Genetic Predisposition to Long-Term Nondiabetic Deteriorations in Glucose Homeostasis

Ten-Year Follow-Up of the GLACIER Study

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OBJECTIVE—To assess whether recently discovered genetic loci associated with hyperglycemia also predict long-term changes in glycemic traits.

RESEARCH DESIGN AND METHODS—Sixteen fasting glucose-raising loci were genotyped in middle-aged adults from the Gene x Lifestyle interactions And Complex traits Involved in Elevated disease Risk (GLACIER) Study, a population-based prospective cohort study from northern Sweden. Genotypes were tested for association with baseline fasting and 2-h postchallenge glycemia (N = 16,330), and for changes in these 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 of 16 variants; 10 variants were also associated with impaired fasting glucose (IFG) and 7 were independently associated with 2-h postchallenge glucose concentrations. In prospective analyses, the effect alleles at four loci (*GCK* rs4607517, *ADRA2A* rs10885122, *DGKB-TMEM195* rs2191349, and *G6PC2* rs560887) were nominally associated with worsening fasting glucose concentrations during 10-years of follow-up. *MTNR1B* rs10830963, which was predictive of elevated fasting glucose concentrations in cross-sectional analyses, was associated with a protective effect on postchallenge glucose concentrations during follow-up; however, this was only when baseline fasting and 2-h 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 0.16 mmol/l ($P = 2.4 \times 10^{-6}$) greater elevation in fasting glucose and a 64% (95% CI: 33–201%) higher risk of developing IFG during 10 years of 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 end points will help establish whether the magnitude of these changes is clinically relevant. ***Diabetes*** **60:345–354, 2011**

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 postchallenge blood glucose concentrations are the cardinal feature of type 2 diabetes. Type 2 diabetes and nondiabetic variations in fasting and postchallenge 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 postchallenge (3,4) glucose-raising loci recently have been 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*, *MTNRIB*, *GCK*, *GCKR*, *G6PC2*, *DGKB-TMEM195*, and *SLC30A8*) that raise fasting glucose concentrations and/or risk of impaired fasting glucose (IFG) (5–17). In the most recent large-scale effort to discover new fasting glucose–related loci, the Meta-Analyses of Glucose and Insulin-Related Traits Consortium (MAGIC) investigators identified a further nine predisposing loci in European whites: rs11708067 (*ADCY5*), rs7944584 (*MADD*), rs10885122 (*ADRA2A*), rs174550 (*FADS1*), rs11605924 (*CRY2*), rs11920090 (*SLC2A2*), rs7034200 (*GLIS3*), rs340874 (*PROX1*), and rs11071657 (*C2CD4B*). To our knowledge, the existing longitudinal epidemiologic studies in which genetic predictors of change in fasting glucose concentrations have been examined have focused on variants in *TCF7L2* (5,18,19), and no longitudinal studies before the present one have examined the entire array of confirmed fasting glucose-raising loci. The extent to which the accumulation of risk alleles influences fasting and postchallenge glucose levels is also unclear.

The purpose of the present study was to examine the cross-sectional and longitudinal relationships between

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previously associated fasting glucose-raising variants, singly and in combination, with fasting and postchallenge glucose concentrations in the Gene x Lifestyle interactions And Complex traits Involved in Elevated disease Risk (GLACIER) Study, a population-based prospective cohort study of adults from northern Sweden.

RESEARCH DESIGN AND METHODS

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 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-h glucose models) to 16,330 (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 center for a clinical examination in 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 16,330 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 kilograms divided by height in meters squared (kg/m^2). Systolic and diastolic blood pressures were measured once, after a 5-min rest, using a mercury-gauge sphygmomanometer with the participant in a supine position. Capillary blood was drawn after an overnight fast, and a second sample was drawn 2 h after a standard 75-g oral glucose load (21). Capillary plasma glucose concentrations were measured with a Reflotron bench-top analyzer (Roche Diagnostics Scandinavia AB). Before the first blood draw, 86% of the cohort had fasted for a minimum of 8 h, ~3% had fasted for between 4 and 8 h, and data were missing for ~11%. Sensitivity analyses (associations of single nucleotide polymorphisms [SNPs] with glucose levels) that included all individuals versus 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 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 the OpenArray SNP Genotyping System (BioTrove, Woburn, MA). One in 10 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. 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 postchallenge glucoses are 0.948 and 1.548, respectively (i.e., 32 risk alleles × the relevant β 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 were 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 subgroups of the population, while 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 Fig. 1).

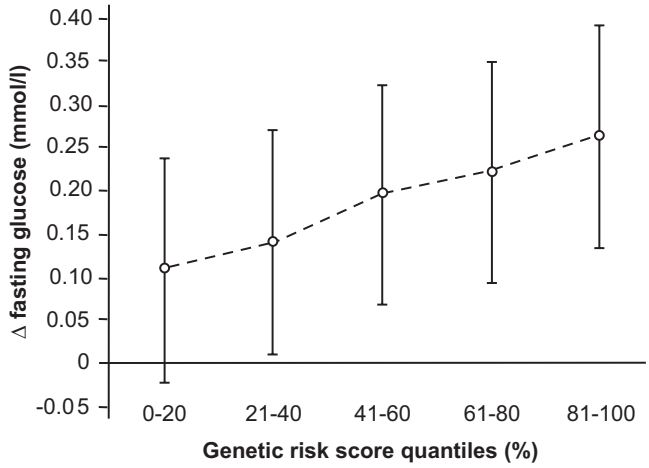

FIG. 1. Association between the weighted genetic risk score and change in fasting glucose concentrations during 10 years of follow-up. The weighted genetic risk score (wGRS) was constructed as described in the RESEARCH DESIGN AND METHODS section. Data are mean (95% CI). Δ glucose levels (follow-up minus baseline) per quintile of the wGRS are adjusted for baseline age, sex, baseline fasting glucose, fasting time at baseline and follow-up, and follow-up time (N = 4,059).

Nongenetic risk score. Two nongenetic risk scores (NGRSs) were computed and used to predict the development of 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-h 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; *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 SAS software (version 9.1, SAS Institute, Cary, NC). A χ^2-test with one degree of freedom 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 the Pearson correlation coefficient. Generalized linear models were used to test cross-sectional associations between each variant and fasting or 2-h glucose concentrations. Associations with changes in fasting and 2-h postchallenge glycemia over the 10-year follow-up period were assessed in two ways: *1*) follow-up glucose (fasting or 2-h) included as the dependent variable and the respective baseline glucose variable as a covariate; *2*) Δ glucose (i.e., the difference between follow-up and the baseline fasting or 2-h glucose concentrations) included as the dependent variable and the respective baseline glucose trait as a covariate. Model *2* yields more easily interpretable coefficients than model *1*, but potentially overadjusts for baseline glucose levels, given that this trait is included both in the calculation of the Δ and as a covariate in the regression model; for these reasons, model *2* was used only to graphically illustrate the results (Fig. 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 adjust-

TABLE 1
Participant characteristics for the GLACIER Study cohort (n = 16,330)

Variable	Mean or n*	SD or %*
Sex (male/female)*	6,526/9,872	40/60
Age (years)	52.3	8.8
BMI (kg/m^2)	25.9	4.1
Fasting glucose (mmol/l)	5.4	0.6
2-h glucose (mmol/l)†	6.7	1.5
NFG vs. IFG*	10,766/5,564	66/34

*Values are n and proportion (%). †2-h glucose concentrations available for 15,828 participants. NFG, normal fasting glucose.

ments 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. The α inflation, owing to multiple hypothesis testing, was controlled for using Holm's procedure (29). The relative predictive accuracy of the genetic and nongenetic risk scores was determined using the area under the receiver operator characteristic curves (ROC AUC) (30). $P < 0.05$ was considered statistically significant.

RESULTS

Table 1 reports baseline characteristics for the 16,330 participants in the full GLACIER cohort. The correlation between fasting and 2-h 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 (± SD) follow-up duration was 9.9 ± 0.3 years and ranged from 6 to 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 postchallenge 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, sex, and fasting time: 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-h glucose concentrations. As shown in Table 3, 8 of the 16 fasting glucose-raising SNPs were associated with 2-h 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-h glucose concentrations in the GLACIER cohort (Table 3).

Although fluctuations in postabsorptive glucose concentrations are primarily determined by the rate of peripheral glucose disposal, the point from which postabsorptive glucose concentrations begin to vary is set by the fasting concentration. Thus, it is possible that associations between fasting glucose-raising loci and 2-h 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-h glucose concentrations (Table 3); the nominal associations observed for *SLC30A8* rs13266634, *SLC2A2* rs11920090, and *FADS1* rs174550 were rendered nonsignificant. 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 postchallenge 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-h glucose concentrations or the development of IFG during 10 years of follow-up.

TABLE 2
Characteristics for GLACIER Study participants in whom baseline and 10-year follow-up exams were performed (n = 4,059)

	Baseline		Follow-up	
Variable	Mean or n	SD or %	Mean or n	SD or %
Sex (male/female)*	1,479/2,580	36/64	—	—
Age (years)	45.2	6.7	55.2	6.7
Family history of T2D*†	697/3,321/41	17/82/1	925/2,980/154	23/73/4
BMI (kg/m^2)‡	25.1	3.7	26.2	4.0
Fasting glucose (mmol/l)‡	5.3	0.6	5.5	0.7
2-h glucose (mmol/l)‡§	6.5	1.3	7.1	1.7
Systolic blood pressure (mmHg)‡	124	16	130	18
Diastolic blood pressure (mmHg)‡	78	10	78	10
Triglycerides (mmol/l)‡¶	1.3	0.8	1.4	0.9
NFG vs. IFG*#	3,087/964	76/24	2,073/1,953	51/49

*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 (except DBP) increased significantly ($P < 0.0001$). Follow-up duration averaged 9.9 ± 0.3 years and ranged from 6 to 13 years. §2-h glucose available for 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 with baseline ($P < 0.0001$). Frequencies were compared between baseline and follow-up using the Mantel-Haenszel χ^2 test (1 degree of freedom). T2D, type 2 diabetes; NFG, normal fasting glucose.

TABLE 3
Baseline associations between each SNP and fasting and 2-h glucose concentrations (n = 16,330)

SNP	Nearest gene (s)	Effect allele (other)	Effect allele frequency	Fasting glucose (mmol/l)		2-h glucose (mmol/l)			
				Effect (SE)*	P*	Effect (SE)*	P*	Effect (SE)†	P†
rs10830963	*MTNR1B*	G(C)	0.28	0.091 (0.008)	2.7×10^{-31}	0.013 (0.019)	0.49	−0.042 (0.018)	0.02
rs560887	*G6PC2*	C(T)	0.71	0.063 (0.008)	1.9×10^{-16}	0.013 (0.018)	0.47	−0.025 (0.018)	0.16
rs4607517	*GCK*	A(G)	0.15	0.054 (0.010)	3.1×10^{-8}	0.083 (0.023)	3.9×10^{-4}	0.050 (0.023)	0.03
rs11605924	*CRY2*	A(C)	0.50	0.033 (0.007)	1.5×10^{-6}	0.062 (0.017)	2.0×10^{-4}	0.042 (0.016)	9.1×10^{-3}
rs2191349	*DGKB-TMEM195*	T(G)	0.51	0.029 (0.007)	2.7×10^{-5}	0.007 (0.017)	0.69	−0.009 (0.016)	0.56
rs13266634	*SLC30A8*	C(T)	0.70	0.029 (0.008)	1.4×10^{-4}	0.044 (0.018)	0.02	0.027 (0.017)	0.12
rs780094	*GCKR*	C(T)	0.71	0.025 (0.008)	1.1×10^{-3}	−0.024 (0.018)	0.20	−0.039 (0.018)	0.03
rs7903146	*TCF7L2*	T(C)	0.20	0.028 (0.009)	1.2×10^{-3}	0.084 (0.021)	6.9×10^{-5}	0.066 (0.020)	1.2×10^{-3}
rs10885122	*ADRA2A*	G(T)	0.89	0.035 (0.011)	1.5×10^{-3}	−0.004 (0.026)	0.89	−0.024 (0.026)	0.36
rs174550	*FADS1*	T(C)	0.66	0.019 (0.007)	9.7×10^{-3}	0.035 (0.017)	0.04	0.024 (0.017)	0.15
rs11920090	*SLC2A2*	T(A)	0.86	0.026 (0.010)	9.7×10^{-3}	0.057 (0.024)	0.02	0.040 (0.023)	0.09
rs340874	*PROX1*	C(T)	0.53	0.016 (0.007)	0.02	0.016 (0.017)	0.33	0.006 (0.016)	0.69
rs7944584	*MADD*	A(T)	0.76	0.014 (0.008)	0.10	0.011 (0.020)	0.57	0.003 (0.019)	0.86
rs7034200	*GLIS3*	A(C)	0.43	0.009 (0.007)	0.18	0.003 (0.017)	0.88	−0.004 (0.016)	0.80
rs11708067	*ADCY5*	A(G)	0.79	0.010 (0.009)	0.24	0.087 (0.021)	2.4×10^{-5}	0.080 (0.020)	5.7×10^{-5}
rs11071657	*C2CD4B*	A(G)	0.60	0.007 (0.007)	0.35	−0.035 (0.017)	0.04	−0.041 (0.016)	0.01

SNPs are ranked by P value for the association with fasting glucose concentrations. Multiple testing was corrected for using the Holm procedure (29). All genotypes are located on the plus strand and coded according to HapMap CEU (phase II + III), release 27, National Center for Biotechnology Information build 36. Two-hour glucose concentrations were measured by a 75-g oral glucose tolerance test in accordance with the World Health Organization guidelines (21). *Adjusted for age, sex, and fasting time. †Adjusted for age, sex, fasting time, and fasting glucose concentrations.

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-h glucose concentrations. After adjustment for baseline age, sex, baseline 2-h glucose, fasting time, and follow-up time, *MTNR1B* rs10830963 and *ADCY5* rs11708067 were associated with change in 2-h 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-h 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-h 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-h 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-h 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 >80th wGRS centile had on average 0.26 mmol/l (CI: 0.23–0.29) higher fasting glucose concentrations than those individuals <20th centile. Adjusting for BMI did not affect the magnitude, but strengthened the statistical significance of the association ($P = 2.3 \times 10^{-79}$).

Each additional unit (risk allele) of the 2-h glucose wGRS was associated with 0.026 mmol/l higher 2-h glucose concentrations ($P = 4.4 \times 10^{-12}$). Including BMI as a covariate in the model slightly reduced the strength and magnitude of the association (β = 0.019 mmol/l, ($P = 1.5 \times 10^{-7}$). Individuals >80th wGRS centile had ~0.17 mmol/l (95% CI: 0.10–0.24) higher 2-h glucose concentrations than those individuals <20th centile.

In the subcohort 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 ($P = 1.9 \times 10^{-7}$) 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 (β = 0.020 mmol/l, $P = 4.5 \times 10^{-8}$). For individuals with a wGRS

TABLE 4
The ability of each SNP to predict changes in fasting and 2-h glucose concentrations over a 10-year follow-up period (n = 4,059)

			Change in fasting glucose (mmol/l)			Change in 2-h glucose (mmol/l)					
SNP	Nearest gene (s)	Effect allele (frequency)	Effect (SE)*	P*	Corrected P*	Effect (SE)†	P†	Corrected P†	Effect‡ (SE)‡	P‡	Corrected P‡
rs4607517	*GCK*	A (0.15)	0.057 (0.022)	0.010	0.17	0.015 (0.048)	0.750	0.99	−0.029 (0.046)	0.528	0.99
rs10885122	*ADRA2A*	G (0.89)	0.062 (0.026)	0.017	0.27	0.042 (0.055)	0.451	0.99	−0.004 (0.052)	0.945	0.99
rs2191349	*DGKB-TMEM195*	T (0.51)	0.037 (0.016)	0.020	0.31	−0.007 (0.034)	0.849	0.99	−0.030 (0.033)	0.362	0.99
rs560887	*G6PC2*	C (0.71)	0.038 (0.017)	0.031	0.43	0.001 (0.038)	0.970	0.99	−0.027 (0.036)	0.454	0.99
rs10830963	*MTNR1B*	G (0.28)	0.034 (0.018)	0.059	0.77	−0.092 (0.039)	0.018	0.29	−0.131 (0.037)	4.0×10^{-4}	6.5×10^{-3}
rs780094	*GCKR*	C (0.71)	−0.030 (0.018)	0.088	0.99	−0.065 (0.038)	0.085	0.99	−0.052 (0.036)	0.144	0.99
rs7903146	*TCF7L2*	T (0.20)	0.028 (0.020)	0.159	0.99	0.044 (0.043)	0.307	0.99	0.027 (0.041)	0.515	0.99
rs11071657	*C2CD4B*	A (0.60)	0.021 (0.016)	0.193	0.99	−0.028 (0.036)	0.426	0.99	−0.046 (0.034)	0.174	0.99
rs11708067	*ADCY5*	A (0.79)	0.014 (0.020)	0.488	0.99	0.100 (0.043)	0.020	0.30	0.092 (0.040)	0.023	0.34
rs11920090	*SLC2A2*	T (0.86)	0.016 (0.024)	0.501	0.99	0.006 (0.051)	0.906	0.99	−0.002 (0.050)	0.959	0.99
rs11605924	*CRY2*	A (0.50)	0.011 (0.016)	0.503	0.99	−0.026 (0.034)	0.453	0.99	−0.032 (0.033)	0.322	0.99
rs340874	*PROX1*	C (0.53)	0.010 (0.016)	0.534	0.99	−0.037 (0.034)	0.278	0.99	−0.057 (0.032)	0.078	0.99
rs174550	*FADS1*	T (0.66)	−0.007 (0.017)	0.668	0.99	−0.006 (0.037)	0.873	0.99	0.002 (0.035)	0.947	0.99
rs7944584	*MADD*	A (0.76)	0.007 (0.019)	0.723	0.99	−0.072 (0.042)	0.085	0.99	−0.081 (0.040)	0.043	0.60
rs13266634	*SLC30A8*	C (0.70)	0.006 (0.017)	0.731	0.99	0.040 (0.037)	0.278	0.99	0.024 (0.035)	0.494	0.99
rs7034200	*GLIS3*	A (0.43)	0.005 (0.016)	0.757	0.99	−0.002 (0.035)	0.948	0.99	−0.003 (0.033)	0.926	0.99

SNPs are ranked by P value of the per-allele effect on change in fasting glucose concentrations. Multiple testing was corrected for using the Holm procedure (29). All genotypes are located on the plus strand and are coded according to HapMap CEU (phase II + III), release 27, National Center for Biotechnology Information build 36. Two-hour glucose concentrations were measured by an oral glucose tolerance test in accordance with the World Health Organization guidelines (21). *Adjusted for baseline age, sex, fasting glycemia, fasting times at baseline and follow-up, and follow-up time. ‡Adjusted for baseline age, sex, 2-h glucose concentrations, fasting times at baseline and follow-up, and follow-up time. †Adjusted for baseline age, sex, 2-h glucose concentrations, fasting times at baseline and follow-up, follow-up time and fasting glucose concentrations at baseline and follow-up.

TABLE 5
Cross-sectional and longitudinal associations between each SNP and IFG

SNP	Nearest gene(s)	Effect allele (freq.)	Cross-sectional§		Longitudinal†	
			OR	95% CI	OR	95% CI
rs10830963	*MTNR1B*	G (0.28)	1.31	(1.24–1.38)*	1.17	(1.05–1.30)*
rs560887	*G6PC2*	C (0.71)	1.21	(1.15–1.28)*	1.13	(1.02–1.25)*
rs4607517	*GCK*	A (0.15)	1.19	(1.11–1.27)*	1.13	(0.99–1.29)
rs10885122	*ADRA2A*	G (0.89)	1.13	(1.05–1.22)*	1.16	(0.99–1.35)
rs2191349	*DGKB-TMEM195*	T (0.51)	1.11	(1.05–1.16)*	1.09	(0.99–1.20)
rs7903146	*TCF7L2*	T (0.20)	1.11	(1.05–1.18)*	1.08	(0.96–1.22)
rs13266634	*SLC30A8*	T (0.70)	1.10	(1.05–1.16)*	1.05	(0.95–1.17)
rs11605924	*CRY2*	A (0.50)	1.09	(1.04–1.14)*	1.09	(0.99–1.20)
rs780094	*GCKR*	C (0.72)	1.08	(1.02–1.13)*	0.92	(0.83–1.02)
rs11920090	*SLC2A2*	T (0.86)	1.06	(0.99–1.13)	1.11	(0.96–1.28)
rs7034200	*GLIS3*	A (0.43)	1.05	(1.00–1.10)*	1.02	(0.93–1.12)
rs11708067	*ADCY5*	A (0.80)	1.05	(0.99–1.12)	0.99	(0.87–1.11)
rs340874	*PROX1*	C (0.53)	1.04	(0.99–1.09)	1.12	(1.02–1.24)*
rs174550	*FADS1*	T (0.66)	1.04	(0.98–1.09)	1.08	(0.97–1.19)
rs7944584	*MADD*	A (0.76)	1.04	(0.98–1.10)	1.00	(0.89–1.12)
rs11071657	*C2CD4B*	A (0.60)	1.01	(0.96–1.06)	1.02	(0.92–1.13)

Main effects are expressed as odds ratios (ORs) (IFG vs. normal glucose regulation) per copy of the effect allele at each locus ranked by effect size on baseline association with IFG. §Models adjusted for age, sex, and fasting time (n = 16,330). †Models adjusted for age and sex, fasting times at baseline and follow-up, follow-up time, and baseline IFG (n = 4,059). *$P < 0.05$.

>80th centile, the increase in fasting glucose concentrations during follow-up was ~0.16 mmol/l greater than for those below the 20th wGRS centile ($P = 2.4 \times 10^{-6}$, Fig. 1).

After correcting for fasting glycemia, no additive effect on longitudinal change in 2-h glucose concentrations was observed in the wGRS model ($\beta = -0.00012$ 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. 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 nongenetic prediction models.

Baseline associations with prevalent IFG. 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 subcohort 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 nongenetic risk scores on incident IFG. Several receiver operating characteristic (ROC) models were compared to estimate the ability of genetic and nongenetic models to predict the development of IFG during a 10-year 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) meta-analysis (for fasting glucose loci) or the Diabetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium 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 in the online appendix available at http://diabetes.

diabetesjournals.org/cgi/content/full/db10-0933/DC1), 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 postchallenge 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-h postchallenge glycemia in the original report, five replicated in the present study. After additionally adjusting the analysis for the influence of fasting glycemia on 2-h postchallenge glucose concentrations, six 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-h 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 nongenetic 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 to 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 calculated 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 β 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 some SNP associations in the GLACIER Study is because of a lack of statistical power.

Although sample size has a demonstrable impact on the statistical power of a 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-h glucose concentrations failed to replicate and three novel SNP associations were observed. It is unclear from their publication whether the 2-h glucose association analyses reported by Dupuis et al. (3) included adjustment for fasting glucose concentrations. Nevertheless, the association analyses reported here for 2-h 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-h 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-h 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-h glucose was similar in the full baseline GLACIER cohort and in the repeated measures subcohort, 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 *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 year. 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 is 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 (25,26,39,40). 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-h glucose concentrations (3). We extended previous studies by identifying four loci (*GCK*, *ADRA2A*, *DGKB-TMEM195*, and *G6PC2*) that may predict worsening glucose control during 10 years of 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 indentify 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.

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F.R. contributed to the analytical plan, assisted with statistical analyses, wrote the manuscript, and critically appraised the manuscript and contributed to draft revisions. I.J. critically appraised the manuscript and contributed to draft revisions. J.C.F. and F.B.H. contributed to the analytical plan, critically appraised the manuscript, and contributed to draft revisions. G.H. and D.S. organized data, critically appraised the manuscript, and contributed to draft revisions. P.W.F. designed and obtained funding for the GLACIER Study, contributed to the analytical plan, organized data, conducted the statistical analyses, wrote the manuscript, and critically appraised the manuscript and contributed to draft revisions.

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REFERENCES

1. Poulsen P, Kyvik KO, Vaag A, Beck-Nielsen H. Heritability of type II (non-insulin-dependent) diabetes mellitus and abnormal glucose tolerance–a population-based twin study. Diabetologia 1999;42:139–145
2. Snieder H, Boomsma DI, van Doornen LJ, Neale MC. Bivariate genetic analysis of fasting insulin and glucose levels. Genet Epidemiol 1999;16:426–446
3. Dupuis J, Langenberg C, Prokopenko I, Saxena R, Soranzo N, Jackson AU, Wheeler E, Glazer NL, Bouatia-Naji N, Gloyn AL, Lindgren CM, Magi R, Morris AP, Randall J, Johnson T, Elliott P, Rybin D, Thorleifsson G, Steinthorsdottir V, Henneman P, Grallert H, Dehghan A, Hottenga JJ, Franklin CS, Navarro P, Song K, Goel A, Perry JR, Egan JM, Lajunen T, Grarup N, Sparso T, Doney A, Voight BF, Stringham HM, Li M, Kanoni S, Shrader P, Cavalcanti-Proenca C, Kumari M, Qi L, Timpson NJ, Gieger C, Zabena C, Rocheleau G, Ingelsson E, An P, O'Connell J, Luan J, Elliott A, McCarroll SA, Payne F, Roccasecca RM, Pattou F, Sethupathy P, Ardlie K, Ariyurek Y, Balkau B, Barter P, Beilby JP, Ben-Shlomo Y, Benediktsson R, Bennett AJ, Bergmann S, Bochud M, Boerwinkle E, Bonnefond A, Bonnycastle LL, Borch-Johnsen K, Bottcher Y, Brunner E, Bumpstead SJ, Charpentier G, Chen YD, Chines P, Clarke R, Coin LJ, Cooper MN, Cornelis M, Crawford G, Crisponi L, Day IN, de Geus EJ, Delplanque J, Dina C, Erdos MR, Fedson AC, Fischer-Rosinsky A, Forouhi NG, Fox CS, Frants R, Franzosi MG, Galan P, Goodarzi MO, Graessler J, Groves CJ, Grundy S, Gwilliam R, Gyllensten U, Hadjadj S, Hallmans G, Hammond N, Han X, Hartikainen AL, Hassanali N, Hayward C, Heath SC, Hercberg S, Herder C, Hicks AA, Hillman DR, Hingorani AD, Hofman A, Hui J, Hung J, Isomaa B, Johnson PR, Jorgensen T, Jula A, Kaakinen M, Kaprio J, Kesaniemi YA, Kivimaki M, Knight B, Koskinen S, Kovacs P, Kyvik KO, Lathrop GM, Lawlor DA, Le Bacquer O, Lecoeur C, Li Y, Lyssenko V, Mahley R, Mangino M, Manning AK, Martinez-Larrad MT, McAteer JB, McCulloch LJ, McPherson R, Meisinger C, Melzer D, Meyre D, Mitchell BD, Morken MA, Mukherjee S, Naitza S, Narisu N, Neville MJ, Oostra BA, Orru M, Pakyz R, Palmer CN, Paolisso G, Pattaro C, Pearson D, Peden JF, Pedersen NL, Perola M, Pfeiffer AF, Pichler I, Polasek O, Posthuma D, Potter SC, Pouta A, Province MA, Psaty BM, Rathmann W, Rayner NW, Rice K, Ripatti S, Rivadeneira F, Roden M, Rolandsson O, Sandbaek A, Sandhu M, Sanna S, Sayer AA, Scheet P, Scott LJ, Seedorf U, Sharp SJ, Shields B, Sigurethsson G, Sijbrands EJ, Silveira A, Simpson L, Singleton A, Smith NL, Sovio U, Swift A, Syddall H, Syvanen AC, Tanaka T, Thorand B, Tichet J, Tonjes A, Tuomi T, Uitterlinden AG, van Dijk KW, van Hoek M, Varma D, Visvikis-Siest S, Vitart V, Vogelzangs N, Waeber G, Wagner PJ, Walley A, Walters GB, Ward KL, Watkins H, Weedon MN, Wild SH, Willemsen G, Witteman JC, Yarnell JW, Zeggini E, Zelenika D, Zethelius B, Zhai G, Zhao JH, Zillikens MC, Borecki IB, Loos RJ, Meneton P, Magnusson PK, Nathan DM, Williams GH, Hattersley AT, Silander K, Salomaa V, Smith GD, Bornstein SR, Schwarz P, Spranger J, Karpe F, Shuldiner AR, Cooper C, Dedoussis GV, Serrano-Rios M, Morris AD, Lind L, Palmer LJ, Hu FB, Franks PW, Ebrahim S, Marmot M, Kao WH, Pankow JS, Sampson MJ, Kuusisto J, Laakso M, Hansen T, Pedersen O, Pramstaller PP, Wichmann HE, Illig T, Rudan I, Wright AF, Stumvoll M, Campbell H, Wilson JF, Bergman RN, Buchanan TA, Collins FS, Mohlke KL, Tuomilehto J, Valle TT, Altshuler D, Rotter JI, Siscovick DS, Penninx BW, Boomsma DI, Deloukas P, Spector TD, Frayling TM, Ferrucci L, Kong A, Thorsteinsdottir U, Stefansson K, van

Duijn CM, Aulchenko YS, Cao A, Scuteri A, Schlessinger D, Uda M, Ruokonen A, Jarvelin MR, Waterworth DM, Vollenweider P, Peltonen L, Mooser V, Abecasis GR, Wareham NJ, Sladek R, Froguel P, Watanabe RM, Meigs JB, Groop L, Boehnke M, McCarthy MI, Florez JC, Barroso I. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet 2010;42:105–116

4. Saxena R, Hivert MF, Langenberg C, Tanaka T, Pankow JS, Vollenweider P, Lyssenko V, Bouatia-Naji N, Dupuis J, Jackson AU, Kao WH, Li M, Glazer NL, Manning AK, Luan J, Stringham HM, Prokopenko I, Johnson T, Grarup N, Boesgaard TW, Lecoeur C, Shrader P, O'Connell J, Ingelsson E, Couper DJ, Rice K, Song K, Andreasen CH, Dina C, Kottgen A, Le Bacquer O, Pattou F, Taneera J, Steinthorsdottir V, Rybin D, Ardlie K, Sampson M, Qi L, van Hoek M, Weedon MN, Aulchenko YS, Voight BF, Grallert H, Balkau B, Bergman RN, Bielinski SJ, Bonnefond A, Bonnycastle LL, Borch-Johnsen K, Bottcher Y, Brunner E, Buchanan TA, Bumpstead SJ, Cavalcanti-Proenca C, Charpentier G, Chen YD, Chines PS, Collins FS, Cornelis M, G JC, Delplanque J, Doney A, Egan JM, Erdos MR, Firmann M, Forouhi NG, Fox CS, Goodarzi MO, Graessler J, Hingorani A, Isomaa B, Jorgensen T, Kivimaki M, Kovacs P, Krohn K, Kumari M, Lauritzen T, Levy-Marchal C, Mayor V, McAteer JB, Meyre D, Mitchell BD, Mohlke KL, Morken MA, Narisu N, Palmer CN, Pakyz R, Pascoe L, Payne F, Pearson D, Rathmann W, Sandbaek A, Sayer AA, Scott LJ, Sharp SJ, Sijbrands E, Singleton A, Siscovick DS, Smith NL, Sparso T, Swift AJ, Syddall H, Thorleifsson G, Tonjes A, Tuomi T, Tuomilehto J, Valle TT, Waeber G, Walley A, Waterworth DM, Zeggini E, Zhao JH, Illig T, Wichmann HE, Wilson JF, van Duijn C, Hu FB, Morris AD, Frayling TM, Hattersley AT, Thorsteinsdottir U, Stefansson K, Nilsson P, Syvanen AC, Shuldiner AR, Walker M, Bornstein SR, Schwarz P, Williams GH, Nathan DM, Kuusisto J, Laakso M, Cooper C, Marmot M, Ferrucci L, Mooser V, Stumvoll M, Loos RJ, Altshuler D, Psaty BM, Rotter JI, Boerwinkle E, Hansen T, Pedersen O, Florez JC, McCarthy MI, Boehnke M, Barroso I, Sladek R, Froguel P, Meigs JB, Groop L, Wareham NJ, Watanabe RM. Genetic variation in GIPR influences the glucose and insulin responses to an oral glucose challenge. Nat Genet 2010;42:142–148
5. Cauchi S, Meyre D, Choquet H, Dina C, Born C, Marre M, Balkau B, Froguel P. TCF7L2 variation predicts hyperglycemia incidence in a French general population: the data from an epidemiological study on the Insulin Resistance Syndrome (DESIR) study. Diabetes 2006;55:3189–3192
6. Korner A, Berndt J, Stumvoll M, Kiess W, Kovacs P. TCF7L2 gene polymorphisms confer an increased risk for early impairment of glucose metabolism and increased height in obese children. J Clin Endocrinol Metab 2007;92:1956–1960
7. Lyssenko V, Nagorny CL, Erdos MR, Wierup N, Jonsson A, Spegel P, Bugliani M, Saxena R, Fex M, Pulizzi N, Isomaa B, Tuomi T, Nilsson P, Kuusisto J, Tuomilehto J, Boehnke M, Altshuler D, Sundler F, Eriksson JG, Jackson AU, Laakso M, Marchetti P, Watanabe RM, Mulder H, Groop L. Common variant in MTNR1B associated with increased risk of type 2 diabetes and impaired early insulin secretion. Nat Genet 2009;41:82–88
8. Prokopenko I, Langenberg C, Florez JC, Saxena R, Soranzo N, Thorleifsson G, Loos RJ, Manning AK, Jackson AU, Aulchenko Y, Potter SC, Erdos MR, Sanna S, Hottenga JJ, Wheeler E, Kaakinen M, Lyssenko V, Chen WM, Ahmadi K, Beckmann JS, Bergman RN, Bochud M, Bonnycastle LL, Buchanan TA, Cao A, Cervino A, Coin L, Collins FS, Crisponi L, de Geus EJ, Dehghan A, Deloukas P, Doney AS, Elliott P, Freimer N, Gateva V, Herder C, Hofman A, Hughes TE, Hunt S, Illig T, Inouye M, Isomaa B, Johnson T, Kong A, Krestyaninova M, Kuusisto J, Laakso M, Lim N, Lindblad U, Lindgren CM, McCann OT, Mohlke KL, Morris AD, Naitza S, Orru M, Palmer CN, Pouta A, Randall J, Rathmann W, Saramies J, Scheet P, Scott LJ, Scuteri A, Sharp S, Sijbrands E, Smit JH, Song K, Steinthorsdottir V, Stringham HM, Tuomi T, Tuomilehto J, Uitterlinden AG, Voight BF, Waterworth D, Wichmann HE, Willemsen G, Witteman JC, Yuan X, Zhao JH, Zeggini E, Schlessinger D, Sandhu M, Boomsma DI, Uda M, Spector TD, Penninx BW, Altshuler D, Vollenweider P, Jarvelin MR, Lakatta E, Waeber G, Fox CS, Peltonen L, Groop LC, Mooser V, Cupples LA, Thorsteinsdottir U, Boehnke M, Barroso I, Van Duijn C, Dupuis J, Watanabe RM, Stefansson K, McCarthy MI, Wareham NJ, Meigs JB, Abecasis GR. Variants in MTNR1B influence fasting glucose levels. Nat Genet 2009;41:77–81
9. Bouatia-Naji N, Bonnefond A, Cavalcanti-Proenca C, Sparso T, Holmkvist J, Marchand M, Delplanque J, Lobbens S, Rocheleau G, Durand E, De Graeve F, Chevre JC, Borch-Johnsen K, Hartikainen AL, Ruokonen A, Tichet J, Marre M, Weill J, Heude B, Tauber M, Lemaire K, Schuit F, Elliott P, Jorgensen T, Charpentier G, Hadjadj S, Cauchi S, Vaxillaire M, Sladek R, Visvikis-Siest S, Balkau B, Levy-Marchal C, Pattou F, Meyre D, Blakemore AI, Jarvelin MR, Walley AJ, Hansen T, Dina C, Pedersen O, Froguel P. A variant near MTNR1B is associated with increased fasting plasma glucose levels and type 2 diabetes risk. Nat Genet 2009;41:89–94
10. Vaxillaire M, Veslot J, Dina C, Proenca C, Cauchi S, Charpentier G, Tichet J, Fumeron F, Marre M, Meyre D, Balkau B, Froguel P. Impact of common type 2 diabetes risk polymorphisms in the DESIR prospective study. Diabetes 2008;57:244–254
11. Orho-Melander M, Melander O, Guiducci C, Perez-Martinez P, Corella D, Roos C, Tewhey R, Rieder MJ, Hall J, Abecasis G, Tai ES, Welch C, Arnett DK, Lyssenko V, Lindholm E, Saxena R, de Bakker PI, Burtt N, Voight BF, Hirschhorn JN, Tucker KL, Hedner T, Tuomi T, Isomaa B, Eriksson KF, Taskinen MR, Wahlstrand B, Hughes TE, Parnell LD, Lai CQ, Berglund G, Peltonen L, Vartiainen E, Jousilahti P, Havulinna AS, Salomaa V, Nilsson P, Groop L, Altshuler D, Ordovas JM, Kathiresan S. Common missense variant in the glucokinase regulatory protein gene is associated with increased plasma triglyceride and C-reactive protein but lower fasting glucose concentrations. Diabetes 2008;57:3112–3121
12. Sparso T, Andersen G, Nielsen T, Burgdorf KS, Gjesing AP, Nielsen AL, Albrechtsen A, Rasmussen SS, Jorgensen T, Borch-Johnsen K, Sandbaek A, Lauritzen T, Madsbad S, Hansen T, Pedersen O. The GCKR rs780094 polymorphism is associated with elevated fasting serum triacylglycerol, reduced fasting and OGTT-related insulinaemia, and reduced risk of type 2 diabetes. Diabetologia 2008;51:70–75
13. Bouatia-Naji N, Rocheleau G, Van Lommel L, Lemaire K, Schuit F, Cavalcanti-Proenca C, Marchand M, Hartikainen AL, Sovio U, De Graeve F, Rung J, Vaxillaire M, Tichet J, Marre M, Balkau B, Weill J, Elliott P, Jarvelin MR, Meyre D, Polychronakos C, Dina C, Sladek R, Froguel P. A polymorphism within the G6PC2 gene is associated with fasting plasma glucose levels. Science 2008;320:1085–1088
14. Chen WM, Erdos MR, Jackson AU, Saxena R, Sanna S, Silver KD, Timpson NJ, Hansen T, Orru M, Grazia Piras M, Bonnycastle LL, Willer CJ, Lyssenko V, Shen H, Kuusisto J, Ebrahim S, Sestu N, Duren WL, Spada MC, Stringham HM, Scott LJ, Olla N, Swift AJ, Najjar S, Mitchell BD, Lawlor DA, Smith GD, Ben-Shlomo Y, Andersen G, Borch-Johnsen K, Jorgensen T, Saramies J, Valle TT, Buchanan TA, Shuldiner AR, Lakatta E, Bergman RN, Uda M, Tuomilehto J, Pedersen O, Cao A, Groop L, Mohlke KL, Laakso M, Schlessinger D, Collins FS, Altshuler D, Abecasis GR, Boehnke M, Scuteri A, Watanabe RM. Variations in the G6PC2/ABCB11 genomic region are associated with fasting glucose levels. J Clin Invest 2008;118:2620–2628
15. Sabatti C, Service SK, Hartikainen AL, Pouta A, Ripatti S, Brodsky J, Jones CG, Zaitlen NA, Varilo T, Kaakinen M, Sovio U, Ruokonen A, Laitinen J, Jakkula E, Coin L, Hoggart C, Collins A, Turunen H, Gabriel S, Elliot P, McCarthy MI, Daly MJ, Jarvelin MR, Freimer NB, Peltonen L. Genome-wide association analysis of metabolic traits in a birth cohort from a founder population. Nat Genet 2009;41:35–46
16. Wu Y, Li H, Loos RJ, Yu Z, Ye X, Chen L, Pan A, Hu FB, Lin X. Common variants in CDKAL1, CDKN2A/B, IGF2BP2, SLC30A8, and HHEX/IDE genes are associated with type 2 diabetes and impaired fasting glucose in a Chinese Han population. Diabetes 2008;57:2834–2842
17. Kelliny C, Ekelund U, Andersen LB, Brage S, Loos RJ, Wareham NJ, Langenberg C. Common genetic determinants of glucose homeostasis in healthy children: the European Youth Heart Study. Diabetes 2009;58:2939–2945
18. Gambino R, Bo S, Gentile L, Musso G, Pagano G, Cavallo-Perin P, Cassader M. Transcription factor 7-like 2 (TCF7L2) polymorphism and hyperglycemia in an adult Italian population-based cohort. Diabetes Care 2010;33:1233–1235
19. Raitakari OT, Ronnemaa T, Huupponen R, Viikari L, Fan M, Marniemi J, Hutri-Kahonen N, Viikari JS, Lehtimaki T. Variation of the transcription factor 7-like 2 (TCF7L2) gene predicts impaired fasting glucose in healthy young adults: the Cardiovascular Risk in Young Finns Study. Diabetes Care 2007;30:2299–2301
20. Hallmans G, Agren A, Johansson G, Johansson A, Stegmayr B, Jansson JH, Lindahl B, Rolandsson O, Soderberg S, Nilsson M, Johansson I, Weinehall L. Cardiovascular disease and diabetes in the Northern Sweden Health and Disease Study Cohort - evaluation of risk factors and their interactions. Scand J Public Health 2003;61(Suppl):18–24
21. World Health Organization. *Definitions, Diagnosis and Classification of Diabetes Mellitus and its Complications: Part 1: Diagnosis & Classification of Diabetes Mellitus*. Geneva, World Health Org., 1999
22. Norberg M, Wall S, Boman K, Weinehall L. The Vasterbotten Intervention Programme: background, design and implications. Glob Health Action 2010;22:3
23. Franks PW, Rolandsson O, Debenham SL, Fawcett KA, Payne F, Dina C, Froguel P, Mohlke KL, Willer C, Olsson T, Wareham NJ, Hallmans G, Barroso I, Sandhu MS. Replication of the association between variants in WFS1 and risk of type 2 diabetes in European populations. Diabetologia 2008;51:458–463
24. Renstrom F, Payne F, Nordstrom A, Brito EC, Rolandsson O, Hallmans G, Barroso I, Nordstrom P, Franks PW. Replication and extension of genome-

wide association study results for obesity in 4923 adults from northern Sweden. Hum Mol Genet 2009;18:1489–1496
25. Cornelis MC, Qi L, Zhang C, Kraft P, Manson J, Cai T, Hunter DJ, Hu FB. Joint effects of common genetic variants on the risk for type 2 diabetes in U.S. men and women of European ancestry. Ann Intern Med 2009;150:541–550
26. Fontaine-Bisson BR, F. Rolandsson O. The MAGIC investigators. Payne, F. Hallmans G Barroso, I. Franks, P.W. Evaluating the discriminative power of multi-trait genetic risk scores for type 2 diabetes in a northern Swedish population. Diabetologia 2010;53:2155–2162.
27. Wilson PW, Meigs JB, Sullivan L, Fox CS, Nathan DM, D'Agostino RB Sr. Prediction of incident diabetes mellitus in middle-aged adults: the Framingham Offspring Study. Arch Intern Med 167:1068–1074, 2007
28. Genuth S, Alberti KG, Bennett P, Buse J, Defronzo R, Kahn R, Kitzmiller J, Knowler WC, Lebovitz H, Lernmark A, Nathan D, Palmer J, Rizza R, Saudek C, Shaw J, Steffes M, Stern M, Tuomilehto J, Zimmet P. Follow-up report on the diagnosis of diabetes mellitus. Diabetes Care 2003;26:3160–3167
29. Holm S. A simple sequentially rejective Bonferroni test procedure. Scan J of Stat 1979;6:65–70
30. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 1988;44:837–845
31. Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PI, Chen H, Roix JJ, Kathiresan S, Hirschhorn JN, Daly MJ, Hughes TE, Groop L, Altshuler D, Almgren P, Florez JC, Meyer J, Ardlie K, Bengtsson Bostrom K, Isomaa B, Lettre G, Lindblad U, Lyon HN, Melander O, Newton-Cheh C, Nilsson P, Orho-Melander M, Rastam L, Speliotes EK, Taskinen MR, Tuomi T, Guiducci C, Berglund A, Carlson J, Gianniny L, Hackett R, Hall L, Holmkvist J, Laurila E, Sjogren M, Sterner M, Surti A, Svensson M, Svensson M, Tewhey R, Blumenstiel B, Parkin M, Defelice M, Barry R, Brodeur W, Camarata J, Chia N, Fava M, Gibbons J, Handsaker B, Healy C, Nguyen K, Gates C, Sougnez C, Gage D, Nizzari M, Gabriel SB, Chirn GW, Ma Q, Parikh H, Richardson D, Ricke D, Purcell S. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 2007;316:1331–1336
32. Einarsdottir E, Egerbladh I, Beckman L, Holmberg D, Escher SA. The genetic population structure of northern Sweden and its implications for mapping genetic diseases. Hereditas 2007;144:171–180
33. Reppert SM, Godson C, Mahle CD, Weaver DR, Slaugenhaupt SA, Gusella JF. Molecular characterization of a second melatonin receptor expressed in human retina and brain: the Mel1b melatonin receptor. Proc Natl Acad Sci U S A 1995;92:8734–8738
34. Peschke E. Melatonin, endocrine pancreas and diabetes. J Pineal Res 2008;44:26–40
35. Ekmekcioglu C. Melatonin receptors in humans: biological role and clinical relevance. Biomed Pharmacother 2006;60:97–108
36. Scheer FA, Hilton MF, Mantzoros CS, Shea SA. Adverse metabolic and cardiovascular consequences of circadian misalignment. Proc Natl Acad Sci U S A 2009;106:4453–4458
37. Peschke E, Frese T, Chankiewitz E, Peschke D, Preiss U, Schneyer U, Spessert R, Muhlbauer E. Diabetic Goto Kakizaki rats as well as type 2 diabetic patients show a decreased diurnal serum melatonin level and an increased pancreatic melatonin-receptor status. J Pineal Res 2006;40:135–143
38. Green CB, Takahashi JS, Bass J. The meter of metabolism. Cell 2008;134:728–742
39. Lyssenko V, Jonsson A, Almgren P, Pulizzi N, Isomaa B, Tuomi T, Berglund G, Altshuler D, Nilsson P, Groop L. Clinical risk factors, DNA variants, and the development of type 2 diabetes. N Engl J Med 2008;359:2220–2232
40. Meigs JB, Shrader P, Sullivan LM, McAteer JB, Fox CS, Dupuis J, Manning AK, Florez JC, Wilson PW, D'Agostino RB Sr, Cupples LA. Genotype score in addition to common risk factors for prediction of type 2 diabetes. N Engl J Med 2008;359:2208–2219