A genetic risk score of 46 type 2 diabetes risk variants associates with changes in plasma glucose and estimates of pancreatic beta-cell function over five years of follow-up

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Short running title: Genetic risk and changes in glycemic traits

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Abstract (word count 240)

More than 40 genetic risk variants for type 2 diabetes have been validated. We aimed to test if a genetic risk score associates with the incidence of type 2 diabetes and with 5-year changes in glycemic traits and if 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 improved their 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 beta-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 beta-cell function. Individuals who gain weight may be more susceptible to the cumulative impact of type 2 diabetes risk variants on fasting plasma glucose.

Introduction: (words 4,131)

Type 2 diabetes is a complex metabolic disorder where both environment and genetic disposition act in concert to cause the disease. Over the last years genome-wide associations studies (GWAS) 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], (reviewed in [2]).

The vast majority of genetic association studies have been performed in a cross-sectional study design. In cross-sectional studies only a snap-shot 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 towards 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]. It has moreover been even less investigated how changes in body mass index (BMI) and lifestyle may interact with genetic factors to modify glucose homeostasis over time [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 present 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; 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 (ClinicalTrials.gov ID-no: NCT00289237) is a population-based non-pharmacological 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 pre-randomised into high-intensity (90%) and low-intensity (10%) intervention groups. 6,784 (52%) attended the baseline health examination (median age 45). All participants received individual lifestyle counselling 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 counselling 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 present study is shown in figure 1. Information on incident type 2 diabetes was collected from the Danish National Diabetes Register [16]. Information included date of inclusion and criteria for inclusion. Data until December 31st 2010 was available. Information on death and emigration was obtained from the Danish Central Person Register until December 31st 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 (kg) and height (cm) were measured in light indoor clothes and without shoes. All participants without previously diagnosed diabetes were characterized by a standardized 75g 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 CV=1.1% and an inter-assay precision of CV=2.3%. Serum insulin (excluding des-31,32 and intact pro-insulin) was measured using the AutoDELFIA insulin kit (Perkin-Elmer/Wallac, Turku, Finland), with an inter-assay precision of CV<6%. The conditions under which plasma glucose and serum insulin was 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 Ethical 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 analysed 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 mislabelled sex (n=11) and individuals with a high discordance rate to previously genotyped SNPs (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, 5 variants were not present on the Metabochip and did not have perfect proxies, and thus these were genotyped by KASPar® (KBioscience, Hoddesdon) with success rates >96% and error rates <0.5%. All variants obeyed Hardy-Weinberg equilibrium (p>0.01), except

HMGA2, rs1531343 (p=0.0043). Overview of the 46 variants genotyped is provided in Online Supplemental Material, Table 1. The 46 variants were chosen based on genome-wide significant ($p<5*10^{-8}$) 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 present study due to the location on the X-chromosome. Variants in or near *FTO* and *MC4R* were not included due to a primary effect on obesity.

Genetic risk score:

Individuals with more than 2 missing genotypes were excluded (n=146). For individuals with 1 (n=588) or 2 (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 Online Supplemental Material, Table 1. All results are from analyses of the simple genetic risk score. The weighted genetic risk score was used to check if weighting each allele would change the results obtained for the simple risk score.

Statistical analyses:

Data was analyzed using the STATA statistical software (version 12.1; StataCorp, College Station, TX, USA) 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 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.

$$5year\ outcome = b0 + b1\ risk + b2\ age + b3\ sex + b4\ baseline\ bmi + b5\ \Delta bmi + b6\ baseline\ value\ of\ outcome\ variable$$

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.

$$5year\ outcome = b0 + b1\ risk + b2\ age + b3\ sex + b4\ baseline\ bmi + b5\ \Delta bmi + b6\ baseline\ value\ of\ outcome\ variable + b7\ risk * \Delta bmi$$

Or

$$5year\ outcome = b0 + b1\ risk + b2\ age + b3\ sex + b4\ baseline\ bmi + b5\ \Delta bmi + b6\ \Delta lifestyle + b7\ baseline\ value\ of\ outcome\ variable + b8\ risk * \Delta lifestyle$$

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 (Figure 2 and Table 1). The cumulative incidence of type 2 diabetes as a function of age increased with tertiles of the genetic risk score (Figure 2; log-rank trend test, p=0.0004). 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], $p=1.1*10^{-4}$ (Table 1, model 3). 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 below 50 years, while it was 1.06 [95%CI 1.02-1.09] in individuals above 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 improved their 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 beta-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 lowered their levels over the 5-year period. Consequently, individuals at increasingly higher genetic risk improved their plasma glucose values less as compared to individuals with a lower genetic risk.

When comparing sub-groups of upper vs. lower decile of genetic risk, the difference evolving over the 5-year period between these two groups was 2.3% for fasting plasma glucose, which averagely corresponds to ~0.14 mmol/l. Extrapolating these results to a follow-up time of 20 years (representing an average year-span in Inter99 from 45 to 65 years of age) and assuming linearity 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 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], $p=1*10^{-4}$ per allele compared with a per allele effect of 0.18% [95%CI, 0.12; 0.24], $p=9*10^{-9}$ 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 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:

To explore 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 (Figure 3). Individuals who gained weight and who were at the highest tertile of genetic risk averagely increased their unadjusted fasting plasma glucose values by 0.19%, whereas individuals who lost weight (or were weight stab~~i~~le) and belonged to the lowest tertile of genetic risk, averagely decreased their unadjusted fasting plasma glucose by -3.3% over 5 years, (Figure 3, Panel A). When dividing the population into quartiles of BMI change, we observed a step-wise increasing effect of the genetic risk score with higher quartiles of BMI change in the fully adjusted model, (Figure 3, Panel B).

No interactions between the genetic risk score and 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 present 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.

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. Of novelty, we found that in the Inter99 population the genetic risk score had measureable effects on changes in plasma glucose and estimates of pancreatic beta-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 in 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 (below 50 years) [5]. When stratifying our study population into age groups below or above 50 years of age, 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 improve 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 the scope of the present 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, and 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 beta-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 beta-cell dysfunction and not through insulin resistance (reviewed in [25]).

The estimated per-allele effect size was slightly lower for the genetic risk score based on all 46 variants compared to a risk score based on only 15 variants from [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 do not infer uncertainty in the statistical models but strengthen 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 6 variants associating with 2-hour glucose values had a stronger impact on levels of 2-hour 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 present study, we did not find differential effects of the genetic risk score on changes in fasting or 2-hour plasma glucose among different age groups, nor did we demonstrate any interactions with age (data not shown). Of note, the Whitehall II study [11] evaluated variants specifically associated with fasting and/or 2- hour glucose values in non-diabetic individuals, which are only partially overlapping those variants found to increase risk of type 2 diabetes investigated in the present 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 a 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 a weight loss, these results suggest that it may be a particularly fruitful intervention for those being 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 GWAS 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 cases compared to obese cases [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 and 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 beta-cells and other organs [28]. It is not known which factors may explain the interaction with the genetic risk observed in the present study, yet, a plausible explanation may be that a high genetic risk of type 2 diabetes renders the pancreatic beta-cells more susceptible to an obesogenic environment.

Strengths and limitations:

The major strengths of the present study are: 1) the longitudinal follow-up in a large well-phenotyped homogenous cohort; 2) investigations of a updated list of type 2 diabetes risk variants genotyped with good genotyping quality and state-of-the-art quality control; 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 if 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 pre-randomized intervention groups in the present 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 in controversy to 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-hour 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 that observed in [3].

In conclusion, a genetic risk score comprising 46 type 2 diabetes risk variants associated strongly with incident type 2 diabetes and changes in plasma glucose and estimates of pancreatic beta-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.

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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-value	$5*10^{-5}$	$9*10^{-6}$	$1*10^{-4}$

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.

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

	Change from baseline to follow-up, mean (95%CI)		*p* for change from baseline to follow-up
Participants, no.	3,727		
BMI, kg/m^2	0.42 (0.36; 0.48)		$1*10^{-45}$
Fasting glucose, mmol/l	-0.08 (-0.09; -0.06)		$3*10^{-24}$
30 min glucose, mmol/l	-0.02 (-0.08; 0.03)		0.3
120 min glucose, mmol/l	-0.13 (-0.19; -0.08)		$1*10^{-6}$
Fasting insulin, %	-8 (-9; -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; -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; 3)		0.4
Disposition index 1, %	8 (6; 12)		$4*10^{-10}$
Disposition index 2, %	5 (3; 8)		$5*10^{-5}$
Weight loss/gain, no.	Weight gain Weight loss (or stable)	2,286 1,441	
Physical activity, no.	Less active No change More active	877 1,748 831	
Dietary score, no.	Unhealthier diet No change Healthier diet	366 2,297 912	
Smoking, no.	More intensive smoking No change Less intensive smoking or cessation	119 3,199 383	

Data is presented for individuals with information on genetic risk who participated in both baseline and follow-up examinations, after excluding 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; no means number.

Table 3: Associations between the genetic risk score and changes in glycemic traits obtained during an oral glucose tolerance tests in the Inter99 cohort (n=3,727)

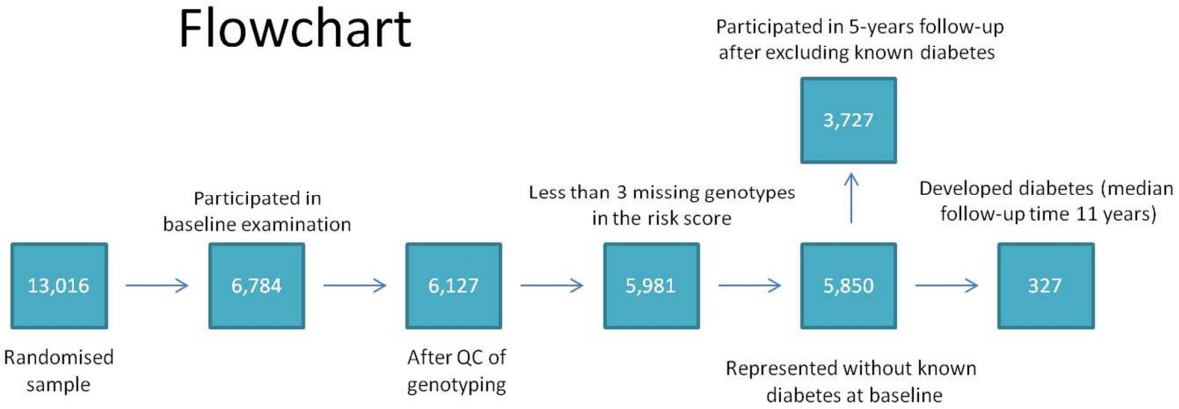
	Effect per risk allele % [95%CI]	*p*
Changes in plasma glucose		
Fasting	0.18 [0.12; 0.24]	$9*10^{-9}$
30 min	0.31 [0.19; 0.44]	$5*10^{-7}$
120 min	0.39 [0.20; 0.58]	$6*10^{-5}$
Changes in serum insulin		
Fasting	0.04 [-0.29; 0.36]	0.8
30 min	-0.36 [-0.72; 0.00]	0.05
120 min	0.14 [-0.36; 0.63]	0.6
Changes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

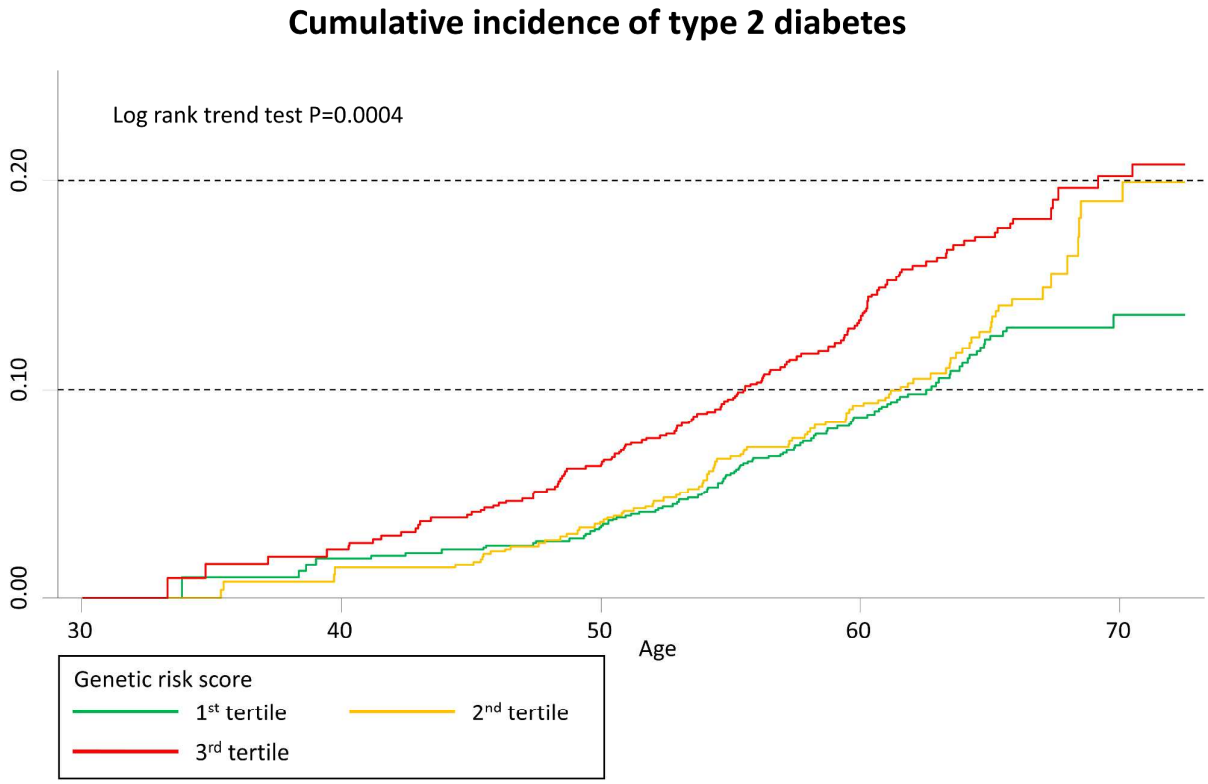
All traits were logarithmically transformed before analyses and effects sizes are given in percentages. All analyses were adjusted for sex, baseline age, baseline BMI, change in BMI and baseline value of the trait analyzed (logarithmical transformed). Insulinogenic index was calculated as (serum insulin at 30 minutes - fasting serum insulin) / (plasma glucose at 30 minutes - fasting plasma glucose). Corrected Insulin Response (CIR) was calculated as (100* serum insulin at 30 minutes)/(plasma glucose at 30 minutes*(plasma glucose at 30 minutes - 3.89)). HOMA index of insulin resistance (HOMA-IR) was calculated as ((fasting plasma glucose*fasting serum insulin) / 135). Matsuda index of insulin sensitivity ($ISI_{Matsuda}$) was calculated as (10,000/√(fasting plasma glucose*fasting serum insulin)*(mean plasma glucose*mean serum insulin during OGTT)). Disposition index 1 was calculated as insulinogenic index/HOMA-IR. Disposition index 2 was calculated as CIR*$ISI_{Matsuda}$.

Figure 1 legend: Flowchart over the number of study participants of the present study

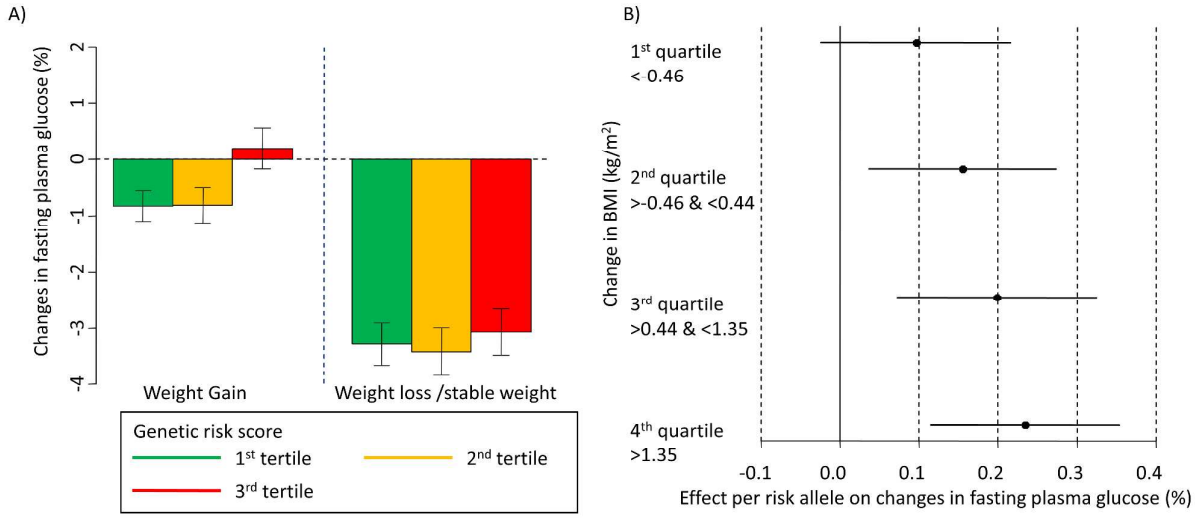
Figure 2 legend: Plot showing the cumulative incidence of type 2 diabetes for tertiles of the genetic risk score (median follow-up 11 years). Green is low genetic risk (1st tertile), yellow is medium genetic risk (2nd tertile), and red is high genetic risk (3rd tertile). (n=5,850, cases=327)

Figure 3 legend: Interaction between the genetic risk score and BMI change on changes in fasting plasma glucose over 5 years. Panel 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. Green is low genetic risk (1st tertile), yellow is medium genetic risk (2nd tertile), and red is high genetic risk (3rd tertile). Panel B) The effect sizes and 95% confidence intervals 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).




Cumulative incidence of type 2 diabetes
Log rank trend test P=0.0004
0.20
0.10
0.00
30
40
50
60
70
Age
Genetic risk score
1st tertile
2nd tertile
3rd tertile

5-year changes in fasting plasma glucose in relation to changes in BMI



Online Supplemental Material:

Table 1: 46 European type 2 diabetes SNPs (or proxies) genotyped in the Inter99 cohort

Nearby gene	Odds ratios used for weighting	SNP or perfect proxy genotyped	References
TCF7L2	1.39	rs7903146	[1-6]
KCNQ1	1.08	rs231362	[6]
KCNQ1	1.09	rs163184	[7]
CDKN2A/2B	1.18	rs10811661	[4, 8, 9]
MTNR1B	1.10	rs10830963	[6, 10]
THADA	1.14	rs7578597	[11]
HHEX/IDE	1.11	rs1111875	[8, 9]
SLC30A8	1.14	rs13266634	[6]
CDKAL1	1.17	rs7756992	[8]
KCNJ11	1.07	rs5219	[8, 11]
IGF2BP2	1.13	rs4402960	[4, 8, 9, 11]
ARAP1	1.11	rs1552224	[6]
WFS1	1.10	rs10010131	[6]
JAZF1	1.11†	rs864745	[11]
CDC123	1.07†	rs12779790 *	[11]
TSPAN8	1.06†	rs7961581 *	[11]
GCK	1.08	rs1799884	[12]
PROX1	1.07	rs340874	[12]
DGKB	1.05	rs2191349	[12]
HNF1B	1.10	rs7501939	[13]
BCL11A	1.07	rs243083	[6]
ADCY5	1.11	rs11708067	[12]
ANK1	1.09	rs516946	[14]
BCAR1	1.12	rs7202877	[14]
IRS1	1.10†	rs2943641	[15]
PPARG	1.13	rs1801282	[8]
ADAMTS9	1.08	rs4607103	[11]
GCKR	1.06†	rs1260326	[12]
KLF14	1.04	rs972283	[6]
RBMS1	1.04	rs4410242	[16]
C2CD4A	1.06†	rs7172432 *	[17]
GRB14	1.07	rs13389219	[14]
ANKRD55	1.08	rs459193	[14]
HMGA2	1.12†	rs1531343 *	[6]
NOTCH2	1.08	rs10923931	[11]

CHCHD9	1.12†	rs13292136 *	[6]
HNF1A	1.08	rs7957197	[6]
ZBED3	1.10	rs4457053	[6]
PRC1	1.07	rs8042680	[6]
TP53INP1	1.05	rs896854	[6]
ZFAND6	1.05	rs11634397	[6]
TLE1	1.07	rs2796441	[14]
ZMIZ1	1.08	rs12571751	[14]
KLHDC5	1.10	rs10842994	[14]
HMG20A	1.08	rs7177055	[14]
CILP2	1.13	rs10401969	[14]

Proxy search was performed based on 1000 Genome Pilot 1 data, linkage disequilibrium was estimated using SNP annotation proxy search (SNAP, http://www.broadinstitute.org/mpg/snap/). *SNPs were genotyped by KBioscience, Hoddesdon. All other SNPs were genotyped in-house using the Illumina Metabochip. †proxy SNP used for weighting if the effect of genotyped SNP is not reported in [18] (all are R^2>0.7).

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