Genetic predisposition to long-term non-diabetic deteriorations in glucose homeostasis: ten-year follow-up of the GLACIER Study

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Abbreviations:

GLACIER, Gene x Lifestyle interactions And Complex traits Involved in Elevated disease Risk

IFG, Impaired fasting glucose

ROC AUC, Area under the receiver operator characteristic curve

SNP, Single Nucleotide Polymorphism
Abstract

Aims/hypothesis: To assess whether recently discovered genetic loci associated with hyperglycemia also predict long-term changes in glycemic traits.

Methods: Sixteen fasting glucose-raising loci were genotyped in middle-aged adults from the GLACIER Study, a population-based prospective cohort study from Northern Sweden. Genotypes were tested for association with baseline fasting and 2-hr post-challenge glycemia (N=16,398), and with change in glycemic traits during a 10 year follow-up period (N=4,059).

Results: Cross-sectional directionally consistent replication with fasting glucose concentrations was achieved for 12/16 variants; nine variants also associated with impaired fasting glucose (IFG) and seven were independently associated with 2-hr post-challenge glucose concentrations. In prospective analyses corrected for multiple testing, the effect alleles at four loci (GCK rs4607517, ADRA2A rs10885122, DGKB-TMEM195 rs2191349, G6PC2 rs560887) were statistically associated with worsening fasting glucose concentrations during 10-years follow-up. MTNR1B rs10830963, which was predictive of elevated fasting glucose concentrations in cross-sectional analyses, was associated with a protective effect on post-challenge glucose concentrations during follow-up; however, this was only when baseline fasting and 2-hr glucoses were adjusted for. An additive effect of multiple risk alleles on glycemic traits was observed: a weighted genetic risk score (80th vs. 20th centiles) was associated with a 0.16mmol/l (P=2.4x10^-6) greater elevation in fasting glucose and a 64% (95% CI:33-201%) higher risk of developing IFG during 10-years follow-up.
Conclusions: Our findings imply that genetic profiling might facilitate the early detection of persons who are genetically susceptible to deteriorating glucose control; studies of incident type 2 diabetes and discrete cardiovascular endpoints will help establish whether the magnitude of these changes is clinically relevant.
Glucose homeostasis in healthy individuals is tightly controlled through a complex pathway of regulatory mechanisms involving multiple organs and tissues. Chronic elevations in fasting and post-challenge blood glucose concentrations are the cardinal feature of type 2 diabetes mellitus. Type 2 diabetes and non-diabetic variations in fasting and post-challenge glucose levels are highly heritable (1; 2); in recent years, the identities of several loci underpinning these heritability estimates have been revealed (3; 4). Because a person’s genotype remains unchanged throughout life, variants that predict deterioration in glucose levels later in life might be used to identify susceptible individuals for early intervention long before changes in glucose concentrations are visible.

Although the cross-sectional relationships between multiple fasting (3) and post-challenge (3; 4) glucose-raising loci have been recently confirmed in large cohort collections, it is unclear whether these loci predict long-term deteriorations in glucose homeostasis. Several cross-sectional studies in European whites or Chinese Han have identified variants within or near one of seven genes (TCF7L2, MTNR1B, GCK, GCKR, G6PC2, DGKB-TMEM195, and SLC30A8) that raise fasting glucose concentrations and/or risk of IFG (5-17). In the most recent large-scale effort to discover new fasting glucose related loci, the MAGIC investigators identified a further nine predisposing loci (rs11708067 (ADCY5), rs7944584 (MADD), rs10885122 (ADRA2A), rs174550 (FADS1), rs11605924 (CRY2), rs11920090 (SLC2A2), rs7034200 (GLIS3), rs340874 (PROX1), and rs11071657 (C2CD4B)) in European whites. To our knowledge, the existing longitudinal epidemiological studies in which genetic predictors of change in fasting glucose
The extent to which the accumulation of risk alleles influences fasting and post-challenge glucose levels is also unclear.

The purpose of the present study was to examine the cross-sectional and longitudinal relationships between previously associated fasting glucose-raising variants, singly and in combination, with fasting and post-challenge glucose concentrations in the GLACIER Study, a population-based prospective cohort study of adults from Northern Sweden.

Methods

Participants

The GLACIER Study is a prospective, population-based cohort study comprising 19,547 adults from the Northern Swedish county of Västerbotten, nested within the Northern Sweden Health and Disease Study (20). Participants were excluded from the current analyses if they reported a physician diagnosis of diabetes at baseline or follow-up, had measured glucose concentrations below 1 mmol/l or above the World Health Organization (WHO) diagnostic thresholds for type 2 diabetes (21), or if >50% of the fasting glucose genotypes were missing. Thus, the maximum number of individuals included in the baseline analyses ranged from 15,483 (for 2-hr glucose models) to 16,398 (for fasting glucose models). All GLACIER participants underwent detailed health and
lifestyle examinations as part of the Västerbotten Intervention Programme, an ongoing population-based prospective cohort study focused on type 2 diabetes, cardiovascular disease, and common cancers (20; 22). Since 1985, all residents of the county of Västerbotten have been invited to visit their primary care centre for a clinical examination within the year of their 40th, 50th, and/or 60th birthday. The protocol is standardized across study centers and conducted by trained research nurses. Baseline examinations for GLACIER participants were undertaken from 1985 through 2004. Of the 16,398 participants free from diabetes and other major chronic diseases at baseline, 4,059 had undergone a 10-year follow-up examination between January 1995 and December 2007 and were included in the longitudinal analyses. The follow-up examination was identical to the baseline examination with the exceptions that waist circumference was measured and modifications had been made to questionnaires on diet and physical activity. All participants gave written informed consent and The Regional Ethical Review Board in Umeå approved all aspects of the study.

Clinical measures

The clinical methods have been described in detail previously (20; 22). Briefly, weight (to the nearest 0.1 kg) and height (to the nearest 1 cm) were measured with a calibrated balance-beam scale and a wall mounted stadiometer, respectively, with participants wearing indoor clothing without shoes. BMI was calculated as weight in kg divided by height in meters squared (kg/m²). Systolic and diastolic blood pressures were measured twice using a mercury-gauge sphygmanometer with the participant seated; the means of the two measurements were used in analyses. Capillary blood was drawn following an
overnight fast and a second sample was drawn 2 hours following a standard 75g oral glucose load (21). Capillary plasma glucose concentrations were measured with a Hemocue bench-top analyzer (Quest Diagnostics, Madison, NJ). Prior to the first blood draw, 86% of the cohort had fasted for a minimum of 8 h, ~3% had fasted for between 4-8 h, and data were missing for ~11%. Sensitivity analyses (associations of SNPs with glucose levels) that included all individuals vs. only those who had fasted > 8 h did not yield materially different results. However, to control for any residual effect that fasting time might have a variable for fasting time was included in the analyses (>8 h vs. 4-8 h vs. unknown fasting duration).

SNP selection and genotyping

Fasting glucose SNPs were identified through participation in the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) (3). The GLACIER Study was not included in the original MAGIC paper (3). DNA was extracted from peripheral white blood cells (23; 24) and genomic DNA samples were diluted to 4 ng/µl. Genotyping was performed using OpenArray™ SNP Genotyping System (BioTrove, Woburn, MA). One in ten GLACIER samples was genotyped in duplicate, in which genotyping concordance was >99%. The genotype success rate was >96% for all SNPs and no departures from Hardy-Weinberg Equilibrium were observed (P > 0.001).

Genetic Risk Score (GRS)

The effect of multiple genetic risk loci on the glycemic traits was studied by constructing two different types of genetic risk score (GRS) for each study participant. The first
assumed an equal magnitude of effect for each risk allele and was generated for each participant by summing the number of risk alleles at each of the 16 SNP loci. Thus, because these are all biallelic loci, the GRS has a maximum possible value of 32 and a minimum possible value of 0. To calculate the second GRS, we used published effect sizes for each SNP (3) to weight the contribution of each risk allele. The weighted alleles were subsequently summed into a single score (wGRS). The maximum value for the wGRS for fasting and post-challenge glucoses are 0.948 and 1.548, respectively (i.e., 32 risk alleles x the relevant beta-coefficient for each allele). To facilitate the interpretation of the results, each individual’s wGRS was divided by the maximum possible wGRS and multiplied by 32 (the maximal number of risk alleles) (25). The purpose of undertaking this last step is to create a variable which is expressed on the same scale as the unweighted GRS, thus facilitating comparisons between these two scores. Missing genotypes were imputed as previously described (26) by replacing each missing genotype with its mean value, which was derived from the fraction of the cohort in which the genotype data was available. Analyses were performed using the wGRS on the continuous scale and on a categorical scale (wGRS quintiles). We used the latter to compare the magnitude of the effects between the top and bottom quintiles of the wGRS. The purpose of this comparison is to illustrate the extent to which having a relatively high genetic burden (i.e. >80% of the wGRS distribution) verses a relatively low genetic burden (i.e. <20% of the wGRS distribution) influences glucose homeostasis. The cut-points were chosen because they allow for the comparison of effects for genetically distinct sub-groups of the population, whilst ensuring these subgroups are sufficiently prevalent to be reasonably generalizable. There was no biologically informed reason for
choosing these cut-points, as the relationship of the wGRS with glucose levels is linear (as illustrated in Figure 1).

Non-genetic Risk Score (NGRS)

Two NGRSs were computed and used to predict the development of impaired fasting glucose (IFG). The first score was comprised of baseline age, sex, and family history of type 2 diabetes (NGRS_1). The second score, which was similar in design to the Framingham Risk Score (27), included these three variables in addition to baseline fasting and 2-hr glucose concentrations, triglycerides, diastolic and systolic blood pressures, and BMI (NGRS_2). The ability to predict IFG for both scores was compared with the predictive accuracy of a genetic model including all 16 fasting glucose loci and 15 additional confirmed type 2 diabetes loci (CDKN2A/B rs10811661; HNF1B rs4430796; PPARG rs1801282; SLC30A8 rs13266634; WFS1 rs10010131; ADAMTS9 rs4607103; CDKAL1 rs7754840; CAMK1D rs12779790; NOTCH2 rs10923931; KCNJ11 rs5912; THADA rs7578597; IGFBP2 rs4402960; JAZF1 rs864745; HHEX rs1111875; KCNQ1 rs2237895; TSPAN8 rs7961581).

Statistical analysis

All analyses were undertaken using the SAS software (version 9.1, SAS Institute, Cary, NC, USA). A $\chi^2$-test with 1 d.f. was used to determine Hardy-Weinberg Equilibrium. A two-sided paired-samples t-test was used to compare mean values at baseline and follow-up. Dependence of the variables was assessed with Pearson’s correlation coefficient.
Generalized linear models were used to test cross-sectional associations between each variant and fasting or 2-hr glucose concentrations. Associations with changes in fasting and 2-hr post-challenge glycemia over the 10-year follow-up period were assessed in two ways: i) follow-up glucose (fasting or 2-hr) included as the dependent variable and the respective baseline glucose variable as a covariate; ii) delta glucose (i.e. the difference between follow-up and the baseline fasting or 2-hr glucose concentrations) included as the dependent variable and the respective baseline glucose trait as a covariate. Model ii yields more easily interpretable coefficients than model i, but potentially over-adjusts for baseline glucose levels, given that this trait is included both in the calculation of the delta and as a covariate in the regression model; for these reasons, model ii was only used to graphically illustrate the results (Figure 1). Logistic regression was used to assess the association between each variant and IFG defined as fasting plasma glucose concentrations >5.6 mmol/l and <7.0 mmol/l according to the ADA guidelines (28). All models were adjusted for age, sex and fasting time, and additional adjustments were made as described in the Results section. As with the study by Dupuis et al. (3), all models assumed an additive mode of inheritance. Alpha inflation owing to multiple hypothesis testing was controlled for using Holm’s procedure (29). The relative predictive accuracy of the genetic and non-genetic risk scores was determined using the area under the receiver operator characteristic curves (ROC AUC) (30). A $P$-value $<0.05$ was considered statistically significant.
Results

Table 1 reports baseline characteristics for the 16,398 participants in the full GLACIER cohort. The correlation between fasting and 2-hr glucose concentrations at baseline was \( r=0.25 \) (\( P<0.0001 \)). The baseline and follow-up characteristics are shown in Table 2 (\( n=4,059 \)). Mean (±s.d.) follow-up duration was 9.9±0.3 years and ranged from 6–13 years. On average, the level of all quantitative variables shown in Table 2 increased significantly between baseline and follow-up (\( P<0.0001 \)).

Replication of genetic associations with fasting and post-challenge glycemia

Baseline fasting glucose concentrations

Table 3 summarizes associations between each of the 16 fasting glucose-raising loci and fasting glycemia after adjustment for age and sex; 12 of the SNPs (\( MTNR1B \) rs10830963, \( G6PC2 \) rs560887, \( GCK \) rs4607517, \( CRY2 \) rs11605924, \( DGKB-TMEM195 \) rs2191349, \( SLC30A8 \) rs13266634, \( GCKR \) rs780094, \( TCF7L2 \) rs7903146, \( ADRA2A \) rs10885122, \( FADS1 \) rs174550, \( SLC2A2 \) rs11920090, and \( PROX1 \) rs340874) were statistically associated with fasting glucose concentrations. The effect estimates from these models are directionally consistent with the original report (3), irrespective of statistical significance. Further adjustment for BMI made no material difference to these results (data not shown).

Baseline 2-hr glucose concentrations
As shown in Table 3, eight of the 16 fasting glucose-raising SNPs were associated with 2-hr glucose concentrations in models adjusted for age, sex, and fasting time. The associations for five of the SNPs (ADCY5 rs11708067, TCF7L2 rs7903146, GCK rs4607517, SLC30A8 rs13266634, and C2CD4B rs11071657) were consistent with the original findings of Dupuis et al. (3). The previously reported associations for MTNR1B rs10830963, GCKR rs780094, and GLIS3 rs7034200 failed to replicate in the GLACIER study cohort but were directionally consistent with the original report. By contrast to the original report, CRY2 rs11605924, FADS1 rs174550 and SLC2A2 rs11920090 were significantly associated with 2-hr glucose concentrations in the GLACIER cohort (Table 3).

Although fluctuations in post-absorptive glucose concentrations are primarily determined by the rate of peripheral glucose disposal, the point from which post-absorptive glucose concentrations begin to vary is set by the fasting concentration. Thus, it is possible that associations between fasting glucose raising loci and 2-hr glucose concentrations might be observed purely because of their correlation with fasting glucose concentrations. Accordingly, we tested a secondary model that was additionally adjusted for fasting glucose concentrations. As hypothesized, these models generally yielded weaker associations between the selected loci and 2-hr glucose concentrations (Table 3); the nominal associations observed for SLC30A8 rs13266634, SLC2A2 rs11920090, and FADS1 rs174550 were rendered non-significant. However, the strength of the associations for C2CD4B rs11071657, MTNR1B rs10830963, and GCKR rs780094 were
all strengthened with adjustment for fasting glucose ($P<0.05$, Table 3). Adjusting the models for BMI did not materially affect the results (data not shown).

**Prospective analyses for fasting and post-challenge glycemia**

To examine the extent to which fasting glucose-raising loci predict longitudinal changes in glucose homeostasis, we modeled the effects of the 16 SNPs on changes in fasting and 2-hr glucose concentrations or the development of IFG during 10 years follow-up.

**Prospective associations with fasting glucose concentrations**

In models adjusted for baseline age, sex, fasting glucose, fasting time, and follow-up time, the effect alleles at the $GCK$ rs4607517, $ADRA2A$ rs10885122, $DGKB-TMEM195$ rs2191349, and $G6PC2$ rs560887 loci were nominally statistically associated with a greater elevation in fasting glucose concentrations at follow-up (Table 4). None of the SNPs remained statistically associated with change in fasting glucose after correction for multiple hypothesis testing (Table 4). Additionally adjusting the models for BMI did not materially alter these results (data not shown).

**Prospective associations with 2-hr glucose concentrations**

After adjustment for baseline age, sex, baseline 2-hr glucose, fasting time, and follow-up time, $MTNR1B$ rs10830963 and $ADCY5$ rs11708067 were associated with change in 2-hr glucose concentrations at a nominal level of statistical significance; neither finding remained significant after correction for multiple testing (Table 4).
Additionally adjusting the models for baseline and follow-up fasting glucose concentrations strengthened the association with change in 2-hr glucose concentrations observed for MTNR1B rs10830963 and the result remained statistically significant after correcting for multiple testing \( P_{\text{corrected}} = 0.0065 \), Table 4. Adjusting the analyses for fasting glucose concentrations did not influence the strength or magnitude of the association for ADCY5 rs11708067 and rendered the association for MADD rs7944584 with change in 2-hr glucose concentrations nominally statistically significant. With the exception of the finding for MTNR1B rs10830963, none of these results remained statistically significantly after correcting for multiple hypothesis testing (Table 4). None of the other fasting glucose-raising SNPs was significantly associated with change in 2-hr glucose concentrations during follow-up. Adding BMI as a covariate to the above analyses made no material difference to the results (data not shown).

**Cumulative effects of the 16 fasting glucose-raising loci on glucose homeostasis**

To examine the cumulative effects of the risk alleles on glucose homeostasis, we tested models incorporating all loci into a single GRS.

**wGRS on fasting and 2-hr glucose concentrations**

Each additional unit of the wGRS (mean=17; range=7–29 risk alleles) was associated with a 0.030 mmol/l \( (P=1.3 \times 10^{-75}) \) higher fasting glucose concentration; individuals >80\textsuperscript{th} wGRS centile had on average 0.26 mmol/l (CI: 0.23-0.29) higher fasting glucose
concentrations than those individuals <20\textsuperscript{th} centile. Adjusting for BMI did not affect the magnitude, but strengthened the statistical significance of the association ($P=2.3\times10^{-79}$).

Each additional unit (risk allele) of the 2-hr glucose wGRS was associated with 0.026 mmol/l higher 2-hr glucose concentrations ($P=4.4\times10^{-12}$). Including BMI as a covariate in the model slightly reduced the strength and magnitude of the association ($\beta=0.019$ mmol/l, $P=1.5\times10^{-7}$). Individuals >80\textsuperscript{th} wGRS centile had ~0.17 mmol/l (95\% CI: 0.10-0.24) higher 2-hr glucose concentrations than those individuals <20\textsuperscript{th} centile.

In the sub-cohort with repeated measures, each unit of the fasting glucose wGRS (mean=17; range=8–27 risk alleles) was associated with a 0.019 mmol/l ($P=1.9\times10^{-7}$) greater elevation in fasting glucose concentrations from baseline to follow-up. Correcting the analysis for BMI did not materially influence the magnitude of the association but strengthened its statistical significance ($\beta=0.020$ mmol/l, $P=4.5\times10^{-8}$). For those individuals with a wGRS > 80\textsuperscript{th} centile, the increase in fasting glucose concentrations during follow-up was ~0.16 mmol/l greater than for those below the 20\textsuperscript{th} wGRS centile ($P=2.4\times10^{-6}$, Figure 1).

After correcting for fasting glycemia, no additive effect on longitudinal change in 2-hr glucose concentrations was observed in the wGRS model ($\beta=-0.000$ mmol/l, $P=0.98$).

The unweighted GRS models yielded similar results to the wGRS models (data not shown).
Associations between fasting glucose-raising loci and IFG

In order to place the associations reported above into a clinical context, we examined the associations between the genetic variants and IFG and compared the power of genetic models to predict the development of IFG during follow-up against non-genetic prediction models.

Baseline associations with prevalent IFG

In order to quantify the magnitude of the association between each SNP and IFG, we began by modeling SNP associations with prevalent IFG. In models adjusted for age, sex and fasting time, 10 of the fasting glucose-raising loci (*MTNR1B* rs10830963, *G6PC2* rs560887, *GCK* rs4607517, *ADRA2A* rs10885122, *DGKB-TMEM195* rs2191349, *TCF7L2* rs7903146, *SLC30A8* rs13266634, *CRY2* rs11605924, *GCKR* rs780094, and *GLIS3* rs7034200) were associated with elevated IFG risk (Table 5).

Prospective associations with incident IFG

Table 5 shows summary results for associations between each of the fasting glucose-raising loci and risk of developing IFG during follow-up, adjusted for baseline age, sex, baseline IFG/NGT, fasting time, and follow-up duration. The glucose-raising alleles at *MTNR1B* rs10830963, *G6PC2* rs560887, and *PROX1* rs340874 were significantly associated with an increased risk of developing IFG during follow-up.

Cumulative effects of the 16 fasting glucose-raising loci on prevalent and incident IFG
Each wGRS unit was associated with a 10% (95% CI: 9-11%) increased risk of IFG. For individuals with a wGRS above the 80th centile the risk of IFG was 2.2 fold higher (95% CI: 2.0-2.5) than for individuals with a wGRS below the 20th centile.

In the sub-cohort with repeated measures, each additional wGRS unit (risk allele) was associated with a 6% (95% CI: 4-9%) greater risk of developing IFG during follow-up. Those above the 80th GRS centile were 64% (95% CI: 33-201%) more likely to develop IFG than those below the 20th centile. Comparable results were obtained in models where the unweighted GRS was included as the predictor variable (data not shown).

Adjusting the above models for BMI did not materially affect these results (data not shown).

Comparison of the predictive accuracy of genetic and non-genetic risk scores on incident IFG

Several ROC models were compared to estimate the ability of genetic and non-genetic models to predict the development of IFG during a 10 y period follow-up and to examine whether combining information from both models significantly improved the power to predict this outcome. The genetic models include all 16 fasting glucose loci outlined above, in addition to 15 independent type 2 diabetes loci. The effect alleles for each of these SNPs were coded in a manner that is consistent with the MAGIC fasting glucose (3) (for fasting glucose loci) or the DIAGRAM type 2 diabetes meta-analysis (for type 2
diabetes loci) (31). All models were adjusted for fasting time. NGRS_1 and NGRS_2 had ROC AUCs of 0.5796 \((P<1.0\times 10^{-20})\) and 0.6536 \((P<1.0\times 10^{-20})\), respectively. The ROC AUC for the genetic model was 0.5933 \((P<1.0\times 10^{-20})\). The predictive accuracy of the genetic model exceeded NGRS_1 \((P=0.033)\), but was less than NGRS_2 \((P=1.7\times 10^{-6})\). Adding the genetic information to NGRS_1 and NGRS_2 significantly improved the predictive accuracy of both models (ROC AUCs from 0.5796 to 0.6133; \(P=1.45\times 10^{-8}\) and 0.6536 to 0.6677, respectively; \(P=1.7\times 10^{-6}\)). Adjusting the models for the amount of time spent fasting slightly inflated the ROC AUCs (supplementary table 1) but in the model where the genetic information was added to the NGRS_2, the ROC AUC did not materially differ (0.6621 vs. 0.6677) irrespective of whether fasting time was or was not controlled for.

**Discussion**

We sought to replicate and extend previously reported associations between genetic loci and fasting and post-challenge glucose concentrations (3) in the GLACIER Study, an ethnically homogenous adult cohort from northern Sweden. Twelve of 16 fasting glucose-raising loci were statistically associated with fasting glycemia in a manner that is directionally consistent with the original report (3). Of the eight loci that were also found to be associated with 2-hr post-challenge glycemia in the original report, five replicated in the present study. After additionally adjusting the analysis for the influence of fasting glycemia on 2-hr post-challenge glucose concentrations, seven of the eight loci replicated, and one novel association was observed (CRY2 rs11605924).
To our knowledge, no studies have yet reported on the longitudinal relationships between all 16 fasting glucose-raising loci recently reported by the MAGIC investigators (3) and quantitative glycemic traits. Our analyses examined the predictive properties of the 16 gene variants singularly and in combination during a decade of follow-up. We found that four variants were nominally associated with change in fasting glucose concentrations, but none remained statistically associated after correction for multiple testing. The MTNR1B rs10830963 variant was associated with a protective effect on elevations in 2-hr glucose concentrations in our study. We also observed a strong combined effect of the risk alleles on glucose concentrations. For example, each wGRS count was associated with a 0.019 mmol/l higher increase in fasting glucose concentration, and a 6% greater risk of developing IFG during a decade of follow-up. When combined with other non-genetic risk factors, the gene variants significantly improved the predictive accuracy for incident IFG.

Four of the variants initially reported as predictive of fasting glycemia (3) failed to replicate in the GLACIER Study materials. These associations were, however, directionally consistent with the original report but of 30-60% lesser magnitude, suggesting that our study might have been underpowered to detect these effects. Indeed, we believe that the provision of confidence intervals is far more informative of whether a study is likely to be underpowered to detect specific effects. Therefore, we calculate the 95% CIs from the standard errors and coefficients provided in the original report from Dupuis et al (3) and compared these with the 95% confidence intervals derived from the GLACIER Study. This comparison showed that the confidence intervals from
the two studies are, without exception, heavily overlapping, meaning that although the beta coefficients may appear to differ in magnitude between studies, these differences are not statistically significant. Given these points, one cannot discount the possibility that the failure to replicate several SNP associations in the GLACIER Study is because of a lack of statistical power.

Whilst sample size has a demonstrable impact on the statistical power of study, the linkage disequilibrium structure of the population from which the GLACIER Study emanates may also have affected the power to detect previously reported effects. For example, as we have previously shown (23; 24; 26), the minor allele frequencies for SNPs associated with complex traits are often lower in northern Swedish populations compared with those in southern Sweden and central Europe. These differences may be because of different founder effects in combination with the low level of genetic admixture in northern Sweden (32). It is possible that the lower minor allele frequencies reflect different patterns of linkage disequilibrium between the observed variants and the unobserved causal variants, which, because of latent misclassification, might diminish power to detect associations between the observed variants and disease traits; this may be the case for the rs7034200 (GLIS3) in the present report. Here, the minor allele frequency for the rs7034200 variant is 43% in the GLACIER Study compared with 49% and 54% reported in the MAGIC and HAPMAP CEU collections, respectively.

In the cross-sectional analyses, three SNP associations with 2-hr glucose concentrations failed to replicate and three novel SNP associations were observed. It is unclear from their paper whether the 2-hr glucose association analyses reported by
Dupuis et al. (3) included adjustment for fasting glucose concentrations. Nevertheless, the association analyses reported here for 2-hr glucose were highly consistent with those reported by Dupuis et al (3) when fasting glucose was controlled for, with the majority of these models being statistically significant. We also identified one novel SNP association with 2-hr glucose concentrations, which was for the CRY2 rs11605924 variant. It is possible that with the low admixture rates and strong founder effects evident in northern Sweden (32), these findings may be population specific.

The protective effect on change in 2-hr glucose concentrations observed for MTNR1B rs10830963 may be false positive given the nominal level of statistical significance and the number of parallel hypotheses tested. However, the magnitude of the inverse associations for MTNR1B and 2-hr glucose was similar in the full baseline GLACIER cohort and in the repeated measures sub-cohort, suggesting that these findings may be reliable. If so, it suggests the involvement of MTNR1B in a complex glucose regulatory feedback-loop or the presence of pleiotropic effects at this locus, either of which would inform our understanding of diabetes pathophysiology. MTNR1B encodes one of two major receptors for the neurohormone melatonin (33) and is expressed in several key tissues involved in regulation of glucose homeostasis: the circadian rhythm control center in the brain, hypothalamus, adipose tissue, liver, kidney and the pancreatic β-cells (9; 34; 35). Aspects of the circadian rhythm (e.g. sleep-awake cycle, feeding and hormone levels) are involved in the regulation of glucose homeostasis. Some have hypothesized that effects of disturbed sleep patterns on risk of metabolic disorders (36; 37) and decreased melatonin secretion might be influence by MTNR1B variation (38).
The recent finding that \textit{MTNR1B} is expressed in the β-cells implies that the gene variant might affect pancreatic glucose sensing and/or insulin release and thereby glucose tolerance (9).

Elevations in glucose, whether defined quantitatively or categorically, are major predictors of type 2 diabetes and cardiovascular disease, and studies that seek to determine the genetic determinants of these traits may hence prove informative for disease prevention. Therefore, the prospective analyses presented here focused on the extent to which the 16 glucose-raising gene variants predict changes in glucose concentrations and the development of IFG. It is important to bear in mind that the longitudinal effects of the risk alleles on worsening glucose control reported here are for a follow-up period of 10 years. If the effects are roughly linear throughout adulthood, the life-long impact of these variants on glucose control is likely to be considerably greater in magnitude. Thus, it may be possible to identify persons at high genetic risk of developing diabetes long before the manifestation of the clinical signs and symptoms. The implication being that for these individuals, early intervention might be beneficial. Studies testing prospective relationships with the development of diabetes complications will be required to determine the clinical value of these genetic prediction models and the potential benefits of early intervention. Unfortunately, such data are presently unavailable in the GLACIER Study.

In the presence of detailed clinical and personal information, the inclusion of genetic information statistically improved the ability to predict IFG. However, the overall predictive accuracy of these models is lower than reported for diabetes incidence (25; 26;
This is unsurprising given that, unlike frank diabetes, IFG is an intermediate state of glucose dyshomeostasis, defined by a relatively narrow range of fasting glucose concentrations from which people often regress; it may, thus, be that the classification of IFG is more prone to regression dilution than the diagnosis of type 2 diabetes.

In conclusion, we have provided detailed replication of many of the genetic effects report previously for fasting and 2-hr glucose concentrations (3). We extended previous studies by identifying four loci (GCK, ADRA2A, DGKB-TMEM195, and G6PC2) that predict worsening glucose control during 10 years follow-up. Somewhat paradoxically, the previously reported risk allele at MTNR1B appears protective of worsening glucose tolerance during follow-up. Future studies testing prospective relationships with glucose homeostasis, as well as with the development of diabetes and its complications will be required to determine the clinical value of these genetic prediction models. Although we were able to identify genetic determinants of hyperglycemia, it is likely that lifestyle factors (e.g. diet, physical activity, and obesity) play more pervasive roles in long-term glucose homeostasis. Thus, studies that examine the interaction of these genetic loci with lifestyle factors may facilitate the application of genetic data to the prevention of type 2 diabetes and its complications.
Author contributions

PWF designed and obtained funding for the GLACIER Study; FR, JCF, FBH, and PWF contributed to the analytical plan; DM, GH, and PWF organized data; FR and PWF conducted the statistical analyses and wrote the manuscript; All authors critically appraised the manuscript and contributed to draft revisions.

Conflicts of interest

JCF has received consulting honoraria from Publicis Healthcare, Merck, bioStrategies, XOMA and Daiichi-Sankyo, and has been a paid invited speaker at internal scientific seminars hosted by Pfizer and Alnylam Pharmaceuticals. PWF has been an invited speaker at an international scientific seminar hosted by Novo Nordisk.

Acknowledgments

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doctoral stipend from the Swedish Heart-Lung Foundation. A list of all MAGIC investigators is shown in the supplementary appendix.
References


Tables

Table 1 Participant characteristics for the GLACIER Study cohort (N=16,398)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean or n*</th>
<th>s.d. or %*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)*</td>
<td>6,526/9,872</td>
<td>40/60</td>
</tr>
<tr>
<td>Age (years)</td>
<td>52.3</td>
<td>8.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.9</td>
<td>4.1</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.4</td>
<td>0.6</td>
</tr>
<tr>
<td>2-hr glucose (mmol/l)†</td>
<td>6.7</td>
<td>1.5</td>
</tr>
<tr>
<td>NFG vs. IFG*</td>
<td>10,766/5,564</td>
<td>66/34</td>
</tr>
</tbody>
</table>

* Values are n and proportion (%) † 2-hr glucose concentrations available in 15,828 participants
Table 2 Characteristics for GLACIER Study participants in whom baseline and 10-year follow-up exams were performed (N=4,059)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline Mean or n</th>
<th>s.d. or %</th>
<th>Follow-up Mean or n</th>
<th>s.d. or %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)*</td>
<td>1,479/2,580</td>
<td>36/64</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.2</td>
<td>6.7</td>
<td>55.2</td>
<td>6.7</td>
</tr>
<tr>
<td>Family history of T2D*†</td>
<td>697/3321/41</td>
<td>17/82/1</td>
<td>925/2980/154</td>
<td>23/73/4</td>
</tr>
<tr>
<td>BMI (kg/m²)*‡</td>
<td>25.1</td>
<td>3.7</td>
<td>26.2</td>
<td>4.0</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)‡</td>
<td>5.3</td>
<td>0.6</td>
<td>5.5</td>
<td>0.7</td>
</tr>
<tr>
<td>2-hr glucose (mmol/l)‡,§</td>
<td>6.5</td>
<td>1.3</td>
<td>7.1</td>
<td>1.7</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)‡</td>
<td>124</td>
<td>16</td>
<td>130</td>
<td>18</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)‡</td>
<td>78</td>
<td>10</td>
<td>78</td>
<td>10</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)‡,¶</td>
<td>1.3</td>
<td>0.8</td>
<td>1.4</td>
<td>0.9</td>
</tr>
<tr>
<td>NFG vs. IFG*#</td>
<td>3,087/964</td>
<td>76/24</td>
<td>2,073/1,953</td>
<td>51/49</td>
</tr>
</tbody>
</table>

* Values are n and proportion (%). † Yes/no/missing information. ‡ A paired samples t-test was used to compare mean values at baseline and follow-up, all of which increased significantly (P<0.0001). Follow-up duration averaged 9.9±0.3 years and ranged from 6-13 years. § 2-hr glucose available in 4,059 participants at follow-up. ¶ Triglycerides available in 3,352 and 4,020 participants at baseline and follow-up, respectively. # The number of individuals with IFG increased significantly at follow-up compared to baseline (P<0.0001). Frequencies were compared between baseline and follow-up using the Mantel-Haenszel χ² test (1df.)