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ARTICLE

Evaluating the discriminative power of multi-trait genetic risk scores for type 2 diabetes in a northern Swedish population

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Abstract
Aims/hypothesis We determined whether single nucleotide polymorphisms (SNPs) previously associated with diabetogenic traits improve the discriminative power of a type 2 diabetes genetic risk score.

Methods Participants (n=2,751) were genotyped for 73 SNPs previously associated with type 2 diabetes, fasting glucose/insulin concentrations, obesity or lipid levels, from which five genetic risk scores (one for each of the four traits and one combining all SNPs) were computed. Type 2 diabetes patients and non-diabetic controls (n=1,327/1,424) were identified using medical records in addition to an independent oral glucose tolerance test.
Results Model 1, including only SNPs associated with type 2 diabetes, had a discriminative power of 0.591 ($p<1.00\times10^{-20}$ vs null model) as estimated by the area under the receiver operator characteristic curve (ROC AUC). Model 2, including only fasting glucose/insulin SNPs, had a significantly higher discriminative power than the null model (ROC AUC 0.543; $p=9.38\times10^{-6}$ vs null model), but lower discriminative power than model 1 ($p=5.92\times10^{-5}$). Model 3, with only lipid-associated SNPs, had significantly higher discriminative power than the null model (ROC AUC 0.565; $p=1.44\times10^{-9}$) and was not statistically different from model 1 (p=0.083). The ROC AUC of model 4, which included only obesity SNPs, was 0.557 ($p=2.30\times10^{-7}$ vs null model) and smaller than model 1 (p=0.025). Finally, the model including all SNPs yielded a significant improvement in discriminative power compared with the null model ($p<1.0\times10^{-20}$) and model 1 ($p=1.32\times10^{-5}$); its ROC AUC was 0.626.
Conclusions/interpretation Adding SNPs previously associated with fasting glucose, insulin, lipids or obesity to a genetic risk score for type 2 diabetes significantly increases the power to discriminate between people with and without clinically manifest type 2 diabetes compared with a model including only conventional type 2 diabetes loci.

Keywords Discriminative power · Genetic risk score · Glucose · Insulin · Lipids · Obesity · Polymorphism · Predictive power · Type 2 diabetes

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Abbreviations

DIAGRAM	Diabetes Genetics Replication and Meta-Analysis
GRS	Genetic risk score
NSHDS	Northern Sweden Health and Disease Study
ROC AUC	Area under the receiver operator characteristic curve
SNP	Single nucleotide polymorphism

Introduction

Type 2 diabetes is a complex disease characterised by chronically elevated fasting or post-challenge systemic glucose concentrations [1]. Heritability studies suggest that genetic factors influence the risk of developing the disease. Indeed, multiple loci pre-disposing to type 2 diabetes have been discovered recently, many of which have emerged from genome-wide association studies [2].

Several studies have examined the clinical value of variants known to predispose to type 2 diabetes by analysing their ability to discriminate between people with or without pre-existing diabetes, or to predict development of the disease [3–5]. Although opinion is divided on the clinical value of these genetic risk scores (GRS), in their present form they do not meaningfully improve the predictive power over risk scores comprised solely of established non-genetic risk factors [3–5].

As with type 2 diabetes, major advances have also been made in identifying gene variants that influence some of the major risk factors for type 2 diabetes, e.g. chronic obesity, dyslipidaemia and hyperglycaemia. Indeed, we have previously studied the level of type 2 diabetes risk associated with several of these loci in the Northern Sweden Health and Disease Study (NSHDS) [6, 7]. However, to our knowledge, the discriminative or predictive power of multi-trait GRSs for type 2 diabetes have not yet been reported on.

The purpose of this study was to test whether gene variants that are not explicitly defined as loci predisposing to type 2 diabetes, but have been shown to influence antecedent traits (i.e. hyperglycaemia, hyperinsulinaemia, dyslipidaemia or obesity) can be used to improve the discriminative power of a GRS for type 2 diabetes compared with a score comprised solely of specific type 2 diabetes loci. We did not seek to establish the comparative power of this score with non-GRSs for type 2 diabetes, in part because cross-sectional studies are inadequate for this purpose.

Methods

Participants Participants (effective n=1,327 type 2 diabetic patients, 1,424 controls) were Swedish adults from the county of Västerbotten in northern Sweden, and selected from the NSHDS, a prospective cohort study of common diseases [8]. All living participants provided written informed consent and the Research Ethics Committee of Umeå University Hospital approved all aspects of the study.

Ascertainment of type 2 diabetes cases and controls The case ascertainment methods have been described in detail previously [7]. In brief, cases were those participants with a documented clinical history of type 2 diabetes in addition to an independent OGTT result consistent with a diagnosis of type 2 diabetes, according to the WHO criteria [1]. Conversely, controls were those participants who did not have a documented clinical diagnosis of diabetes (of any type), were not taking glucose-lowering medications, and who had fasting and 2 h glucose values below the diagnostic thresholds for diabetes [1].

Clinical measures The clinical methods have been described in detail previously [8]. Briefly, height, weight, glucose concentrations and lipid fractions were measured using standard methods (Table 1). The purpose of providing this information is to emphasise that type 2 diabetic patients and controls differed significantly in levels of the traits related to the single nucleotide polymorphisms (SNPs) focused on in this study. Blood was drawn after an overnight fast from an antecubital vein; a second sample was drawn 2 h after a 75 g oral glucose load.

Selection of SNPs and genetic analyses The type 2 diabetes and lipid SNPs are those for which replication results were in the public domain as of May 2008 (Fig. 1a–d). Additional obesity and fasting glucose/insulin SNPs were identified through participation in the Genetic Investigation of ANthropometric Traits consortium [9, 10] and the Meta-Analyses of Glucose and Insulin-related traits Consortium [11], respectively. Thus, because of the timing of genotyping relative to progress in the field, and to a limited extent because of assay design limitations, several previously replicated SNPs could not be included.

DNA was extracted from peripheral white blood cells [6, 7]. Genomic DNA samples were subsequently diluted to 4 ng/μl. Genotyping was performed using Taqman MGB chemistry (Applied Biosystems, Foster City, CA, USA) or Sequenom iPLEX (Sequenom, Hamburg, Germany). Genotyping success rates were >95%.

Statistical analysis Analyses were conducted in SAS version 9.2 (SAS Institute, Cary, NC, USA). A likelihood ratio test with 1 *df* was used to test Hardy–Weinberg equilibrium (all SNPs fulfilled Hardy–Weinberg expectations; Bonferroni corrected p>0.05). SNPs were individ-

Table 1 Participant characteristics stratified by case and control status

Variable	Non-diabetes controls		Type 2 diabetes cases		*p* value for difference
	n	Mean (SE)	*n*	Mean (SE)	
Age (years)	1,424	53.1 (0.2)	1,327	53.6 (0.2)	NS
Sex[a] (*n*)	715/709		775/552		<0.0001
BMI (kg/m^2)	1,423	25.8 (0.1)	1,327	29.5 (0.1)	<0.0001
Fasting glucose (mmol/l)	1,417	5.27 (0.02)	1,305	8.04 (0.09)	<0.0001
2 h glucose (mmol/l)	1,380	6.55 (0.04)	805	10.48 (0.15)	<0.0001
Total cholesterol (mmol/l)	1,413	5.99 (0.03)	1,312	6.12 (0.04)	0.0048
Triacylglycerol (mmol/l)	945	1.63 (0.02)	935	2.36 (0.05)	<0.0001

[a] Male/Female

Test of difference of means between groups (*p* for difference) was performed with an independent samples *t* test; for sex distributions, between-group differences were tested using the Mantel–Haenszel χ^2 statistic

ually tested (additive SNP models) for association with type 2 diabetes using unconditional logistic regression from which ORs and 95% CI were estimated (Fig. 1a–d). In the discriminative power comparison models, effect alleles for all SNPs are coded in a manner consistent with the Diabetes Genetics Replication and Meta-Analysis (DIAGRAM) database [12] or the findings from MAGIC [11]. Regression models were adjusted for age and sex. In an ethnically homogeneous population such as this, biological traits such as obesity and dyslipidaemia are unlikely to confound the effects of gene variants on diabetes risk, once age has been accounted for. Therefore, because we sought to exploit such effects, no adjustments for intermediate diabetes risk factors were made. The basic genetic model (model 1) included 17 variants previously associated with type 2 diabetes. Comparison models included previously associated fasting glucose/insulin (n=13) (model 2), lipid (n=26) (model 3) [13] or obesity (n=17) (model 4) [9, 10, 14, 15] SNPs. Finally, a model containing all 73 SNPs (model 5) was compared with the null model and with model 1. The discriminative power of the five different SNP models was estimated by comparing the area under the receiver operator characteristic curves (ROC AUC) for each model. Because the majority of SNPs studied here are in low linkage disequilibrium, we were unable to accurately impute missing genotypes using methods based on linkage disequilibrium. Therefore, we calculated the mean genotype at each locus in cases and controls separately, and exchanged missing genotypes for the relevant mean value for that SNP. Alleles were rounded to the nearest whole unit. Prior to imputing genotypes, we tested whether genotyping failure rates differed between the type 2 diabetes group and controls, as this could have biased tests of association using imputed data. There was no evidence of such selection bias (association of missing genotypes with diabetes: OR 1.00, 95% CI 0.98–1.01). ROC AUCs were compared using the methods described by DeLong et al. [16]. In these analyses, the null model included no predictor variables. Prior to entering the SNPs into the ROC models, we ensured each risk allele was coded in a manner consistent with the DIAGRAM database [12] and used the relevant random effects ORs from this dataset to derive weightings for each risk allele. This was achieved by multiplying each risk allele by the log of its OR in the DIAGRAM dataset. Four SNPs were unavailable in DIAGRAM. In these cases, we used the average effect estimate for the SNPs within the relevant trait group (i.e. diabetes, glucose, lipid or obesity SNPs). The GRSs were computed by summing the weighted risk alleles across all loci for each trait (or for the full model for all traits). Overall, weighting SNP models did not materially alter the discriminative power compared with the unweighted models.

Results

Participant characteristics are shown in Table 1. Figure 1a–d shows ORs (95% CIs) for each of the 73 SNPs. In general, the risk estimates in this cohort for SNPs previously associated with type 2 diabetes were directionally consistent with previous reports. As shown in Fig. 1a–d, few SNPs were individually statistically associated with type 2 diabetes (at p<0.05).

Figure 2 shows the relationships between each GRS (expressed in quartiles of the GRS) and type 2 diabetes risk. For each of the GRSs, statistically significant relationships with type 2 diabetes risk were observed (p<0.05). The odds of type 2 diabetes per quartile of the score was: for the type 2 diabetes GRS OR 1.25 (95% CI 1.17–1.34); for the glucose GRS OR 1.08 (95% CI 1.01–1.15); for the lipid GRS OR 1.07 (95% CI 1.00–1.14); for the obesity GRS OR 1.14 (95% CI 1.07–1.22); and for the full GRS OR 1.33 (95% CI 1.24–1.43). With the exceptions of the glucose and lipid SNP GRSs (p=0.09 and p=0.06, respectively), individuals in the highest quartile of each GRS were at statistically greater risk of type 2 diabetes than those in the first quartile. For example, those in the highest quartile of the full GRS had a 2.40-fold higher odds of type 2 diabetes

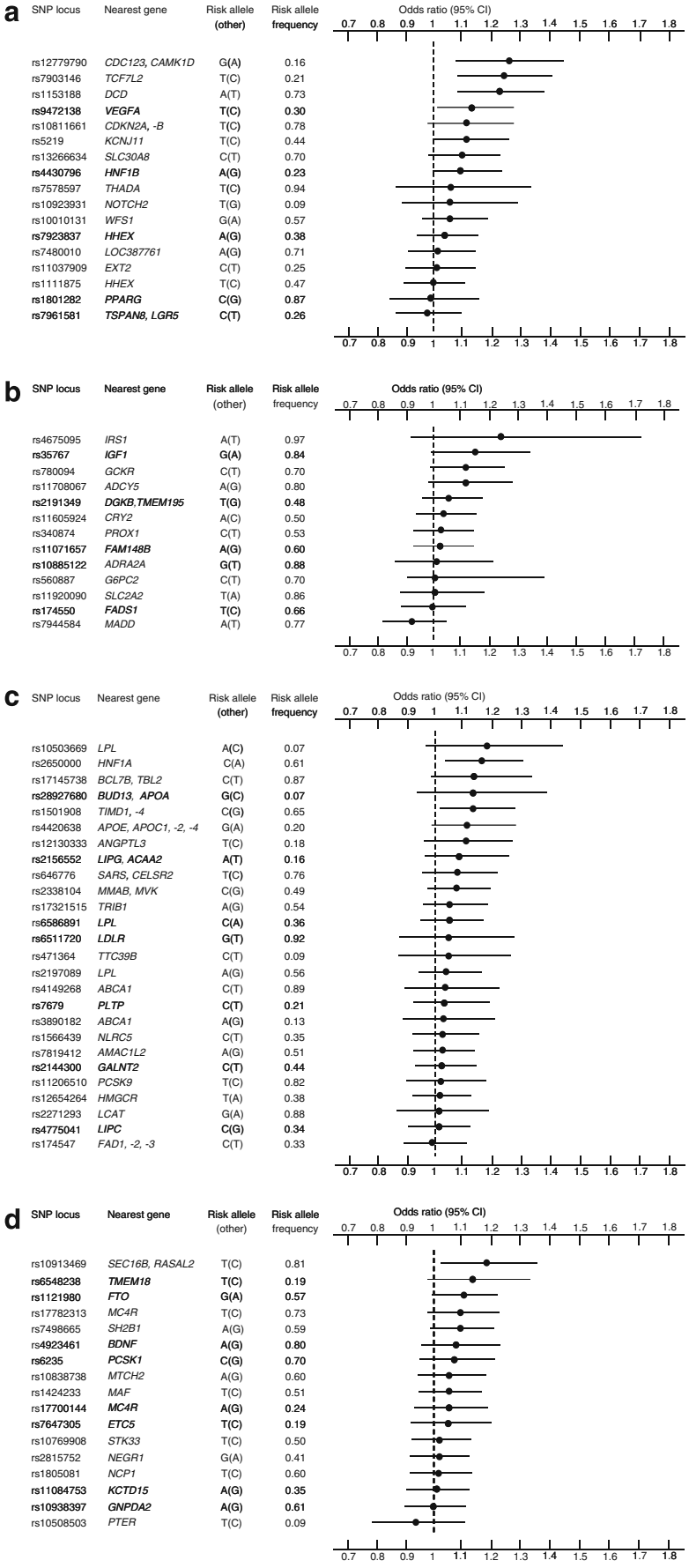

Fig. 1 Individual odds ratios (95% CIs) for type 2 diabetes for each of the **a** type 2 diabetes (n=17), **b** fasting glucose/insulin (n=13), **c** dyslipidaemia (n=26) and **d** obesity (n=17) SNPs included in these analyses (n=73). Allele frequencies were calculated in the control group. All SNPs are located on the plus strand (HapMap CEU, Phase II +III, release 27, NCBI build 36). *LOC287761* is discontinued, but was included here as it was documented as a replicated locus when this study began. Data were adjusted for age and sex. Odds ratios between the *WFS1* rs10010131 and most obesity SNPs with type 2 diabetes have been previously reported for this sample (7, 8). The association between the *TCF7L2* SNP and type 2 diabetes has been previously reported for a sub-sample of the case–control cohort examined here [35]

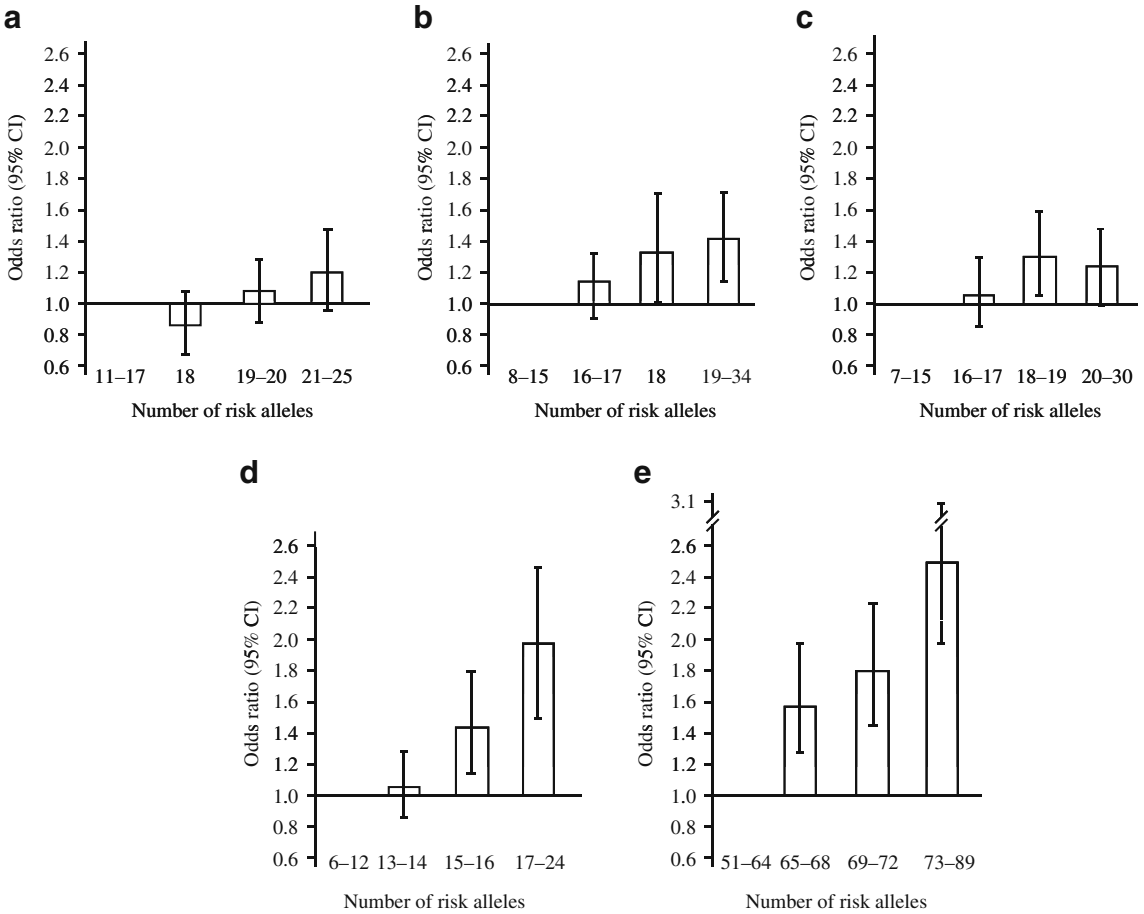

Fig. 2 Odds ratios (95% CI) for type 2 diabetes relative to the number of risk alleles across 73 SNP loci. **a** Glucose and insulin SNPs, **b** obesity SNPs, **c** lipid SNPs, **d** type 2 diabetes SNPs and **e** all SNPs. Data are adjusted for age and sex. Missing genotypes were imputed as described in the Methods. Type 2 diabetes patients, $n=1{,}327$, controls, $n=1{,}424$

than those in the lowest quartile ($p=3.50\times10^{-16}$); for the type 2 diabetes GRS, the respective odds of type 2 diabetes was 1.96 ($p=3.42\times10^{-8}$).

Five separate ROC models were run to compare the discriminative power of the different SNP sets. Model 1, including only type 2 diabetes-associated SNPs, had a discriminative power of 0.591 ($p<1.00\times10^{-20}$ vs null model) as estimated by the ROC AUC. Model 2, including only fasting glucose/insulin SNPs, had significantly higher discriminative power than the null model (ROC AUC 0.543; $p=9.38\times10^{-6}$ vs null model), but lower discriminative power than model 1 ($p=5.92\times10^{-5}$ vs model 1). Model 3, with only lipid-associated SNPs, had significantly higher discriminative power than the null model (ROC AUC 0.565; $p=1.44\times10^{-9}$) and was not statistically different from model 1 ($p=0.083$). The ROC AUC of model 4, which included only obesity SNPs, was 0.557 ($p=2.30\times10^{-7}$ vs null model), which was smaller than model 1 ($p=0.025$). Finally, the model including all SNPs yielded a significant improvement in discriminative power compared with the null model ($p<1.0\times10^{-20}$) and model 1 ($p=1.32\times10^{-5}$); its ROC AUC was 0.626. Figure 3 shows the ROC AUCs for all SNPs compared with only conventional type 2 diabetes SNPs.

Discussion

Our findings show that inclusion of genetic information from loci previously associated with quantitative risk factors for type 2 diabetes, but not primarily with diabetes, significantly increases the power to discriminate between people with and without clinically manifest type 2 diabetes. This emphasises the multi-factorial nature of type 2 diabetes and highlights the important potential role in disease development played by loci that do not reach a level of genome-wide significance in type 2 diabetes scans.

Our study was based on the premise that some loci capable of influencing diabetes risk and thus contributing to the discriminative power of type 2 diabetes GRSs have weak effects on type 2 diabetes individually, falling, as a result, below the stringent significance thresholds used in

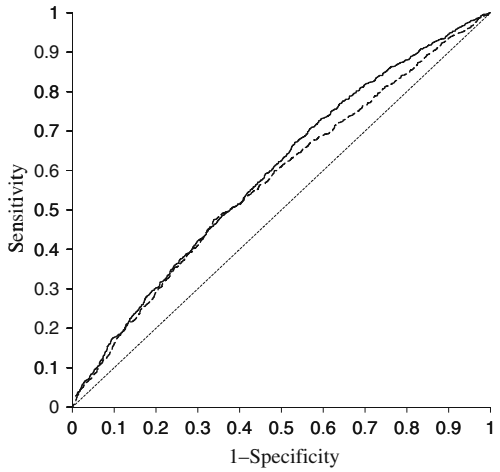

Fig. 3 Power to discriminate between type 2 diabetes cases and controls for GRSs comprised of type 2 diabetes variants (dashed line) or all variants (solid line) expressed as ROC AUCs. ROC AUCs for type 2 diabetes loci and for all loci are 0.591 and 0.626, respectively ($p=1.32\times10^{-5}$ for difference). Data are unadjusted

genome-wide scans, which means they have not previously been identified as diabetes-predisposing loci. We hypothesised that some of the loci reliably associated with traits that predispose to type 2 diabetes might, by virtue of this association, also raise the risk of type 2 diabetes. Hyperglycaemia is the cardinal feature of type 2 diabetes, providing sufficient justification for including glucose-raising alleles in a GRS for type 2 diabetes. However, it is worth noting that not all glucose-raising loci appear to influence type 2 diabetes risk [11], possibly because some loci may cause modest elevations in glucose concentrations that do not worsen over time, as observed in maturity-onset diabetes of the young [17]. Obesity is also a well established risk factor for diabetes, as illustrated in clinical trials where weight loss interventions have substantially reduced the incidence of the disease in high risk individuals [18, 19]. For dyslipidaemia, the mechanisms of association with type 2 diabetes primarily involve insulin resistance caused by the infiltration of insulin-sensitive tissues by triacylglycerol and other lipid metabolites [20, 21]. Two important organs in this regard are muscle and liver, the former being important because of its predominance as a site for glucose uptake and metabolism, and the latter because of its major role in glucose production. Studies in non-obese individuals with a strong family history of type 2 diabetes have provided experimental evidence that elevations in NEFA directly impair muscle glycogen synthesis and glucose uptake, and induce muscle, hepatic and adipose tissue insulin resistance in a genetically determined manner [3]. Prospective epidemiological studies indicate that dyslipidaemia early in life [22, 23] or during adulthood [24] raises the risk of developing type 2 diabetes later in life, but such associations may be driven by obesity [22] rather than a lipid-specific genetic defect. Nevertheless, animal and human studies suggest a shared genetic basis for diabetes and dyslipidaemia. For example, expression of the HDL-associated apolipoprotein M is completely abolished in the liver of mice lacking the *HNF1A* gene [25]; mutations in *HNF1A* also cause maturity-onset diabetes of the young class 3 [26]. Epidemiological studies have also identified genetic loci that influence dyslipidaemia and glucose homeostasis or type 2 diabetes [25, 27–30]. Although these joint relationships are unlikely to result from confounding, it remains unclear whether they reflect causal relationships between dyslipidaemia and diabetes, or pure genetic pleiotropy. Similarly, one cannot easily determine whether the cumulative association between lipid loci and diabetes in the present study is attributable to (1) dyslipidaemia mediating the effects of the genotypes on diabetes risk; (2) purely pleiotropic effects; or (3) a combination of these explanations. Notwithstanding these limitations of interpretation, the use of a priori biological information to help filter genome-wide scan results minimises the multiple testing burden inherent in hypothesis-free whole-genome genetic association studies and may raise the prior probability of association, hence helping to preserve statistical power.

To minimise over-fitting of our models, prior evidence of association from the DIAGRAM dataset [12] was used to code the effect alleles in the ROC analyses presented here. Fitting the alleles in this way did not result in markedly different ROC AUCs than when alleles were fitted directly to the current dataset, indicating that our data are unlikely to be markedly over- or under-fitted. We were unable to include all currently identified risk alleles for the traits of interest, partly because the rate at which new risk variants have been discovered out-paced our study and partly because resources were limited. Although initially presumed otherwise [31], it is unlikely that *LOC387761* is a true diabetes locus and could thus have been excluded from our models without diminishing the discriminative power. It is also important to highlight that there are many other antecedent traits for type 2 diabetes beyond those studied here, e.g. HbA_{1c}, fibrinogen and adiponectin; if variants associated with such traits were to be included in a GRS, the discriminative power would probably increase further.

The derivation of GRSs using the approach applied here requires complete genotype data in the population in which the score is computed. Because the genotype success rates were less than perfect in our study (as in virtually all studies) and genotyping failures were randomly distributed across the selection of SNPs in this cohort, it was necessary and appropriate to impute missing genotypes. The alternative would have been to use a sample set in which directly genotyped data were available for all SNPs. However, because missing genotype data were random across the

study sample, around half of all participants were missing data on at least one of the 73 SNPs. Thus, use of only the complete directly genotyped subgroups would have resulted in a considerable loss of statistical power and could have led to biased conclusions about the magnitude of association for the GRSs.

A further consideration is whether our findings are likely to be attributable to confounding. With the exception of linkage disequilibrium between the non-functional observed and functional unobserved loci, statistical associations between germline genetic variants such as SNPs and phenotypes are generally robust to confounding in ethnically homogeneous cohorts such as that studied here. Therefore, the associations reported here are unlikely to be prone to confounding.

Our study is clearly a hypothesis-generating effort and robust type 2 diabetes effect sizes for most of the GRSs of interest in this report are absent from the published literature. As such, meaningful a priori power calculations could not be performed for this study and post-hoc power calculations would be inappropriate, as discussed at length elsewhere [32–34]. The fact that most of the associations reported for the GRS models are highly statistically significant indicates that our study was well powered to detect the observed effects (which is a circular argument and one important reason why post hoc power calculations are often discouraged).

Finally, owing to the cross-sectional study design, we were unable to calculate the reclassification index attributable to the different genetic models, which would be valuable when considering a possible clinical application. One should also consider that in cross-sectional studies, in which cases and controls are phenotypically highly distinct, estimates of discriminative power may exceed estimates of predictive power derived from prospective studies.

In conclusion, polymorphisms that affect diabetogenic traits, but which are not conventionally considered to be diabetes-predisposing loci, significantly improve the discriminative power of a conventional GRS for type 2 diabetes. This is the case even though, on an individual basis, most variants have weak effects that were not statistically associated with type 2 diabetes in our study. Nevertheless, the discriminative power of the GRS remains below a level many would consider clinically useful; thus, validated non-genetic prediction algorithms remain the most appropriate tools for predicting type 2 diabetes in the clinical setting.

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Duality of interest I. Barroso owns stock in Incyte and GlaxoSmithkline. All other authors declare that there is no duality of interest associated with this manuscript.

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