Genetic Risk Score of 46 Type 2 Diabetes Risk Variants Associates With Changes in Plasma Glucose and Estimates of Pancreatic β-Cell Function Over 5 Years of Follow-Up

Ehm A. Andersson,[1] Kristine H. Allin,[1] Camilla H. Sandholt,[1] Anders Borglykke,[2] Cathrine J. Lau,[2] Rasmus Ribel-Madsen,[1] Thomas Sparsø,[1] Johanne M. Justesen,[1] Marie N. Harder,[1] Marit E. Jørgensen,[3] Torben Jørgensen,[2,4,5] Torben Hansen,[1,6] and Oluf Pedersen[1,3,7]

More than 40 genetic risk variants for type 2 diabetes have been validated. We aimed to test whether a genetic risk score associates with the incidence of type 2 diabetes and with 5-year changes in glycemic traits and whether the effects were modulated by changes in BMI and lifestyle. The Inter99 study population was genotyped for 46 variants, and a genetic risk score was constructed. During a median follow-up of 11 years, 327 of 5,850 individuals developed diabetes. Physical examinations and oral glucose tolerance tests were performed at baseline and after 5 years (n = 3,727). The risk of incident type 2 diabetes was increased with a hazard ratio of 1.06 (95% CI 1.03–1.08) per risk allele. While the population in general had improved glucose regulation during the 5-year follow-up period, each additional allele in the genetic risk score was associated with a relative increase in fasting, 30-min, and 120-min plasma glucose values and a relative decrease in measures of β-cell function over the 5-year period, whereas indices of insulin sensitivity were unaffected. The effect of the genetic risk score on 5-year changes in fasting plasma glucose was stronger in individuals who increased their BMI. In conclusion, a genetic risk score based on 46 variants associated strongly with incident type 2 diabetes and 5-year changes in plasma glucose and β-cell function. Individuals who gain weight may be more susceptible to the cumulative impact of type 2 diabetes risk variants on fasting plasma glucose. ***Diabetes*** **62:3610–3617, 2013**

Type 2 diabetes is a complex metabolic disorder where both environment and genetic disposition act in concert to cause the disease. Over the last few years, genome-wide association studies and large-scale genotyping studies using the Metabochip array have identified close to 50 variants associating with type 2 diabetes in cross-sectional studies of whites (1 [rev. in 2]).

From the [1]Novo Nordisk Foundation Center for Basic Metabolic Research, Section of Metabolic Genetics, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; the [2]Research Centre for Prevention and Health, Glostrup University Hospital, Glostrup, Denmark; [3]Steno Diabetes Center A/S, Gentofte, Denmark; the [4]Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; the [5]Faculty of Medicine, Aalborg University, Aalborg, Denmark; the [6]Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark; and the [7]Faculty of Health Sciences, University of Aarhus, Aarhus, Denmark.

Corresponding author: Ehm A. Andersson, ehm.andersson@sund.ku.dk.

Received 3 March 2013 and accepted 26 June 2013.

DOI: 10.2337/db13-0362

This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db13-0362/-/DC1.

The vast majority of genetic association studies have been performed in a cross-sectional study design. In cross-sectional studies, only a snapshot of the effect can be evaluated, and it is not clear from these studies to what extent the genetic risk variants may affect changes over time. It is of relevance to address the fluctuations in glycemic traits in order to obtain improved understanding of the timing and cause of progression toward disease. Whereas recent prospective studies in populations of varying age, metabolic status, and ethnicity have shown that genetic risk is associated with incidence of type 2 diabetes (3–8), very few studies have investigated the effect of genetic variants on changes in quantitative glycemic traits over time in large study samples (3,9–11). How changes in BMI and lifestyle may interact with genetic factors to modify glucose homeostasis over time has, moreover, been even less investigated (6,12). Since the effects of common single variants are generally modest and of limited clinical significance, it is more likely that risk assessment of a combination of variants will be useful to identify subgroups at increased risk of type 2 diabetes that would require more aggressive intervention and monitoring.

In the current study of the Danish population-based Inter99 study sample, we aimed to investigate *1*) the association between a genetic risk score of 46 validated type 2 diabetes risk variants and the incidence of type 2 diabetes, *2*) the association between the genetic risk score and changes in oral glucose tolerance test (OGTT)-derived glycemic traits over 5 years, and *3*) whether specific changes in BMI and lifestyle factors, including smoking, physical activity, and diet, may interact with the effect of the genetic risk score on observed changes in glycemic traits.

RESEARCH DESIGN AND METHODS

Inter99 study population. The Inter99 study (clinical trial reg. no. NCT00289237, clinicaltrials.gov) is a population-based nonpharmacological intervention study for ischemic heart disease conducted at the Research Centre for Prevention and Health in Glostrup, Denmark (www.inter99.dk). A randomized sample of 13,016 individuals living in Copenhagen County (30–60 years) was drawn from the Civil Registration System and further prerandomized into high-intensity (90%) and low-intensity (10%) intervention groups. A total of 6,784 (52%) subjects attended the baseline health examination (median age 45 years). All participants received individual lifestyle counseling at the baseline examination, focused on habits of smoking, physical activity, dietary intake, and use of alcohol. The high-intensity group was in addition offered group-based lifestyle counseling if considered at high risk for ischemic heart disease. Follow-up examinations were conducted after 5 years with a participation rate of 66% (n = 4,511) (13–15).

Incident type 2 diabetes. A flowchart of the current study is shown in Fig. 1. Information on incident type 2 diabetes was collected from the Danish National Diabetes Register (16). Information included date of inclusion and

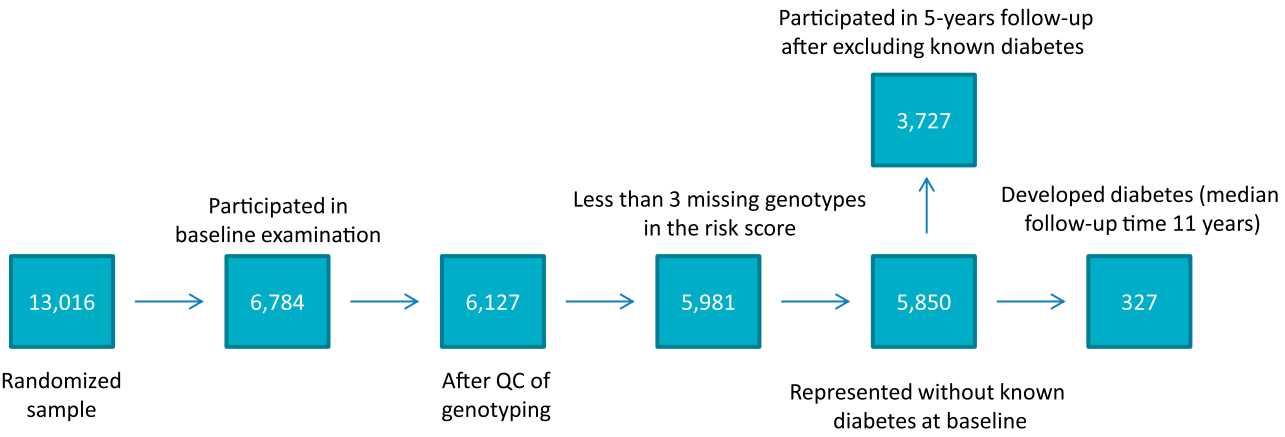

FIG. 1. Flowchart over the number of study participants of the current study.

criteria for inclusion. Data until 31 December 2010 were available. Information on death and emigration was obtained from the Danish Central Person Register until 31 December 2010.

Anthropometrics, biochemical measurements, and lifestyle factors. At the baseline and 5-year follow-up examination, anthropometric and biochemical measures were obtained after an overnight fast. Weight (kilograms) and height (centimeters) were measured in light indoor clothes and without shoes. All participants without previously diagnosed diabetes were characterized by a standardized 75-g OGTT with plasma glucose and serum insulin measured at fasting and 30 and 120 min after the oral glucose load. Plasma glucose was analyzed using the hexokinase/glucose-6-phosphate dehydrogenase technique (Boehringer Mannheim, Germany), which has an intra-assay precision of coefficient of variation (CV) 1.1% and an interassay precision of CV 2.3%. Serum insulin (excluding des-31,32 and intact proinsulin) was measured using the AutoDELFIA insulin kit (Perkin-Elmer/Wallac, Turku, Finland), with an interassay precision of CV <6%. The conditions under which plasma glucose and serum insulin were measured were identical at baseline and at the 5-year follow-up examination.

Screen-detected diabetes was defined according to World Health Organization 1999 criteria (17). All lifestyle factors were estimated from self-reported questionnaire data as previously described (18–20). Written informed consent was obtained from all participants. The study was approved by the regional ethics committee of Copenhagen and is in accordance with the principles of the Declaration of Helsinki II.

Genotyping. Genotyping was performed with the Metabochip (21) using the Illumina HiScan (Illumina, San Diego, CA). Genotypes were called using the genotyping module (version 1.9.4) of GenomeStudio software (version 2011.1; Illumina), and custom cluster data generated from ~6,000 Danish DNA samples were analyzed on the same Illumina HiScan. Quality control removed 1st- and 2nd-degree related individuals (n = 119), individuals with an extreme inbreeding coefficient ($F > 0.1$ or $F < 0.1$, n = 25), individuals with a low genotype call rate (call rate <90%, n = 30), individuals with mislabeled sex (n = 11), and individuals with a high discordance rate to previously genotyped single nucleotide polymorphisms (concordance <80%, n = 65), leaving 6,127 individuals who passed all quality-control criteria. The average call rate for all SNPs on the Metabochip was 99.0%. Of the previously identified type 2 diabetes risk variants, five were not present on the Metabochip and did not have perfect proxies, and thus, these were genotyped by KASPar (KBioscience, Hoddesdon, U.K.) with success rates >96% and error rates <0.5%. All variants obeyed Hardy-Weinberg equilibrium ($P > 0.01$), except *HMGA2*, rs1531343 (P = 0.0043). An overview of the 46 variants genotyped is provided in Supplementary Table 1. The 46 variants were chosen based on genome-wide significant ($P < 5 \times 10^{-8}$) association signals for the risk of type 2 diabetes identified or confirmed in European populations. Variants only found to be associated with type 2 diabetes in Asian populations were not included. The risk variant in *DUSP* is not included in the current study because of the location on the X-chromosome. Variants in or near *FTO* and *MC4R* were not included owing to a primary effect on obesity.

Genetic risk score. Individuals with more than two missing genotypes were excluded (n = 146). For individuals with one (n = 588) or two (n = 109) missing genotypes, genotypes were imputed by assigning the most common genotype in Inter99 for the missing variants. A simple genetic risk score was constructed by summing up the number of risk alleles over 46 variants for each individual. The median risk score was 50, ranging from 31 to 66. A weighted genetic risk score was created as previously described (22) and by weighting each risk allele with the effect size (the natural log of the odds ratios) for risk of type 2 diabetes reported by the largest meta-analyses performed (1). Effect sizes used for weighting for the specific variants or the best proxies can be seen in Supplementary Table 1. All results are from analyses of the simple genetic risk score. The weighted genetic risk score was used to check whether weighting each allele would change the results obtained for the simple risk score.

Statistical analyses. Data were analyzed using the STATA statistical software (version 12.1; StataCorp, College Station, TX) and RGui, version 2.13.2 (http://www.r-project.org/). The Kaplan-Meier method was used to plot cumulative incidence of type 2 diabetes against age, and the log-rank trend test was used to test for differences between tertiles of the genetic risk score. Cox proportional hazards regression models were used to address the risk of incident type 2 diabetes. Individuals with self-reported diabetes at the baseline examination as well as individuals present in the Danish National Diabetes Registry before the baseline examination were excluded from the present analyses of incident type 2 diabetes. To automatically adjust for age, we used left truncation and age as a time scale. We tested the assumption of proportional hazards graphically by plotting log(cumulative hazard) as a function of age and with a test based on Schoenfeld residuals. We detected no major violations of the proportional hazards assumption.

Paired t tests were used to test for differences in quantitative traits at baseline and at follow-up. Linear regression was used to model the effect of the genetic risk score on 5-year changes in glycemic traits. Individuals with known diabetes at the baseline or at the follow-up examination were excluded from the present analyses of glycemic traits. Values of plasma glucose, serum insulin, and all indices were logarithmically transformed before analyses to obtain a normal distribution. Changes in lifestyle measures of physical activity, smoking, and diet were individually defined into three classes; healthier, unhealthier, or no change.

The fully adjusted regression model included the additive genetic risk score, baseline age, sex, baseline BMI, change in BMI, and baseline value of the trait analyzed.

$$\begin{aligned}\text{5-year outcome} = {} & b_0 + b_1\,\text{risk} + b_2\,\text{age} + b_3\,\text{sex} + b_4\text{baseline BMI} \\ & + b_5\,\Delta\text{BMI} + b_6\,\text{baseline value of outcome variable}\end{aligned}$$

Additive interactions between the genetic risk score and changes in BMI and changes in lifestyle factors (physical activity, diet, and smoking) were tested by including the interaction term "change in BMI × risk score" or "change in lifestyle factor × risk score," where BMI change is a continuous variable and change of lifestyle is a three-class variable, into the fully adjusted regression model.

$$\begin{aligned}\text{5-year outcome} = {} & b_0 + b_1\,\text{risk} + b_2\,\text{age} + b_3\,\text{sex} + b_4\,\text{baseline BMI} \\ & + b_5\,\Delta\text{BMI} + b_6\,\text{baseline value of outcome variable} \\ & + b_7\,\text{risk} \times \Delta\text{BMI}\end{aligned}$$

or

$$\begin{aligned}\text{5-year outcome} = {} & b_0 + b_1\,\text{risk} + b_2\,\text{age} + b_3\,\text{sex} + b_4\,\text{baseline BMI} \\ & + b_5\,\Delta\text{BMI} + b_6\,\Delta\text{lifestyle} \\ & + b_7\,\text{baseline value of outcome variable} \\ & + b_8\,\text{risk} \times \Delta\text{lifestyle}\end{aligned}$$

RESULTS

Incidence of diabetes. A total of 5,850 individuals had information on the genetic risk score and did not have known diabetes at the baseline examination. These individuals were followed with a median follow-up time of 11 years, and 327 individuals developed type 2 diabetes. The genetic risk score was strongly associated with incident diabetes (Fig. 2 and Table 1). The cumulative incidence of type 2 diabetes as a function of age increased with tertiles of the genetic risk score (log-rank trend test, $P = 0.0004$) (Fig. 2). Each additional allele increased the risk of type 2 diabetes with a multifactor-adjusted hazard ratio of 1.06 (95% CI 1.03–1.09, $P = 1.1 \times 10^{-4}$) (Table 1). To elucidate differential effects over age, we stratified the population into two age-groups; below or above 50 years of age at censoring. In a Cox regression model adjusted for age, sex, and BMI, the hazard ratio was 1.10 (95% CI 1.04–1.16) in individuals age <50 years, while it was 1.06 (95% CI 1.02–1.09) in individuals >50 years. No interaction with age-group was observed ($P = 0.19$).

Five-year changes in quantitative glycemic traits. To address the underlying phenotypes causing the increased risk of diabetes, we tested whether the genetic risk score had measureable effects on changes in quantitative glycemic traits during the 5 years of follow-up. A total of 3,727 had information on the genetic risk score and did not have known diabetes at the baseline or at the follow-up examination. On average, after 5 years the population had improved glycemic regulation (15). They experienced an average decrease in fasting, 30-min, and 120-min plasma glucose values, as well as in fasting serum insulin values. They were less insulin resistant and increased their disposition index, although there was an average weight gain in the population. A borderline improvement of corrected insulin response occurred, but no changes in the insulinogenic index were observed (Table 2).

In the fully adjusted model, each additional allele in the genetic risk score associated with a relative increase in plasma glucose at fasting and during an OGTT over the 5-year follow-up period (Table 3). Moreover, the genetic risk score associated with relative decrements in insulinogenic index, corrected insulin response, and disposition indices—all surrogate measures of pancreatic β-cell function. No associations with changes in values of serum insulin or indices of insulin sensitivity were observed (Table 3). Similar results were seen when changes in lifestyle factors were included in the regression model (data not shown). Although the genetic risk score associated with increases in plasma glucose per allele, the average individual still had levels lower over the 5-year period. Consequently, individuals at increasingly higher genetic risk improved their plasma glucose values less compared with individuals with a lower genetic risk.

When subgroups of upper versus lower decile of genetic risk were compared, the difference evolving over the 5-year period between these two groups was 2.3% for fasting plasma glucose, which on average corresponds to ~0.14 mmol/L. With these results extrapolated to a follow-up time of 20 years (representing an average year span in Inter99 from 45 to 65 years of age) and with linearity assumed in the genetic effects over the 20 years, this would correspond to a difference between the groups of ~0.54 mmol/L for fasting plasma glucose. For comparison, the effect of a one-unit BMI increase over 5 years is associated with a 1% (~0.05 mmol/L) relative increase in fasting plasma glucose in this population.

To understand the effect of adding more risk variants to the genetic risk score, we calculated the effect of a genetic risk score comprising 15 variants overlapping the study by Lyssenko et al. (3). When we included only these 15 variants in the genetic risk score, the effect on changes in fasting plasma glucose was 0.22% (95% CI 0.11–0.32, $P = 1 \times 10^{-4}$ per allele) compared with a per allele effect of 0.18% (95% CI 0.12–0.24, $P = 9 \times 10^{-9}$) when including 46 variants in the genetic risk score. Based on the estimated effect sizes and the number of variants included in two genetic risk scores, a theoretical maximum difference between individuals carrying no risk alleles and individuals

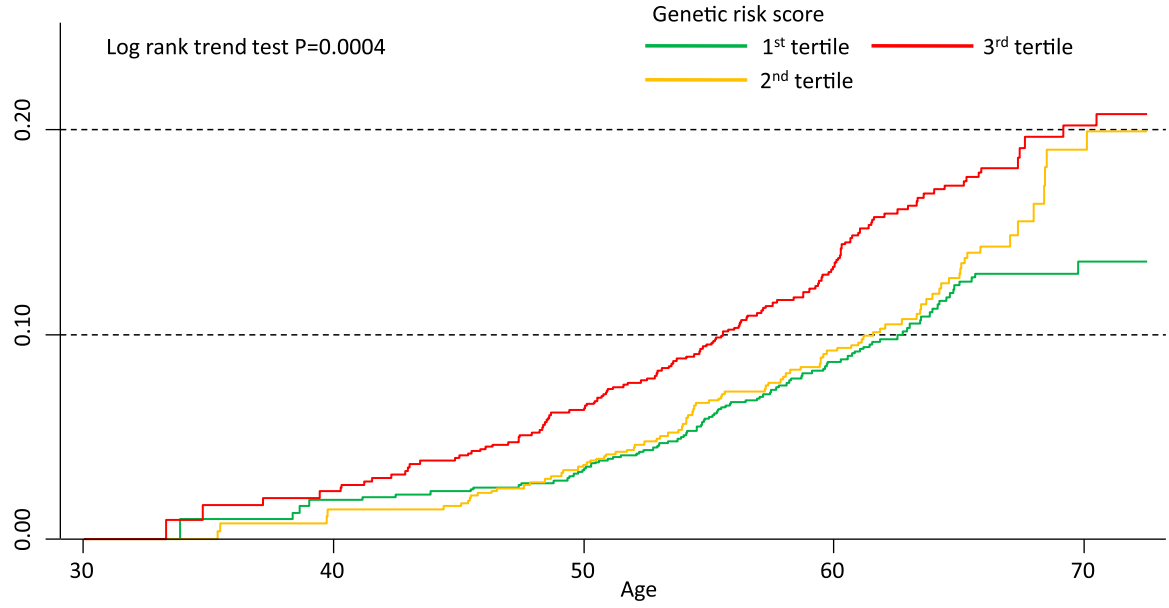

FIG. 2. Plot showing the cumulative incidence of type 2 diabetes for tertiles of the genetic risk score (median follow-up 11 years). The first tertile is low genetic risk, and the third is high genetic risk. (n = 5,850, cases = 327.)

TABLE 1
Associations between the genetic risk score and the risk of incident late-onset diabetes in the Inter99 cohort (median follow-up time 11 years) (n = 5,850)

	Model 1	Model 2	Model 3
Hazard ratio per risk allele (95% CI)	1.06 (1.03–1.08)	1.06 (1.03–1.09)	1.06 (1.03–1.09)
P	5×10^{-5}	9×10^{-6}	1×10^{-4}

Data are hazard ratios per risk allele and P values of three different models. All models are adjusted for age using left truncation and age as time scale. Three models were analyzed: model 1, a simple model including only the genetic risk score, sex, and age; model 2, a model including genetic risk score, sex, age, and baseline BMI; model 3, a fully adjusted model including genetic risk score, sex, age, baseline BMI, baseline smoking habits, baseline measure of physical activity, and baseline measure of diet.

carrying the maximum number of risk alleles (30 or 92) was calculated for changes in fasting plasma glucose. This corresponded to a theoretical maximum change of ~7% for the risk score comprising 15 variants and ~17% for the updated score comprising 46 variants.

Interactions between genetic risk score and changes in BMI and lifestyle factors. For exploration of whether the effect of the genetic risk score on changes in glycemic traits was affected by changes in BMI or lifestyle factors, we tested for potential interactions. Since the genetic risk score had the strongest statistical association with fasting plasma glucose, potential interactions were only assessed for this outcome to achieve the highest statistical power.

We found an interaction between the genetic risk score and BMI changes over 5 years in relation to changes in fasting plasma glucose (P = 0.004). An increased BMI over the 5-year period resulted in a larger effect of the genetic risk score on changes in fasting plasma glucose (Fig. 3). Individuals who gained weight and who were in the highest tertile of genetic risk on average increased their unadjusted fasting plasma glucose values by 0.19%, whereas individuals who lost weight (or were weight stabile) and belonged to the lowest tertile of genetic risk on average decreased their unadjusted fasting plasma glucose by −3.3% over 5 years (Fig. 3*A*). When dividing the population into quartiles of BMI change, we observed a stepwise increasing effect of

TABLE 2
Overview of the average changes from baseline to 5-year follow-up examinations in the Inter99 cohort (n = 3,727)

	Change from baseline to follow-up	P for change from baseline to follow-up
Participants (n)	3,727	
BMI (kg/m^2)	0.42 (0.36–0.48)	1×10^{-45}
Fasting glucose (mmol/L)	−0.08 (−0.09 to −0.06)	3×10^{-24}
30-min glucose (mmol/L)	−0.02 (−0.08 to 0.03)	0.3
120-min glucose (mmol/L)	−0.13 (−0.19 to −0.08)	1×10^{-6}
Fasting insulin (%)	−8 (−9 to −6)	2×10^{-16}
30-min insulin (%)	2 (1–4)	0.009
120-min insulin (%)	6 (3–8)	2×10^{-6}
HOMA index of insulin resistance (%)	−9 (−12 to −7)	2×10^{-20}
Matsuda index of insulin sensitivity (%)	3 (1–4)	0.002
Corrected insulin response (%)	2 (0–5)	0.04
Insulinogenic index (%)	1 (−1 to 3)	0.4
Disposition index 1 (%)	8 (6–12)	4×10^{-10}
Disposition index 2 (%)	5 (3–8)	5×10^{-5}
Weight loss/gain (n)		
Weight gain	2,286	
Weight loss (or stable)	1,441	
Physical activity (n)		
Less active	877	
No change	1,748	
More active	831	
Dietary score (n)		
Unhealthier diet	366	
No change	2,297	
Healthier diet	912	
Smoking (n)		
More intensive smoking	119	
No change	3,199	
Less intensive smoking or cessation	383	

Data are means (95% CI) unless otherwise indicated. Data are presented for individuals with information on genetic risk who participated in both baseline and follow-up examinations after exclusion of individuals with known diabetes. Paired t tests were used to test for differences in quantitative traits at baseline and at follow-up. Values of serum insulin and derived indices were logarithmically transformed, and the change is shown on the log scale.

TABLE 3
Associations between the genetic risk score and changes in glycemic traits obtained during OGTTs in the Inter99 cohort (n = 3,727)

	Effect per risk allele % (95% CI)	P
Changes in plasma glucose		
Fasting	0.18 (0.12–0.24)	9×10^{-9}
30 min	0.31 (0.19–0.44)	5×10^{-7}
120 min	0.39 (0.20–0.58)	6×10^{-5}
Changes in serum insulin		
Fasting	0.04 (−0.29 to 0.36)	0.8
30 min	−0.36 (−0.72 to 0.00)	0.05
120 min	0.14 (−0.36 to 0.63)	0.6
Changes in measures of β-cell function		
Insulinogenic index	−0.92 (−1.4 to −0.43)	3×10^{-4}
Corrected insulin response	−1.2 (−1.7 to −0.77)	2×10^{-7}
Changes in measures of insulin sensitivity		
HOMA index of insulin resistance	0.23 (−0.12 to 0.58)	0.2
Matsuda index of insulin sensitivity	−0.09 (−0.42 to 0.24)	0.6
Changes in disposition indices		
Disposition index 1	−1.3 (−1.8 to −0.68)	2×10^{-5}
Disposition index 2	−1.4 (−2.0 to −0.89)	2×10^{-7}

All traits were logarithmically transformed before analyses, and effects sizes are given in %. All analyses were adjusted for sex, baseline age, baseline BMI, change in BMI, and baseline value of the trait analyzed (logarithmically transformed). Insulinogenic index was calculated as follows: (serum insulin at 30 min − fasting serum insulin)/(plasma glucose at 30 min − fasting plasma glucose). Corrected insulin response was calculated as follows: (100 × serum insulin at 30 min)/[plasma glucose at 30 min × (plasma glucose at 30 min − 3.89)]. Homeostasis model assessment index of insulin resistance was calculated as follows: [(fasting plasma glucose × fasting serum insulin)/135]. Matsuda index of insulin sensitivity was calculated as {10,000/$\sqrt{}$[(fasting plasma glucose × fasting serum insulin) × (mean plasma glucose × mean serum insulin during OGTT)]}. Disposition index 1 was calculated as insulinogenic index/homeostasis model assessment (HOMA) of insulin resistance. Disposition index 2 was calculated as corrected insulin response × Matsuda index of insulin sensitivity.

the genetic risk score with higher quartiles of BMI change in the fully adjusted model (Fig. 3*B*).

No interactions between the genetic risk score or changes in smoking, physical activity, or diet were observed (P = 0.20–0.44). Throughout all analyses, similar results were obtained for the weighted genetic risk score (data not shown).

DISCUSSION

In the current study, we have performed prospective analyses of a genetic risk score comprising 46 genetic risk variants for type 2 diabetes in the Danish Inter99 population of middle-aged to elderly people without accounting for single variant associations or gene-gene interactions.

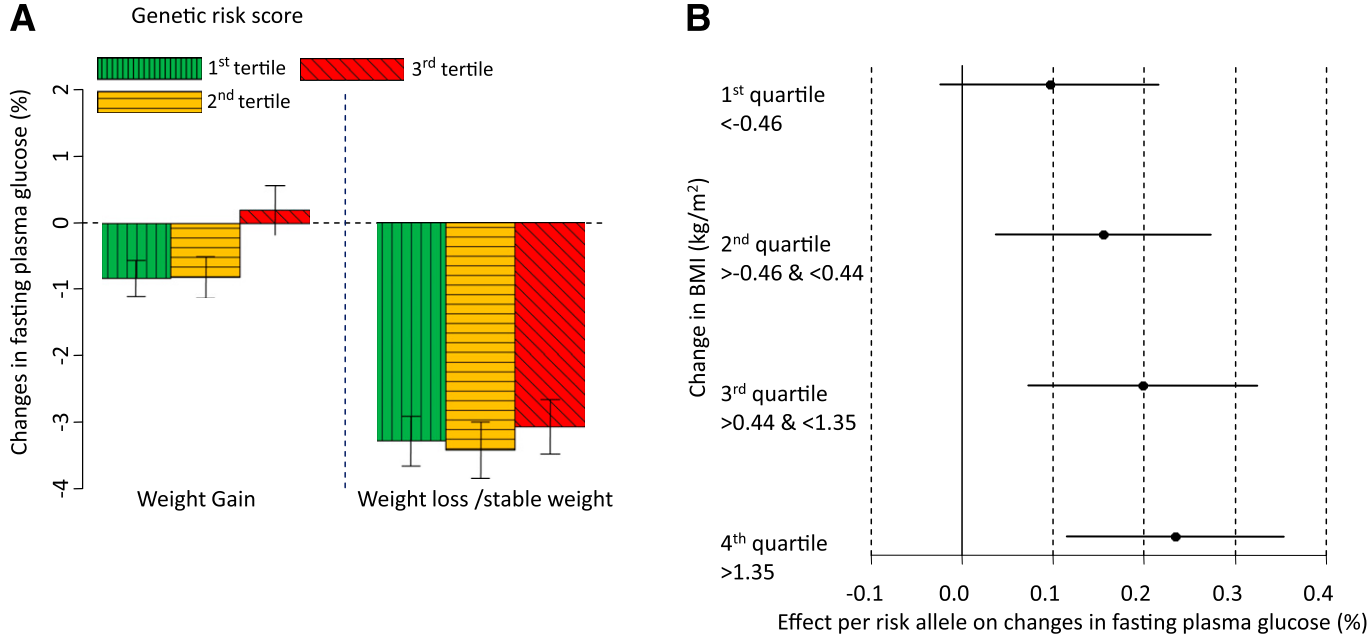

FIG. 3. Interaction between the genetic risk score and BMI change on changes in fasting plasma glucose over 5 years. *A*: The effect of tertiles of the genetic risk score are given on changes in fasting plasma glucose in individuals who gained weight (n = 2,286) and in individuals who lost weight or were weight stable (n = 1,441) over 5 years. The first tertile is low genetic risk, and the third is high genetic risk. *B*: The effect sizes and 95% CIs per allele of the genetic risk score on changes in fasting plasma glucose in the fully adjusted model are shown for different quartiles of BMI changes in the population (n = 3,727).

In line with previous studies, we found that an updated genetic risk score was strongly associated with incident type 2 diabetes (3–8), although effect sizes were modest. What is new, we found that in the Inter99 population the genetic risk score had measureable effects on changes in plasma glucose and estimates of pancreatic β-cell function during 5 years of follow-up. Of interest, we report an interaction suggesting that individuals increasing their BMI are more susceptible to the effect of the genetic risk.

Incident diabetes. Our reported hazard ratio of 1.06 per allele resides in the lower end of the spectrum previously reported (3–8). One explanation may be that Inter99 is a lifestyle intervention study and it has been suggested that lifestyle intervention may attenuate the effect of genetic risk. This has been seen particularly for the variant in *TCF7L2* in the Diabetes Prevention Program (23) and Finnish Diabetes Prevention Study (24) and was also suggested when a genetic risk score of 34 variants was investigated (6). Longer follow-up times have also been suggested to increase the effect of genetic variants. Moreover, it has recently been observed that the effects of genetic variants are stronger with younger age (<50 years) (5). When stratifying our study population into age-groups below or above 50 years, we observed a higher hazard ratio in the younger group, thus suggesting that also in Inter99 the genetic risk of type 2 diabetes may be slightly stronger with younger age onset; however, there was no significant interaction.

A number of reports have lately addressed the predictive ability of genetic risk scores in the progression to type 2 diabetes and collectively conclude that genetic risk assessment at best modestly improves prediction and reclassification over conventional risk factors such as age, BMI, and family history (3–8). Some of these reports have longer follow-up times and include more individuals than our study, and thus, it was not in the scope of the current study to investigate this further.

Five-year changes in quantitative glycemic traits. It was not known whether an updated genetic risk score of 46 type 2 diabetes risk variants would affect changes in glycemic traits during 5 years of follow-up or how the genetic risk may interact with changes in BMI and lifestyle factors. A previous study in the Botnia cohort including 2,444 Scandinavian individuals evaluated the effect of a genetic risk score, comprising 16 type 2 diabetes risk variants, on changes in OGTT-derived glycemic measures during 8 years of follow-up. The authors found that this genetic risk score associated with decreased insulin secretion and disposition index, whereas it did not affect changes in insulin sensitivity over time (3). In line with the results from the Botnia study, we found that despite a 5-year lifestyle intervention a genetic risk score comprising 46 risk variants associated with 5-year changes in plasma glucose and measures of β-cell function, but it did not associate with changes in measures of insulin sensitivity. In accordance with our results, the majority of variants included in the risk score have in cross-sectional studies been suggested to modulate the risk of type 2 diabetes through β-cell dysfunction and not through insulin resistance (rev. in 25).

The estimated per-allele effect size was slightly lower for the genetic risk score based on all 46 variants compared with a risk score based on only 15 variants from the study by Lyssenko et al. (3), while the theoretical difference in change in fasting plasma glucose between individuals carrying no risk alleles and maximal number of risk alleles was notably larger for the risk score based on all 46 variants. Together, these results suggest that inclusion of more risk variants with relatively lower individual effect sizes observed at the cross-sectional level does not infer uncertainty in the statistical models but strengthens the association between a genetic risk score and changes in fasting plasma glucose.

A recent study using the Whitehall II population reported that a genetic risk score of six variants associating with 2-h glucose values had a stronger impact on levels of 2-h glucose with increasing age, while a genetic risk score of 16 fasting plasma glucose variants had constant effects on fasting plasma glucose over the age span investigated (11). In the current study, we did not find differential effects of the genetic risk score on changes in fasting or 2-h plasma glucose among different age-groups, and we did not demonstrate any interactions with age (data not shown). Of note, the Whitehall II study (11) evaluated variants specifically associated with fasting or 2-h glucose values in nondiabetic individuals, which are only partially overlapping those variants found to increase risk of type 2 diabetes investigated in the current study.

Interactions between genetic risk score and changes in BMI and lifestyle factors. We observed an interaction between the genetic risk score and changes in BMI, suggesting that individuals increasing their BMI may be more susceptible to the effects of type 2 diabetes risk variants. From a different point of view, this suggests that weight loss may attenuate the effect of the genetic risk, since the genetic risk score had a smaller effect in the group of individuals who decreased their BMI. Even though a large part of the general population will benefit from weight loss, these results suggest that it may be a particularly fruitful intervention for those at increased genetic risk of type 2 diabetes. In line with this, a recent study reported that intensive lifestyle intervention was effective in preventing type 2 diabetes at any genetic risk but with suggestive evidence that it may be more effective in the group with highest genetic risk (6).

The influence of obesity on genetic risk of type 2 diabetes has been debated. A large genome-wide association study stratifying lean and obese cases found that the majority of previously validated type 2 diabetes risk variants had a higher risk estimate for type 2 diabetes among lean case compared with obese case subjects (26). In contrast, a smaller study suggested that a genetic risk score had a stronger effect among obese participants (22), and it has also been reported that the effect of a genetic risk score on risk of impaired glucose tolerance was not evident in lean or insulin-sensitive persons (27). These reported interactions clearly illustrate the diversity underlying genetic risk of complex type 2 diabetes, and further well-powered studies investigating potential interactions are highly warranted.

We were not able to show any interactions with changes in lifestyle factors, which may be due to a modest statistical power to exploit the potential modifying impact of these factors. It is, however, likely that a large part of lifestyle changes may be "summarized" in the BMI change, thus explaining why BMI change may be a statistically more powerful factor to include in the model.

Obesity is believed to contribute to the risk of type 2 diabetes through several mechanisms including insulin resistance, inflammation, and lipotoxicity of the pancreatic β-cells and other organs (28). It is not known which factors may explain the interaction with the genetic risk observed

in the current study; yet, a plausible explanation may be that a high genetic risk of type 2 diabetes renders the pancreatic β-cells more susceptible to an obesogenic environment.

Strengths and limitations. The major strengths of the current study are as follows: *1*) the longitudinal follow-up in a large well-phenotyped homogenous cohort, *2*) investigations of an updated list of type 2 diabetes risk variants genotyped with good genotyping quality and state-of-the-art quality control, and *3*) the repeated examinations including anthropometric measurements, OGTTs, and extensive characterization of lifestyle, which provide a wide collection of glycemic traits and relevant modifying factors.

Since Inter99 is a lifestyle intervention study, it is not known whether our results are directly transferrable to other populations, and further evidence is highly warranted. It has been described previously that in the Inter99 study, the intensity of the lifestyle intervention (high vs. low intensity) did not have any differential effect on 5-year changes in fasting plasma glucose (15), and we obtained similar results when adjusting for prerandomized intervention groups in the current study (data not shown). Interestingly, the group of individuals attending the follow-up examination overall improved their glycemic regulation, although their body weight increased. This seems to contrast with results in previous studies. Nonetheless, the group of individuals belonging to the highest tertile of genetic risk and who also gained weight did increase their values of plasma glucose over 5 years (fasting plasma glucose, 0.02 mmol/L; 2-h glucose, 0.14 mmol/L). The general glycemic improvement may be caused by enrollment in the Inter99 intervention study itself, and attendees for follow-up may also be those most motivated for improvement of their lifestyle (15). An alternative explanation may be that unidentified bias has skewed the distribution from the baseline to 5-year examination. This will, however, not affect the validity of our results, as the genotypic distribution can be assumed to be random over such potential bias, and our results are in line with those observed in the study by Lyssenko et al. (3).

In conclusion, a genetic risk score comprising 46 type 2 diabetes risk variants associated strongly with incident type 2 diabetes, changes in plasma glucose, and estimates of pancreatic β-cell function over a 5-year period in the Inter99 population of middle-aged to elderly Danish people. Individuals who increased their BMI were more susceptible to the cumulative impact of risk variants on fasting plasma glucose. This suggests that especially individuals at high genetic risk may benefit from weight loss intervention; however, this hypothesis remains to be tested.

ACKNOWLEDGMENTS

This project was funded by the Lundbeck Foundation and produced by the Lundbeck Foundation Centre for Applied Medical Genomics in Personalised Disease Prediction, Prevention and Care (LuCamp) (www.lucamp.org). The Novo Nordisk Foundation Center for Basic Metabolic Research is an independent Research Center at the University of Copenhagen partially funded by an unrestricted donation from the Novo Nordisk Foundation (www.metabol.ku.dk). Further funding came from the Danish Council for Independent Research (Medical Sciences).

No potential conflicts of interest relevant to this article were reported.

E.A.A. drafted the manuscript, conceived and designed the experiments, analyzed data, revised the manuscript, and contributed to discussions and interpretations. K.H.A. and C.H.S. conceived and designed the experiments, analyzed data, revised the manuscript, and contributed to discussions and interpretations. R.R.-M., T.S., J.M.J., and M.N.H. performed the genotyping experiments and the quality control, revised the manuscript, and contributed to discussions and interpretations. T.J., T.H., and O.P. conceived and designed the experiments, revised the manuscript, and contributed to discussions and interpretations. E.A.A. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

The authors thank A. Forman, T. Lorentzen, B. Andersen, M. Andersen, and G. Klavsen for technical assistance and A. Nielsen, P. Sandbeck, G. Lademann, and M.M.H. Kristensen for management assistance (all of Novo Nordisk Foundation Center for Basic Metabolic Research).

APPENDIX

The Inter99 study was initiated by T.J. (principal investigator [PI]) (Research Centre for Prevention and Health), Knut Borch-Johnsen (co-PI) (Steno Diabetes Center A/S), Troels Thomsen (Research Centre for Prevention and Health), and Hans Ibsen (Division of Cardiology, Holbæk University Hospital, Holbæk, Denmark). The present steering group comprises T.J., Knut Borch-Johnsen, and Charlotta Pisinger (Research Centre for Prevention and Health).

REFERENCES

1. Morris AP, Voight BF, Teslovich TM, et al. Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. Nat Genet 2012;44:981–990
2. Visscher PM, Brown MA, McCarthy MI, Yang J. Five years of GWAS discovery. Am J Hum Genet 2012;90:7–24
3. Lyssenko V, Jonsson A, Almgren P, et al. Clinical risk factors, DNA variants, and the development of type 2 diabetes. N Engl J Med 2008;359:2220–2232
4. Meigs JB, Shrader P, Sullivan LM, et al. Genotype score in addition to common risk factors for prediction of type 2 diabetes. N Engl J Med 2008;359:2208–2219
5. de Miguel-Yanes JM, Shrader P, Pencina MJ, et al.; MAGIC Investigators; DIAGRAM+ Investigators. Genetic risk reclassification for type 2 diabetes by age below or above 50 years using 40 type 2 diabetes risk single nucleotide polymorphisms. Diabetes Care 2011;34:121–125
6. Hivert MF, Jablonski KA, Perreault L, et al.; DIAGRAM Consortium; Diabetes Prevention Program Research Group. Updated genetic score based on 34 confirmed type 2 diabetes Loci is associated with diabetes incidence and regression to normoglycemia in the diabetes prevention program. Diabetes 2011;60:1340–1348
7. Vassy JL, Durant NH, Kabagambe EK, et al. A genotype risk score predicts type 2 diabetes from young adulthood: the CARDIA study. Diabetologia 2012;55:2604–2612
8. Vassy JL, Dasmahapatra P, Meigs JB, et al. Genotype prediction of adult type 2 diabetes from adolescence in a multiracial population. Pediatrics 2012;130:e1235–e1242
9. Vaxillaire M, Veslot J, Dina C, et al.; DESIR Study Group. Impact of common type 2 diabetes risk polymorphisms in the DESIR prospective study. Diabetes 2008;57:244–254
10. Renström F, Shungin D, Johansson I, et al.; MAGIC Investigators. Genetic predisposition to long-term nondiabetic deteriorations in glucose homeostasis: Ten-year follow-up of the GLACIER study. Diabetes 2011;60:345–354
11. Jensen AC, Barker A, Kumari M, et al. Associations of common genetic variants with age-related changes in fasting and postload glucose: evidence from 18 years of follow-up of the Whitehall II cohort. Diabetes 2011;60:1617–1623

12. Florez JC, Jablonski KA, McAteer JB, et al.; Diabetes Prevention Program Research Group. Effects of genetic variants previously associated with fasting glucose and insulin in the Diabetes Prevention Program. PLoS ONE 2012;7:e44424
13. Jørgensen T, Borch-Johnsen K, Thomsen TF, Ibsen H, Glümer C, Pisinger C. A randomized non-pharmacological intervention study for prevention of ischaemic heart disease: baseline results Inter99. Eur J Cardiovasc Prev Rehabil 2003;10:377–386
14. Glümer C, Jørgensen T, Borch-Johnsen K; Inter99 study. Prevalences of diabetes and impaired glucose regulation in a Danish population: the Inter99 study. Diabetes Care 2003;26:2335–2340
15. Lau C, Vistisen D, Toft U, et al. The effects of adding group-based lifestyle counselling to individual counselling on changes in plasma glucose levels in a randomized controlled trial: the Inter99 study. Diabetes Metab 2011;37:546–552
16. Carstensen B, Kristensen JK, Marcussen MM, Borch-Johnsen K. The National Diabetes Register. Scand J Public Health 2011;39(Suppl.):58–61
17. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15:539–553
18. von Huth Smith L, Borch-Johnsen K, Jørgensen T. Commuting physical activity is favourably associated with biological risk factors for cardiovascular disease. Eur J Epidemiol 2007;22:771–779
19. Pisinger C, Glümer C, Toft U, et al. High risk strategy in smoking cessation is feasible on a population-based level. The Inter99 study. Prev Med 2008;46:579–584
20. Toft U, Kristoffersen L, Ladelund S, et al. Relative validity of a food frequency questionnaire used in the Inter99 study. Eur J Clin Nutr 2008;62:1038–1046
21. Voight BF, Kang HM, Ding J, et al. The metabochip, a custom genotyping array for genetic studies of metabolic, cardiovascular, and anthropometric traits. PLoS Genet 2012;8:e1002793
22. Cornelis MC, Qi L, Zhang C, et al. 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
23. Florez JC, Jablonski KA, Bayley N, et al.; Diabetes Prevention Program Research Group. TCF7L2 polymorphisms and progression to diabetes in the Diabetes Prevention Program. N Engl J Med 2006;355:241–250
24. Wang J, Kuusisto J, Vänttinen M, et al. Variants of transcription factor 7-like 2 (TCF7L2) gene predict conversion to type 2 diabetes in the Finnish Diabetes Prevention Study and are associated with impaired glucose regulation and impaired insulin secretion. Diabetologia 2007;50:1192–1200
25. Grarup N, Sparsø T, Hansen T. Physiologic characterization of type 2 diabetes-related loci. Curr Diab Rep 2010;10:485–497
26. Perry JR, Voight BF, Yengo L, et al.; MAGIC; DIAGRAM Consortium; GIANT Consortium. Stratifying type 2 diabetes cases by BMI identifies genetic risk variants in LAMA1 and enrichment for risk variants in lean compared to obese cases. PLoS Genet 2012;8:e1002741
27. Linder K, Wagner R, Hatziagelaki E, et al. Allele summation of diabetes risk genes predicts impaired glucose tolerance in female and obese individuals. PLoS ONE 2012;7:e38224
28. DeFronzo RA. Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard Lecture 2009. Diabetologia 2010;53:1270–1287