.[1]

To date, T2D risk prediction in the clinical practice has focussed on the classical risk factors of sex, age, obesity, family history, hypertension, cholesterol levels and lifestyle factors [2–5]. Recent advances in omics technologies have allowed exploring the risk factors of T2D in more detail, opening possibilities for more precise biomarkers and thus, better identification of people at risk for T2D in the future. A large number of metabolites, including amino acids, especially branched-chain amino acids (BRACA) and aromatic amino acids, fatty acids, glycerophospholipids, ketone bodies and mannose have been associated with T2D incidence[6–8]. However, whether metabolites can be effective and reliable T2D predictors, remains unclear.

During the past decade, genome-wide association studies (GWAS) have enlightened the genetic risk of developing T2D. Currently, 403 independent DNA variants are established for T2D risk by GWAS meta-analyses[9,10] as well as dozens of loci have been associated with quantitative glycaemic traits in individuals without T2D, including fasting glucose (FG)[11], fasting insulin (FI)[11], FG adjusted for body-mass-index (BMI)[11], FI adjusted for BMI[11], 2 hour post-prandial or post oral glucose-tolerance test glucose (2hGluc) [12] and glycaeted haemoglobin (HbA1c)[13]. Besides genetics, environment and lifestyle are likely to have a large contribution to T2D risk[14]. Environmental factors can affect gene expression by an addition of a methyl group on a CpG-dinucleotide site of DNA. This is called DNA methylation and is the most widely studied type of epigenetic modification[15,16]. Studies in peripheral blood have found a mean absolute difference of 0.5-1.1% in methylation levels between individuals with and without T2D[17]. Epigenome-wide association studies have reported associations at 65 methylation

markers for T2D[17,18] and provided support for overlap in epigenetic effects between T2D and glycaemic traits[18,19]. The epigenetic effect on variability of glycaemic traits is magnified in the presence of obesity[19]. The investigation of the link between BMI and methylation levels demonstrates that methylation at the majority of CpG sites in blood is consequential to higher BMI[20]. Interestingly, a weighted methylation risk score calculated from 187 markers was shown to have an even stronger effect on incident T2D[20] than the traditional risk factors of overweight, central obesity, phenylalanine, tyrosine, isoleucine, FG, FI and C-reactive protein. The methylation risk score remained associated with incident T2D even after adjustment for age, sex, BMI, FI, FG and central obesity[20].

In the era of multi-omics data and millions of measurable variables, it is challenging to identify the best biomarkers for clinical use. Machine learning approaches are ideal for such high-dimensional data due to their ability to learn from the data and identify patterns without knowledge of the joint distribution of the variables[21]. Recently, the studies of T2D risk have leveraged association analyses as well as machine learning algorithms for the prediction of binary T2D phenotypes. Thus far, machine learning used for T2D classification include logistic regression with and without Lasso regularization[17,20,22–26], Regularized least-squares (RLS)[27], Cox regression[23], naïve Bayes[25] and J48-decision tree[25]. In predictive studies using machine learning models, classical risk factors, genetic risk scores (GRS)[22], methylation risk scores (MRS)[17,20] and metabolomic data[24–27] have been used as predictors of T2D incidence after a follow-up window of two to fourteen years. A few studies have suggested that metabolites improve prediction performance[23,25–27], while others have reported negligible to no improvement in prediction[24]. GRS have been shown to bring no incremental value over classical non-invasive factors and metabolic markers[22]. MRS combining CpG loci have been found to be associated with future T2D incidence[17,20]. All the previous studies have focused on the binary disease status as the outcome which may greatly reduce the power of the analyses. To our knowledge, there are no machine learning studies on predictors of continuous glycaemic traits relevant for T2D pathophysiology. In addition, most of the studies on T2D have used a single pre-selected machine learning approach and have not

compared their performance in terms of predictive capacities. With the present study, we aimed to address these shortcomings, as well as to shed light on the contribution of longitudinal predictors, especially metabolomic and methylation markers by using data from two well-characterised population-based studies and by applying and comparing six different machine learning approaches.

Results

We focused on epigenetic and metabolic markers (**Supplementary Table 1**, **Supplementary Table 2**) from the Northern Finland Birth Cohort 1966 (NFBC1966) measured at 31 (T1) and 46 (T2) years for prediction of HbA1c, FG, 2hGluc, FI and two-hours post-oral glucose tolerance test insulin (2hIns) at T2 in individuals free from T2D diagnosis or medication at T1. We implemented and compared the results obtained from six machine learning approaches: Boosted trees (BT), Random forest (RF) and support vector regression (SVR) with Linear Kernel with L2 regularization and with L1 and L1/L2 loss functions (SVR-L2Linear-L1, SVR-L2Linear-L1L2, respectively), with Polynomial Kernel (SVR-Polynomial) and with Radial Basis function Kernel (SVR-RBF). The algorithms were chosen for their ability to handle a large number of predictors, to account for multi-collinearity, non-linear relationships, the absence of the assumption regarding data distribution, and for their computational times. We also tested different input data combinations (**Figure 1**). We further validated our approach in an independent French cohort (Data from an Epidemiological Study on the Insulin Resistance syndrome, DESIR) that shared 48 variables with the NFBC1966 cohort (**Supplementary Table 3**).

Best predicted outcome variables

We compared the performance of the six models for each of the outcomes (HbA1c, FG, 2hGluc, FI and 2hIns) with varying input data combinations, including omics data in their raw or scored forms (**Methods, Supplementary Table 4**). With the usage of metabolic data as the minimal input, we observed that performance was the best for FI prediction (**Figure 2A**). In the context of the model with

metabolic raw data (Mb-R), models with different outcomes were ranked as follows: FI > FG > 2hIns > 2hGlc > HbA1c ($P_{TukeyHSD}$<5.0x10^{-5} for all other comparisons except for 2hIns>2hGluc for which $P_{TukeyHSD}$=0.19, **Supplementary Table 5**). The average coefficients of determination R^2 over all machine learning algorithms were 0.53, 0.38, 0.29, 0.25 and 0.14 (Mb-R) for FI, FG, 2hIns, 2hGlc, HBA1c respectively (**Supplementary Table 6**).

Best predictor data combinations

Regarding the predictors, we found that models that included at least some metabolic data, either in their raw format (Mb-R) or transformed into scores (Mb-S) (**Methods**) had the best performance reaching a maximum R^2=0.56 (**Figure 2A, Supplementary Table 7**). In contrast, models with methylation data only as predictors (Mh-R and Mh-S), reached R^2 values of up to 0.20. Thus, metabolic models performed significantly better than pure epigenomic models ($P_{TukeyHSD}$<1.7×10^{-9}) (**Table 1**, Comparison 1-4; all comparisons are provided in **Supplementary Table 8**). When metabolic and methylation data were combined, Mb-R performed better for FI and FG than Mb-R + Mh-R ($P_{TukeyHSD}$ <0.04). Adding Mh-S to the model did not alter the model performance for any of the outcomes ($P_{TukeyHSD}$>0.99) (**Table 1**, Comparison 5-6). These results suggest that addition of methylation information does not increase the predictive ability of the tested models. When exploring the effect of transforming the original variables into scores (**Table 1**, comparison 7-8), we observed no significant differences for any of the outcomes when comparing Mb-R vs. Mb-S ($P_{TukeyHSD}$>0.29). This was true also for Mh-R vs. Mh-S, except for the prediction of FI, where Mh-R performed better than Mh-S ($P_{TukeyHSD}$=1.6×10^{-4}). Therefore, based on these results we are unable to generalise better performance of the scored data as compared to raw data. Finally, we found that Mb-S + Mh-S model performed significantly better than the model with Mb-S + Mh-R ($P_{TukeyHSD}$<0.02) for all outcomes except for 2hGluc. This observation reflects the decrease in performance of the models upon inclusion of a large number of weak predictors.

Adjustment for Measures of Adiposity

To understand the influence of the measures of adiposity in the models, we adjusted all the outcomes for T1-BMI, T2-BMI, T1-WHR and T2-WHR and performed the machine learning models on these adjusted outcomes. All adjusted models exhibited an R^2<0.13 (**Figure 2B, Supplementary Figure 1** with a zoomed-in scale for R^2, **Supplementary Table 9**), including models predicting FI and FG. Therefore, the measures of adiposity at T1 and T2 are the main drivers of prediction for FI and FG.

Variable Importance

We investigated the contribution of metabolic and epigenomic variables to the prediction of glycaemic traits. We discuss predictors importance only in the context of FG and FI outcomes, for which prediction algorithms reached the best R^2 (**Figure 2A**). FG prediction was mostly explained by metabolic variables: valine, leucine, isoleucine, tyrosine, BMI and WHR, HDLs and VLDL, glycerol, alanine, SBP and DBP at T2, WHR and FG at T1 and sex (**Figure 3** bottom right). FI prediction was explained by BMI, WHR, HDL, VLDL, BRACA, phenylalanine, leucine, glycerol, lactate, tyrosine, valine at T2, and FI at T1 (**Figure 3** top left). Once we adjusted the outcomes for the measures of adiposity, the top predictor for FI was measurement of FI at T1, followed by alanine and other variables at T2 already observed as important before adjustment for measurements of adiposity (**Supplementary Figure 2**). The top variables driving the prediction of FG were, similarly to FI, valine at T2 and FG at T1 (**Supplementary Figure 2**). The metabolic models with scored variables were driven by variables that mirrored the top raw predictors. Overall, the model with scored variables for FI supported the importance of the former variables, as well as ketone bodies (acetoacetate and 3-hydroxybutyrate) at T2 (**Figure 3**, top right and bottom left). Importantly, even though we previously observed that adding methylation data does not improve model prediction, a number of methylation probes were among the top predictors for both FG and FI when combined with scored metabolic variables (**Figure 3**).

Association Analysis for Effect Sizes, Direction and Variance Explained

Linear regression analyses between ln(FI)/FG and each of the top 25 predictors identified by machine learning indicated that increases in HDL cholesterol were predictive of decreased values of FI and FG (**Table 2**). The same was true for free cholesterol to total lipids ratio in IDL and cholesterol esters to total lipids ratio in medium VLDL. Similarly, female gender and higher sum score of ketone bodies, i.e. acetoacetate and 3-hydroxybutyrate, predicted lower FI and FG levels, as did higher methylation levels of the probes T2_cg00574958, T2_cg17058475, and T2_cg08309687. In the models with scored variables, the adjusted R^2 for ln(FI) when including all 25 top predictors was 0.58. After exclusion of methylation markers, it decreased to 0.54 and finally, after excluding all other variables than measures of adiposity, the variance explained was 0.40. The same figures for FG were 0.45, 0.42 and 0.31, respectively.

Prediction from Variables at T1

To test whether variables already 15 years beforehand, i.e. at T1 only, can provide information about glycaemic traits at T2, we restricted the prediction variables to those measured at T1 only. For example, under the well-performing Mb-R in the full model with predictors both from T1 and T2 and unadjusted for measures of adiposity, FI, FG, 2hIns, 2hGlc, HBA1c were predicted with R^2 values of 0.53, 0.38, 0.29, 0.25 and 0.14, respectively. The restriction to T1 variables caused a drop in R^2 to 0.25, 0.22, 0.15, 0.06, 0.06, respectively, when averaging over all machine learning methods. This suggests that prediction from T1 variables only is not achievable in our dataset.

Performance of the machine learning algorithms

When at least metabolic data in either form (Mb-R or -S + any other data) were included as input, we found no statistically significant differences between the performances of RF and BT for all phenotypes ($P_{TukeyHSD}$>0.91, **Supplementary Table 10**). In addition, no significant difference was found between SVR-L2Linear-L1L2 and RF or BT models ($P_{TukeyHSD}$>0.29, **Supplementary Table 10**). Among SVR models, we found that SVR-L2Linear-L1L2 either performed equally or outperformed the other SVRs,

depending on the input dataset. In particular, for datasets with a large number of predictors SVR-L2Linear-L1L2 was the best performing SVR ($P_{TukeyHSD}$<0.05, **Supplementary Table 10**). SVR-L2Linear-L1 in turn showed lower performance ($P_{TukeyHSD}$<0.05, **Supplementary Table 10**) at several occasions when compared to that of the other algorithms. Even though both SVR-L2Linear-L1 and SVR-L2Linear-L1L2 both use the L2 regularization, the first uses only the L1 loss function whereas the latter optimizes over both L1 and L2 loss functions (**Supplementary Material**). We observed that in 78.5% of the time the L2 loss function was chosen over L1 in the SVR-L2Linear-L1L2 analyses (data not shown). We investigated whether the better performance of the SVR-L2Linear-L1L2 algorithm over the SVR-L2Linear-L1 was due to the evaluation criterion used, namely R^2 which computes a scaled measure based on the quadratic loss function. For this purpose, we assessed the model performance in terms of Mean Absolute Error (MAE), i.e. based on L1 loss. By definition we aim to maximize R^2 while we aim to minimize MAE. Evaluations based on MAE did not show improved performance of the predictions based on SVR-L2Linear-L1 (**Supplementary Figure 3**, **Supplementary Table 11**).

Validation of the Machine Learning models in the French DESIR cohort

For the replication analysis, we first trained and tested the data in the NFBC1966 using the set of 48 variables common to both NFBC1966 and DESIR. Next, we trained the data in the NFBC1966 and tested in DESIR. We predicted only FI and FG levels using the top three performing algorithms: RF, BT and SVR-L2Linear-L1L2 (**Methods**). We were able to predict FI and FG levels in the DESIR data with the average R^2 values of 0.30 and 0.17, respectively (**Figure 4**, **Supplementary Table 12**). The values were decreased as compared to those when trained and tested in the same data, i.e. NFBC1966 (R^2 for FI=0.55, FG=0.41, **Supplementary Table 12**). RF and SVR-L2Linear-L1L2 produced a smaller change in the predictions (FI: RF=0.18, SVR-L2Linear-L1L2=0.22; FG: RF=0.21, SVR-L2Linear-L1L2=0.19), while BT was less stable in its performance (change in R^2 0.35 for FI, 0.32 for FG). Having a larger sample size in the training data (**Methods**) produced less variable R^2 values than those resulting from the use of a smaller training dataset in all models (**Figure 4A** vs. **4B**). While we still observed a drop in the R^2 values

for both FG and FI when tested in the external data, RF and SVR-L2Linear-L1L2 produced smaller decreases than BT, as before (**Supplementary Table 12**).

Discussion

To our knowledge, this is the first multi-omics study implementing machine learning to predict continuous glycaemic traits over time. We dissected the predictive value of methylation and metabolic, including metabolomic, data from two time points for individual's glycaemic health. We compared six machine learning approaches, while most previous studies have usually focussed on only one selected approach. We detected the best predictive ability of our models for FI and FG levels out of the five glycaemic traits tested, with raw or scored metabolic data as predictors and, BT, RF, or SVR-L2Linear-L1L2 as the algorithms. We identified metabolic variables that drove the prediction of the models. We showed that measures of adiposity are the most important contributors to glycaemic health. We also found that methylation probes accounted for 4 and 3 percentage points of the variance explained for FI (58%) and FG (45%), respectively. Finally, replication of the approach in an external European descent dataset (DESIR) using a subset of variables common to both cohorts, suggested that RF and SVR-L2Linear-L1L2 are more stable than BT in their performance.

Most of the published studies have targeted T2D onset prediction as a discrete value and rely on the categorization of individuals based on diagnosis thresholds for HbA1c, FG, 2hGluc and random glucose. The cut-off points for prediction analyses may vary across studies, and more generally, information loss is rather large when data is categorised[28,29]. Continuous phenotypes, on the contrary, have the potential to reflect the progressive onset of a disease without assuming a discontinuity in the underlying phenomenon. In addition, focussing on the prediction of continuous glycaemic phenotypes themselves allows removing them from the set of predictors for T2D and may reveal more modest effects of other variables[24]. Indeed, FG[23–27] and 2hGluc[24,25] have been shown to be good predictors of

T2D. Our study indicated that out of the five glycaemic traits we used, FG and FI were best predictable. This is expected as fasting values are tightly regulated. However, from the clinical practice point of view regarding the prediction of developing T2D, especially FI measurements have less relevance as compared to HbA1c measurements, for example. This suggests that future efforts should be directed towards improving the prediction of the other glycaemic indices than FG and FI.

Our study leverages machine learning ability to perform variable selection independently of a pre-filtering. To date, RLS (a variant of SVR-L2Linear algorithms)[27], J48-decision tree[25], and logistic regression with regularization[23,26] have highlighted the importance of specific metabolites consistent with our findings. Indeed, branched-chain amino acids (Leucine, Valine, Isoleucine)[25,26], HDL, VLDL, glycerol, ApoA and Apo B, 3-hydroxybutyrate[26], aromatic amino acids (phenylalanine, tyrosine)[23,26] are established as important predictors by machine learning algorithms, as also shown by our study. Moreover, in this study, we report glycoprotein acetyls and acetoacetate as good predictors of glycaemic trait levels. These markers have previously been associated with T2D[30–32]; however, for the first time here, we show that they are not only associated, but are also predictors of glycaemic health.

In addition to specific metabolites, the machine learning algorithms assigned a high rank (first 25) to several established metabolic health-associated methylation probes in the prediction of FI and FG when collapsing metabolic predictors into scores but keeping methylation probes as such. The probes included for instance those within the genes *CPT1A* and *SREBF1*, where the first, *CPT1* (Carnitine palmitoyltransferase I) is involved in fatty acid metabolism (RefSeq, Jul 2008) , and the latter, *SREBF1* (Sterol regulatory element-binding transcription factor 1) regulates genes required for glucose metabolism as well as fatty acid and lipid production, and its expression is regulated by insulin[33]. Previously, methylation at *CPT1A* and *SREBF1* has been associated with 2hIns[19], BMI[20], FG[18] and T2D[18]. In our study, hypomethylation at *CPT1A* (cg17058475) at T2 predicted higher FI and FG levels, whereas hypermethylation at *SREBF1* (cg11024682) at T1 predicted higher FI levels. The

303 predictive power of the methylation probes was modest, 0.04 out of 0.58 for FI and 0.03 out of 0.45
304 for FG. However, for both glycaemic traits the ranking of 15 methylation probes among the top 25
305 predictors when using scored metabolic data, sets the ground for larger studies of this kind, similarly
306 to the work that has been achieved through large-scale GWAS. A recent study aggregating information
307 over millions of genetic markers into a score showed that genetic risk scores for common diseases can
308 identify people at risk equivalent to monogenic mutations[34]. On that account, the findings from our
309 study encourage further exploration of methylation scores consisting of thousands or even millions of
310 probes in glycaemic trait level prediction.
311
312 Measures of body adiposity and those of obesity are established risk factors for T2D[35] and have a well-
313 known impact on glycaemic trait variability[36]. In all six machine learning approaches and within all data
314 combinations, we confirmed the high predictive value of BMI and WHR already 15 years beforehand.
315 Indeed, when we calculated the variance explained from linear regression with the top 25 predictors,
316 BMI and WHR accounted for the most part of it for both FI (0.40 out of 0.58) and FG (0.31 out of 0.45).
317 When the outcomes were adjusted for the measures of adiposity, FG and FI levels at T1 gained more
318 weight as predictors. These findings emphasize the importance of classical risk factors in T2D
319 prediction but also show that the tracking is relevant already 15 years beforehand. Taken together, it
320 is clear that classical risk factors will remain as valuable tools in the clinical practice for predicting
321 future glycaemic health. However, more detailed biomarkers, for example certain metabolites as
322 shown in the present study, genetic risk factors as shown recently[34] and possibly methylation markers
323 will open avenues for more precise prediction.
324
325 From the algorithm point of view, our analyses showed that the highest prediction performance was
326 achieved with BT, RF and SVR-L2Linear-L1L2 algorithms. This is consistent with the literature as BT and
327 RF have shown to perform well in the prediction studies of various types of data[37,38] . The SVR-L2Linear-
328 L1L2 (LIBLINEAR library in R) algorithm performed significantly better than the SVR-L2LinearL1

329 (kernelab library in R). This difference can be explained by the use of the L2-loss over the L1-loss in the
330 SVR-L2Linear-L1L2 in 78.5% of the cases (i.e. across all external cross-validations, all phenotypes and
331 all data type combinations). We further investigated whether the better performance was due to the
332 choice of the evaluation criterion, namely R^2 which evaluates the performance in squared terms,
333 similar to L2 loss, rather than in absolute terms, as do MAE and L1. Our analyses showed that SVR-
334 L2Linear-L1 performed worse, regardless of the evaluation criterion used. These observations highlight
335 the importance of loss function choice when using the SVR linear with L2-regularisation, independent
336 of the model performance evaluation criterion. The SVR-Polynomial and SVR-RBF showed slightly
337 lower predictions of the phenotypes, but there were no statistically significant differences when their
338 performances were compared to those of the top three algorithms.
339
340 Our analysis has some limitations that warrant discussion. First, the relatively small sample size (513
341 subjects) is a drawback for taking full advantage of machine learning prediction with high-dimensional
342 multi-omics data layers. However, our data is the largest to our knowledge featuring both longitudinal
343 data and a comprehensive set of multi-omics data. UK Biobank has expressed its plans to acquire
344 methylation data on its participants[39]. These data will be an important resource for future methylation
345 studies. Nevertheless, the data will be cross-sectional and will not allow investigation of changes in
346 methylation as the data used in the current study data does. Second, parameter tuning and drawing
347 of a threshold regarding variable importance in our machine learning models are not trivial. Longer
348 parameter tuning times might have resulted in more precise predictions and better performance of
349 some or all of the algorithms. Variable importance in turn will depend on the number of variables
350 resampled by the algorithms or the regularization parameters chosen. Overall, we restricted our
351 analyses to six machine learning algorithms in total. It was out of the scope of this study to explore
352 other potentially relevant algorithms, which will remain of future research interest. Third, regarding
353 the samples and the study design, the use of whole blood only for methylation markers, and the
354 relatively young age of the participants, 46 years old at the measurement time of the outcome

355 variables is a limitation. The latter might alternatively represent a positive feature, since blood is the
356 easiest tissue to obtain for any study, while the trend of deteriorating glycaemic health in younger
357 adults is growing in all human populations. Finally, our replication effort also has some limitations. We
358 were able to integrate only a small number of variables shared by both cohorts, and the time between
359 the measurements differed between the Finnish and French studies, as did the age of the participants
360 at recruitment. Despite these limitations the tested machine learning models showed promising
361 consistency between the two cohorts, and we would expect better performance would the
362 abovementioned limitations be addressed better.
363

364 Conclusions

365 With the use of six different machine learning algorithms, we have identified clinically relevant sets of
366 predictors of glycaemic traits from large multi-omics datasets and highlighted the potential of
367 methylation markers and longitudinal changes in prediction. In the future, we expect that
368 improvements in study sizes, methylation score computation, finer model tuning and replication in
369 more similar external datasets will improve predictive ability of our models for glycaemic traits and
370 will unveil novel prognostic omics biomarkers for T2D endophenotypes.
371

372 Methods

373 Study Populations

374 NFBC1966

375 Northern Finland Birth Cohort 1966 (NFBC1966) comprises participants from the two northernmost
376 provinces of Finland with expected dates of birth falling in 1966 (N=12,058 births)[40]. From the medical
377 examination at 31 (T1, N=6,007) and 46 years (T2, N=5,861) we included participants with
378 demographic, medication, epidemiological, blood biochemical, metabolomic and epigenetic

information available at both time points (N=626). Consent was obtained and the study was approved by the ethical committees of the University of Oulu and Imperial College London (Approval:18IC4421).

DESIR

The longitudinal DESIR study (Data from an Epidemiological Study on the Insulin Resistance syndrome) included 5,212 participants from the French general population. Clinical and biological evaluations were performed at inclusion and after three, six, and nine years, as previously described[41,42]. We used the data from 769 individuals aged from 30 to 65 years old at time of inclusion (T1) and nine years after inclusion (T2). Written informed consent was obtained from all participants. The study was approved by the Ethics Committee for the Protection of Subjects for Biomedical Research of Bicêtre Hospital, France.

Epidemiological, Blood Biochemical and Metabolomic Data in the NFBC1966

Height, weight, waist and hip circumference, and systolic and diastolic blood pressure (SBP, DBP, each measured in triplicate) were measured according to standard study protocols at the clinical examinations at T1 and T2. We used the measured height and weight; however, if unavailable, data from postal questionnaire were used. Body mass index (BMI) was calculated from height and weight and waist-hip-ratio (WHR) from waist and hip measurements accordingly. The biochemical assays[43,44], oral glucose[45], and HbA1c measurements[46] are detailed elsewhere. Metabolites were quantified by a high-throughput serum nuclear magnetic resonance (NMR) platform[47–50]. Imputation of epidemiological, biochemical and metabolomic variables was performed jointly with random forest (MisForest in R[51]) (**Supplementary Methods**). Post imputation, for individuals with diagnosed T2D at T2 (N=18), we corrected their potentially T2D medication-induced and thus artificially normal FG values to 7 mmol/l, HbA1c values to 48 mmol/l (6.5%) and 2hGluc values to 11.1 mmol/l. Detailed descriptions of all the exclusions and corrections are given in the **Supplementary Methods**. Finally, all the predictor variables were normalised using inverse-normal transformation. The epidemiological,

405 blood biochemical and metabolomic variables used in the predictions are listed in **Supplementary**
406 **Table 1**.
407
408 **Epigenomic Data in NFBC1966**
409 DNA methylation was measured in whole blood from 807 randomly selected individuals after
410 overnight fasting. At T2, DNA methylation was measured for 758 selected subjects that attended the
411 clinical examination, completed the questionnaire and had DNA methylation data from the previous
412 clinical examination available. IlluminaInfiniumHumanMethylation450 Beadchip and EPIC arrays were
413 used at T1 and T2, respectively. Methylation data was quality controlled according to study protocol
414 (**Supplementary Material**) and pre-processed on genome build CGCh37/hg19. Imputation of
415 methylation data was performed with random forest (MisForest in R[51]) using the methylation residuals
416 corrected for sex, and blood cell type (**Supplementary Material**). We limited our analysis to the
417 methylation probes previously associated with seven phenotypes: 187 probes associated with BMI[20],
418 21 with FG[18], 11 with HbA1c[18] and 68 with T2D[18], one with 2hGluc[19], eight with FI[19], 21 with 2hIns[19]
419 (**Supplementary Table 2**).
420
421 **Epidemiological, Blood Biochemical and Metabolomic Data in DESIR**
422 A total of 48 predictor variables overlapped between NFBC1966 and DESIR data. These included sex,
423 measures of adiposity, biochemical data (triglycerides, total cholesterol, high and low-density
424 lipoprotein cholesterol (HDL-C and LDL-C) at T1 and T2, insulin and glucose at T1), and metabolomic
425 data (32 variables, 17 at T1 and 15 at T2). A full list of the included variables is given in **Supplementary**
426 **Table 3**. The data were imputed with the package MisForest in R[51] (missingness rate < 1%)
427 (**Supplementary Material**). The values of FG were set to 7 mmol/l if the individual had diagnosed T2D.
428 Finally, all the predictor variables were normalised using inverse-normal transformation.
429
430

Individuals and Study Variables in the Machine Learning Models

In total, 513 individuals in the NFBC1966 were included in the machine learning analysis. HbA1c, 2hGluc, 2hIns, FG, FI levels were used as continuous outcomes to predict. A total of 1,001 variables from T1 and T2 were used as predictors in the NFBC1966. Metabolic predictors included: epidemiological data - sex, measures of adiposity (BMI and waist-to-hip ratio), SBP and DBP, biochemical data - ten blood measurements of triglycerides, total cholesterol, high and low-density lipoprotein cholesterol (HDL-C and LDL-C), metabolomic data - 454 metabolites (228 at T1 and 226 at T2) (**Supplementary Table 1**). The methylation dataset included 528 unique probes, including 264 at T1 and 264 at T2 (**Supplementary Table 2**).

For the replication analysis we chose to predict only FG and FI, for which the predictions worked the best in the NFBC1966 analyses. We included 48 variables common to both cohorts (**Supplementary Table 3**). The DESIR Cohort did not encompass methylation data. Therefore, before testing the prediction ability of our models in 769 individuals from DESIR, we could train the algorithms in the NFBC1966 either with the 513 individuals as previously, or with all 3,056 individuals who had metabolomics data available. This allowed us to test whether prediction in the external cohort was improved by increasing the sample size of the training data.

Predictor Combinations and Prediction Frameworks

Metabolic (Mb) and Methylation (Mh) data were combined as their individual (or raw, denoted here as R) values or transformed into scores (S) (**Supplementary Material**). Methylation scores were formed according to the traits the probes have been previously associated with (**Supplementary Table 2)** and metabolic scores according to specific categories (**Supplementary Table 4**). We refer to Mb-R/Mh-R when the input data are all represented as individual values and to Mb-S/Mh-S when all the input data are combined in scores. Sex was kept separate and included in the model in addition to the scored variables. The following combinations were tested: Mb-R/ Mb-S/ Mh-R / Mh-S/ Mb-R + Mh-R/ Mb-R +

Mh-S/ Mb-S + Mh-R/ Mb-S + Mh-S (**Figure 1**). Methylation and Metabolic data were either adjusted for BMI and waist-hip-ratio at T1 and T2, or kept unadjusted.

Machine Learning Approaches

Three machine learning methods were used for regression analysis: Boosted trees (BT), Random Forest (RF) and Support Vector Regression (SVR) (**Supplementary Material**). SVR was implemented with Linear Kernel with L2 regularization and with L1 and L1/L2 loss functions (SVR-L2Linear-L1, SVR-L2Linear-L1L2, respectively), with Polynomial Kernel (SVR-Polynomial) and with Radial Basis function Kernel (SVR-RBF) (**Figure 1**). For all the analyses, the packages kernelab, LIBLINEAR, RandomForest and xgboost in R[51] were used with Caret as a wrapper.

Optimization of the Machine Learning Algorithms

Nested cross validation was implemented. The data set was split into a training (80%) and testing set (20%) with a 5-fold cross validation. The performance of the machine learning models was estimated on the testing set, while parameter tuning was implemented on the training set by splitting it further into a 5-fold cross validation (nested). Random search method was used to find the model parameter combination (**Supplementary Table 13**) which minimized the error of the model. The Root Mean Square Error (RMSE) was used to assess model performance during training. Both Rsquared (R^2) and RMSE were computed in the testing set to estimate performance. For additional checks we used the Mean Absolute Error (MAE) to assess model performance.

Variable Importance in the Machine Learning Models

In BT, the information gain was used as a measure of importance. Gain is based on the decrease in entropy after a dataset is split on a feature j at a branch of the tree. RF variables were ranked with the Increase in Mean Square Error (MSE). It estimates the increase of prediction error when the values of

482 the feature j are randomly permuted. For SVR, each feature is evaluated based on its independent
483 association with the outcome. The slope of the regression is used to rank the features.
484
485 **Statistical Analysis for Comparing Model Performance**
486 The performance of each model was computed as the average R^2 over the 5 testing folds of the cross
487 validation. In the Results section, we report the R^2 pooled for the six machine learning algorithms.
488 Comparison of the models was performed with a one-way ANOVA and post-hoc Tukey honest
489 significance test (HSD) test. We use *P*-value<0.05 to denote statistical significance.
490
491 **Association Analysis to Obtain Effect Size, Direction and Variance Explained**
492 We performed linear regression analyses between the best predicted outcomes (FG and FI) and each
493 of the top 25 predictors suggested by the machine learning models to assess the effect sizes and
494 directions of the associations. The analyses were conducted in R[51]. We report betas with their standard
495 errors and related *P*-values. Additionally, we performed linear regression analyses for ln(FI)/FG by
496 including all the top 25 predictors in the same model and then by removing metabolites/ methylation
497 markers from the set of predictors to evaluate the variance explained and the contribution of
498 metabolites/methylation markers in it. We report the adjusted R^2 from these analyses.
499
500 **Replication analysis**
501 For the replication analysis we used the three most consistently performing machine learning
502 approaches: BT, RF, and SVR-L2Linear-L1L2. First, we estimated the performance of the algorithms in
503 the NFBC1966 using a restricted set of 48 predictors. We selected the 48 input variables as overlapping
504 with our validation cohort variables. This performance estimation was done by splitting the NFBC1966
505 dataset of 513 individuals into 80% for training and 20% for testing (nested cross validation for tuning
506 as described above). Second, we "re-trained" the model with the maximum number of individuals in
507 the NFBC1966, i.e. 513 individuals which represent 100% of the previous dataset with methylation and

metabolomics data, or all 3,056 individuals with metabolomics data only. Then we tested for the ability of this model to predict accurately glycaemic traits when given an independent population (DESIR) with the same input variables. Practically, we used either 513 or 3,056 individuals from the NFBC1966 for training and used the resulting models to predict FI and FG in 769 individuals of the DESIR cohort. Performance was evaluated with R2 as described above.

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Author contributions

IP conceived the study idea. IP, MK, LP, HD and ZB defined the phenotypes and quality control criteria. LP, HD, MDA, MW and MK performed the analyses. LY, BB, RR, SS, MAK, PF and MRJ provided the data. LP, MK and IP formed the central writing group. All authors participated in the critical revision of the manuscript and approved the final version.

Competing Interests

The authors declare no competing interests.

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671
672

673 **Figure legends**

674

675 **Figure 1. Experimental set-up for Machine learning analysis.** We applied machine learning to multi-
676 omics data based on blood samples and data collections from the Northern Finland Birth Cohort 1966
677 at 31 and 46 years. Fasting glucose/insulin (FG/FI), glycated haemoglobin (HbA1c) and 2-hour
678 glucose/insulin (2hGlu/2hIns) phenotypes at T2 were predicted in 513 individuals using up to 991
679 variables from T1 and T2: Body-mass-index (BMI), waist-hip-ratio, systolic and diastolic blood pressure
680 (SBP and DBP), sex; 10 blood plasma measurements; 453 NMR-based metabolites; 528 methylation
681 probes established for BMI, FG, FI, HbA1c, 2hGlu, 2hIns or Type 2 diabetes. Six machine learning
682 approaches were used: random forest, boosted trees and support vector regression (SVR) with the
683 kernels of linear function with L2 regularization and L1 loss function (L2Linear-L1), linear with L2
684 regularization and L1/L2 loss function (L2Linear-L1L2), polynomial and radial basis function (RBF).

685

686 **Figure 2. Performance as measured in R^2 of the different machine learning models.** A. Unadjusted for
687 measurements of adiposity (Waist-to-hip-ratio and Body mass index) at T1 and T2; B. Adjusted for
688 measurements of adiposity (Waist-to-hip-ratio and Body mass index) at T1 and T2. Training of the
689 algorithm was performed with a nested cross validation (5-folds outer, and 5-folds inner cross
690 validation) and the R^2 of 5 outer testing folds is displayed for each machine learning model. Metabolic
691 predictors include epidemiological, biochemical and metabolomic data. SVR: Support Vector
692 Regression with the kernels of linear function with L2 regularization and L1 loss function (L2Linear-L1),
693 linear with L2 regularization and L1/L2 loss function (L2Linear-L1L2), polynomial and radial basis
694 function (RBF).

695

696 **Figure 3. Variable Importance for fasting glucose and fasting insulin prediction from two different**
697 **combinations of predictor data.** For each Machine learning method, the normalized variable
698 importance over five outer fold of cross validation was averaged into the "Variable-Model-Importance"

699 (var.mod.Imp). Then for each of the six machine learning models, the variables were ranked based on
700 the var.mod.Imp. The rank was averaged over the six models to obtain the "mean variable rank". The
701 latter was used to select top 25 variables for display. For these variables, we display the variable
702 importance after (1) weighting the var.mod.Imp by the R^2 obtained for each of the individual machine
703 learning algorithms (2) averaging variable importance across the six machine learning models.
704 FI: Fasting Insulin; FG: Fasting Glucose; RF: random forest; BT: boosted trees, SVR: support vector
705 regression models with the kernels of linear function with L2 regularization and L1 loss function
706 (L2Linear-L1), linear with L2 regularization and L1/L2 loss function (L2Linear-L1L2), polynomial and
707 radial basis function (RBF). Metabolic predictors include epidemiological data, biochemical data and
708 metabolomic data. T2: 46 years old, T1: 31 years old. BMI: Body Mass Index according to clinical
709 examination, postal questionnaire if missing; WHR: Waist-to-hip ratio. Metabolite name descriptions
710 are provided in **Supplementary Table 1**.
711
712 **Figure 4. Performance as measured in R^2 of the different machine learning models with 48 variables**
713 **shared between NFBC1966 and DESIR.** A. Using NFBC1966 data with individuals having methylation
714 data (N=513) and all available DESIR individuals (N=769). B. Using NFBC1966 data with all available
715 individuals (N=3,056) and all available DESIR individuals (N=769). Training of the algorithm was
716 performed with a nested cross validation (5-folds outer, and 5-folds inner cross validation) and the R^2
717 of 5 outer testing folds is displayed for each machine learning model. Metabolic predictors include
718 epidemiological, biochemical and metabolomic data. SVR: Support Vector Regression with the kernel
719 of linear function with L2 regularization and L1/L2 loss function (L2Linear-L1L2).
720

721 **Tables**

722 **Table 1. Effect of the input dataset on the prediction performance of the five glycaemic traits.**

Comparison	Model	Input dataset				Number of outcome variables for which the model (A or B) performs the best
		Mb-R	Mb-S	Mh-R	Mh-S	
1	A	x				5/5, $P<2.3\times10^{-10}$
	B			x		0/5
2	A	x				5/5, $P<1.8\times10^{-11}$
	B				x	0/5
3	A		x			5/5, $P<1.7\times10^{-9}$
	B			x		0/5
4	A		x			5/5, $P<6.0\times10^{-14}$
	B				x	0/5
5	A	x				2/5, $P<0.04$ (equal performance for 3/5)
	B	x		x		0/5
6	A	x				0/5 (equal performance for all)
	B	x			x	0/5
7	A		x			0/5 (equal performance for all)
	B	x				0/5
8	A			x		1/5, $P<1.6\times10^{-4}$ (equal performance for 4/5)
	B				x	0/5
9	A		x		x	4/5, $P<0.02$ (equal performance for 1/5)
	B		x	x		0/5

723

724 Selected comparisons of models in pairs are displayed to illustrate different scenarios. 1-4) Comparison
725 between models with metabolic or methylation data only; 5-6) The effect of combining two data
726 types.; 7-8) The effect of variable transformations into scores; 9) The decrease in performance of the
727 models upon inclusion of a large number of predictors. Mb-R: Metabolic Raw Variables, Mb-S:
728 Metabolic Scored Variables, Mh-R: Methylation Raw Variables, Mh-S: Methylation Scored Variables.
729 Metabolic predictors include epidemiological data, biochemical data and metabolomic data. FG/FI:
730 Fasting glucose/insulin; HbA1c: glycated haemoglobin; 2hGlu/2hIns: 2-hour glucose/insulin.
731 The performance of all machine learning algorithms upon inclusion of different datatypes was
732 evaluated. For a given phenotype (FG, FI, HbA1c, 2hGlu or 2hIns), the effect of an input dataset was
733 assessed. Column 4 shows which model performed the best, and the number of outcomes for which
734 this pattern is observed. The *P*-value is the maximum *P*-value observed when comparing model A
735 against model B for each of the five outcomes. Models were compared with Tukey HSD test following

736 a one-way ANOVA. To test the effect of a given dataset, we run all six machine learning algorithms in
737 a nested cross validation framework (5 outer, 5 inner folds), thereby each group compared included
738 six (machine learning algorithms) x five (testing errors) = 30 R^2 measures.
739

740 **Table 2. Linear regression analysis results for ln(FI)/FG and the top 25 predictors.**

Mb-R + Mh-R	beta	SE	*P*-value	Mb-S + Mh-R	beta	SE	*P*-value
ln(FI)				**ln(FI)**			
T2_BMI	0.08	0.00	3.29×10^{-55}	T2_MeasuresOfAdiposity	0.08	0.00	5.75×10^{-56}
T2_Large_HDL_FreeChol_%	-0.11	0.01	1.48×10^{-40}	T2_BRACA	3.76	0.29	1.17×10^{-32}
T2_GlycoproteinAcetyls	1.32	0.10	5.88×10^{-37}	T1_MeasuresOfAdiposity	0.07	0.01	7.39×10^{-26}
T2_TrigyceridestoPhosphoglycerides	1.13	0.09	8.73×10^{-35}	T2_BloodProteins	1.32	0.10	3.36×10^{-37}
T2_WHR	3.79	0.27	3.12×10^{-38}	T2_Carbohydrates	0.73	0.06	1.62×10^{-26}
T2_Phenylalanine	29.08	2.32	1.62×10^{-31}	T2_cg00574958	-9.28	1.48	8.62×10^{-10}
T2_Large_HDL_FreeChol	-4.91	0.39	1.03×10^{-31}	T2_OtherAminoAcids	0.66	0.18	3.42×10^{-4}
T2_Large_HDL_TotChol_%	-0.04	0.00	2.04×10^{-34}	T2_cg06898549	3.01	0.63	2.54×10^{-6}
T2_Isoleucine	15.32	1.23	2.52×10^{-31}	T2_cg17058475	-4.67	1.12	3.45×10^{-5}
T2_Large_HDL_TotChol	-1.14	0.09	4.61×10^{-30}	T1_CarbohydratesAndInsulin	0.10	0.02	1.68×10^{-9}
T2_Leucine	13.13	1.07	1.09×10^{-30}	T2_cg08309687	-3.51	0.68	3.24×10^{-7}
T1_FI	0.80	0.07	1.19×10^{-27}	T2_KetonBodies	-0.70	0.29	1.52×10^{-2}
T2_Large_HDL_CholEsters	-1.47	0.12	1.86×10^{-29}	T2_cg10927968	3.01	0.64	3.23×10^{-6}
T2_Large_VLDL_PhosphoLipids	4.59	0.41	1.76×10^{-26}	T1_cg08309687	-2.44	0.63	1.10×10^{-4}
T2_Glycerol	11.03	0.94	3.94×10^{-28}	T2_Lipoparticules	0.00	0.00	4.99×10^{-9}
T2_VLDL_Trigycerides	0.40	0.03	6.03×10^{-28}	T1_cg26361535	1.76	0.70	1.29×10^{-2}
T2_Lactate	0.74	0.07	2.34×10^{-25}	T2_cg25217710	4.83	1.27	1.51×10^{-4}
T2_Tyrosine	20.79	1.87	5.98×10^{-26}	T1_cg11832534	3.30	1.13	3.54×10^{-3}
T2_XL_HDL_PhosphoLipids	-1.30	0.11	1.81×10^{-28}	T1_cg11024682	3.92	1.02	1.33×10^{-4}
T2_Large_HDL_Trigycerides_%	0.10	0.01	3.53×10^{-26}	T1_cg09469355	-3.98	0.93	2.11×10^{-5}
T2_XL_VLDL_Trigycerides	4.04	0.38	3.47×10^{-24}	T1_cg07728579	1.87	1.00	6.18×10^{-2}
T2_Valine	5.83	0.54	1.48×10^{-24}	T2_cg25096107	-3.36	1.40	1.68×10^{-2}
T2_Large_HDL_Particules	-375767.70	31515.32	4.35×10^{-29}	T2_cg26804423	4.02	1.10	2.96×10^{-4}
T2_XL_HDL_PhosphoLipids_%	-0.02	0.00	2.03×10^{-26}	T2_cg04524040	-3.21	0.89	3.59×10^{-4}
T2_Medium_VLDL_TotChol	2.45	0.22	6.10×10^{-26}	T1_BloodProteins	0.39	0.08	1.52×10^{-6}
FG				**FG**			

T2_Valine	7.43	0.56	7.64×10^{-35}	T2_BRACA	4.52	0.31	2.79×10^{-41}
T2_Leucine	15.38	1.12	1.26×10^{-36}	T2_MeasuresOfAdiposity	0.06	0.01	2.57×10^{-28}
T2_WHR	3.73	0.30	4.59×10^{-31}	T1_MeasuresOfAdiposity	0.06	0.01	2.56×10^{-14}
T2_BMI	0.06	0.01	8.60×10^{-28}	T2_BloodProteins	1.05	0.11	1.42×10^{-19}
T2_Isoleucine	16.56	1.33	2.53×10^{-31}	Sex	-0.41	0.05	1.37×10^{-14}
T2_Tyrosine	20.74	2.05	5.73×10^{-22}	T2_OtherAminoAcids	0.96	0.19	1.08×10^{-6}
T2_SystolicBloodPressure	0.01	0.00	2.82×10^{-20}	T1_BRACA	1.88	0.31	4.10×10^{-9}
T1_WHR	2.80	0.30	7.01×10^{-19}	T1_CarbohydratesAndInsulin	0.13	0.02	4.46×10^{-13}
T1_FG	0.48	0.05	1.85×10^{-18}	T2_cg11080651	-8.53	1.94	1.39×10^{-5}
T2_Large_HDL_PhosphoLipids_%	0.05	0.01	1.04×10^{-19}	T2_cg19695507	4.06	1.13	3.57×10^{-4}
T2_Large_HDL_FreeChol_%	-0.09	0.01	2.80×10^{-20}	T2_cg19693031	-2.79	0.73	1.53×10^{-4}
T2_Small_HDL_Trigycerides	10.63	1.52	7.58×10^{-12}	T2_Lipoparticules	0.00	0.00	9.06×10^{-8}
T2_DiastolicBloodPressure	0.02	0.00	1.00×10^{-16}	T2_cg26403843	2.07	0.60	6.44×10^{-4}
T2_Medium_VLDL_Trigycerides_%	0.03	0.00	7.61×10^{-12}	T2_cg25217710	4.17	1.38	2.58×10^{-3}
T2_TrigyceridestoPhosphoglycerides	0.88	0.10	1.77×10^{-17}	T2_cg09777883	2.20	1.09	4.32×10^{-2}
T2_Glycerol	8.74	1.08	4.71×10^{-15}	T1_cg04816311	1.75	0.67	8.93×10^{-3}
T2_Alanine	2.89	0.35	8.41×10^{-16}	T2_Carbohydrates	0.51	0.07	2.37×10^{-11}
T2_XL_HDL_Particules	-768070.10	98342.58	3.27×10^{-14}	T1_cg03497652	1.96	0.70	5.47×10^{-3}
T2_IDL_FreeChol_%	-0.18	0.02	2.22×10^{-15}	T1_cg11183227	3.85	1.10	5.01×10^{-4}
T2_Large_HDL_CholEsters	-1.15	0.14	3.18×10^{-15}	T1_cg23906191	4.99	1.89	8.39×10^{-3}
T2_XL_HDL_CholEsters_%	0.02	0.00	7.27×10^{-12}	T1_cg00574958	-4.21	1.64	1.06×10^{-2}
T2_Large_HDL_TotChol	-0.89	0.11	9.31×10^{-16}	T1_cg02059849	5.61	1.36	4.20×10^{-5}
T2_Medium_VLDL_CholEsters_%	-0.04	0.01	8.94×10^{-13}	T2_cg00634542	2.90	1.30	2.65×10^{-2}
Sex	-0.41	0.05	1.37×10^{-14}	T2_cg17058475	-3.86	1.22	1.60×10^{-3}
T2_Small_VLDL_Trigycerides_%	0.03	0.00	1.58×10^{-12}	T2_cg06898549	2.47	0.69	3.89×10^{-4}

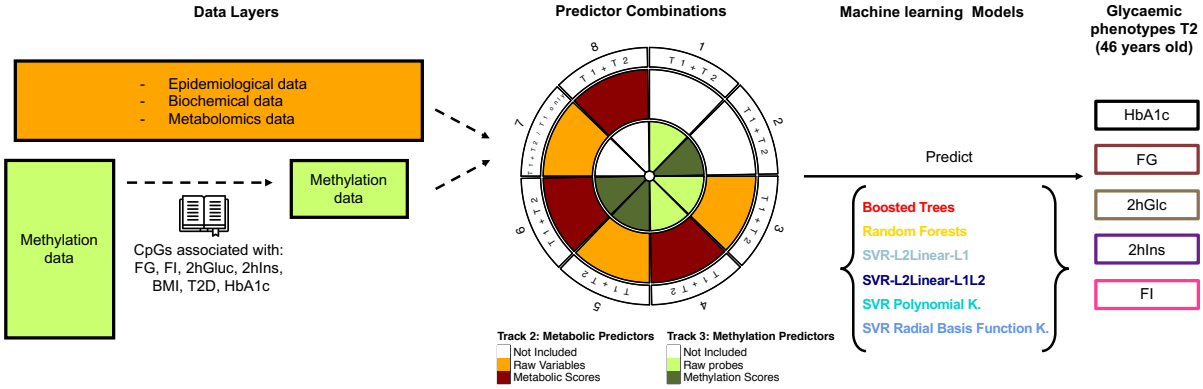
741 The models were fitted for each predictor separately. Left panel shows the results when the predictors were in their raw format (Metabolic-Raw, Mb-R +

742 Methylation-Raw, Mh-R) and right panel shows when the metabolic predictors were transformed into scores but methylation data kept in raw format

743 (Metabolic-Score, Mb-S + Methylation-Raw, Mh-R).

744 **Figures**

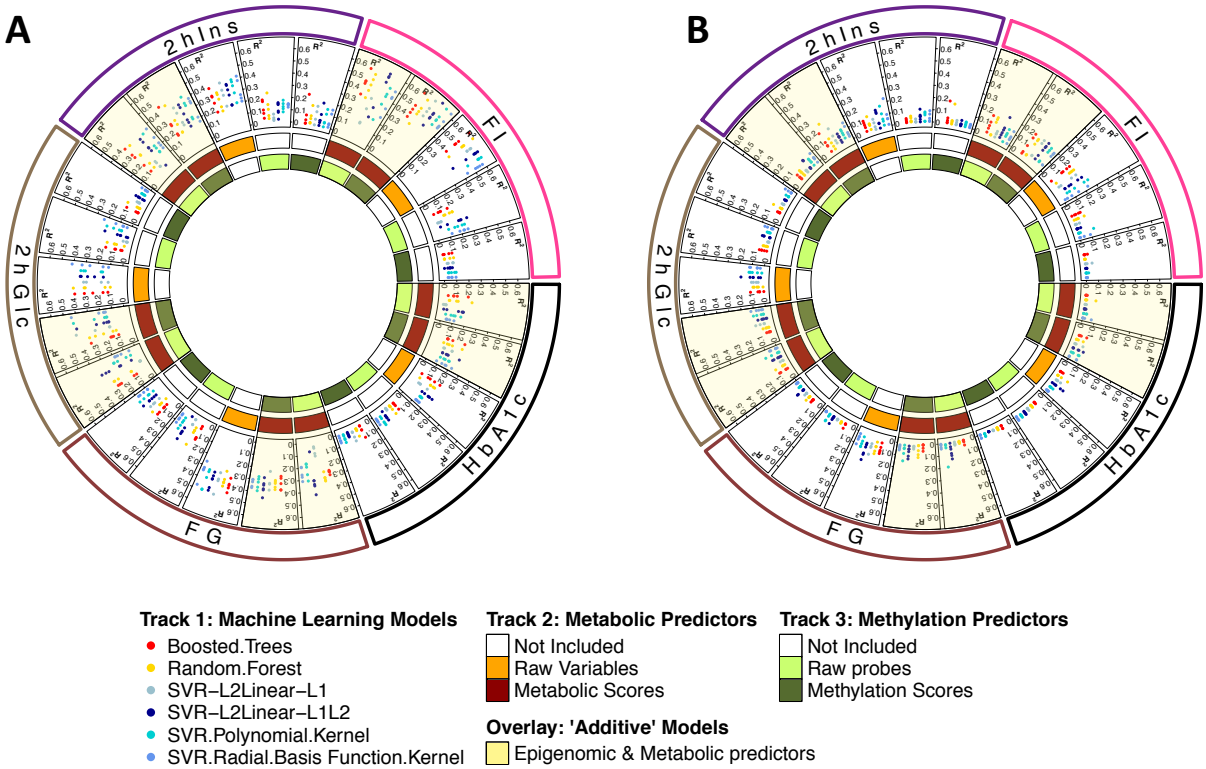
745 **Figure 1.**



746

747 **Figure 2.**

748



749

Figure 3.

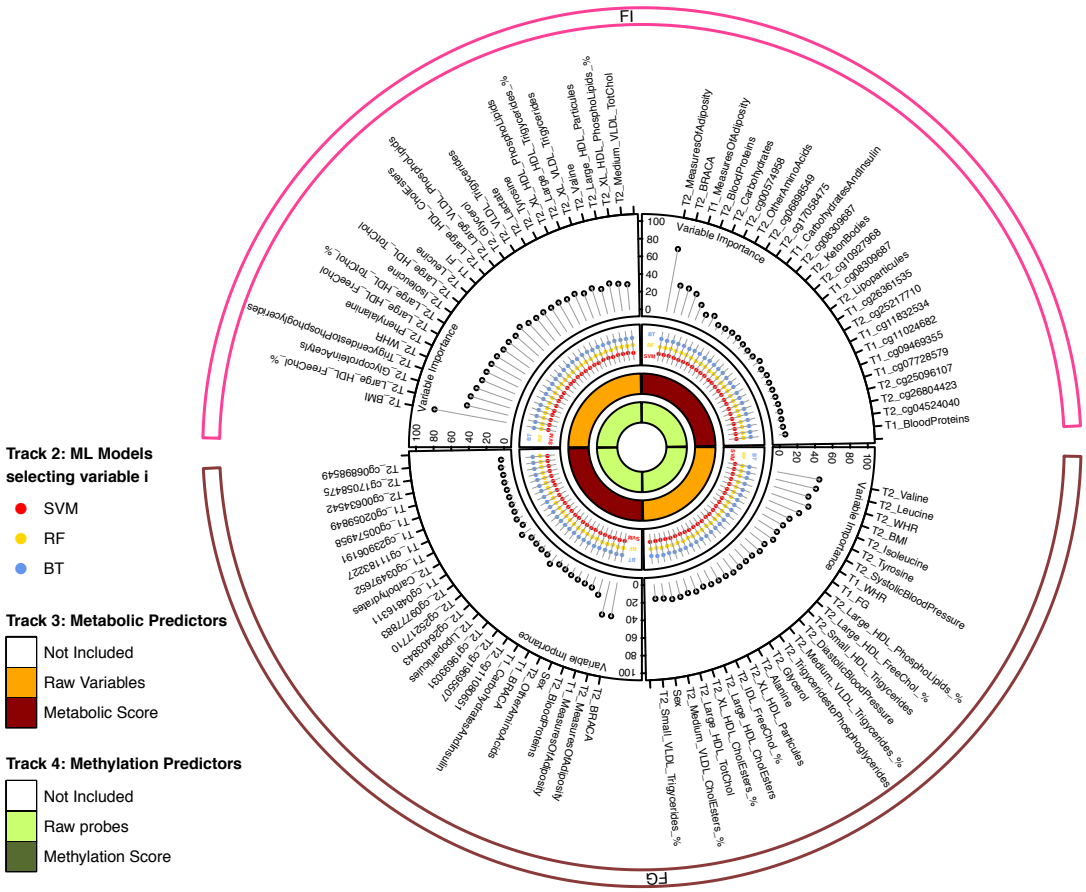


Figure 4.