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Genetic Variation in the Glucose-Dependent Insulinotropic Polypeptide Receptor Modifies the Association between Carbohydrate and Fat Intake and Risk of Type 2 Diabetes in the Malmö Diet and Cancer Cohort

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Context: A common genetic variant (rs10423928, A-allele) in the glucose-dependent insulinotropic polypeptide receptor gene (*GIPR*) is associated with decreased insulin secretion. Glucose-dependent insulinotropic polypeptide is secreted after food consumption and *gipr* knockout mice fed a high-fat diet are protected against obesity and disturbances in glucose homeostasis.

Objective: Our objective was to examine the interactions between rs10423928 and macronutrients and fiber intakes on body mass index and type 2 diabetes risk.

Design, Setting, and Participants: Among nondiabetic subjects in the Swedish population-based Malmö Diet and Cancer cohort ($n = 24,840$; 45–74 yr), 1541 diabetes cases were identified during 12 yr of follow-up. Dietary intakes were assessed using a diet history method.

Main Outcome Measure: Incident type 2 diabetes was identified through registers.

Results: There was no indication that dietary intakes significantly modify the association between *GIPR* genotype and body mass index (P interaction >0.08). We observed significant interactions between *GIPR* genotype and quintiles of carbohydrate ($P = 0.0005$) and fat intake ($P = 0.0006$) on incident type 2 diabetes. The TT-genotype carriers within the highest compared with the lowest carbohydrate quintile were at 23% (95% confidence interval = 5–39%) decreased type 2 diabetes risk. In contrast, AA-genotype carriers in the highest compared with the lowest fat quintile were at 69% (95% confidence interval = 29–86%) decreased risk.

Conclusions: Our prospective, observational study indicates that the type 2 diabetes risk by dietary intake of carbohydrate and fat may be dependent on *GIPR* genotype. In line with results in *gipr* knockout mice, AA-genotype carriers consuming high-fat low-carbohydrate diets had reduced type 2 diabetes risk, whereas high-carbohydrate low-fat diets benefitted the two thirds of population homozygous for the T-allele. (*J Clin Endocrinol Metab* 97: E810–E818, 2012)

Glucose-dependent insulinotropic polypeptide (GIP) is released from intestinal K cells after food ingestion. GIP is, together with glucagon-like peptide-1, an incretin hormone that is responsible for the majority of the insulin secreted after a meal. The release of GIP is influenced mainly by dietary intake of carbohydrates and fat (1), but

specific proteins are also able to increase GIP concentration (2, 3). The GIP receptor (GIPR) is widely expressed in the body, including pancreas, adipocytes, bone, and vascular endothelial cells (4). A meta-analysis of several genome-wide association studies identified a genetic variant in intron 12 of *GIPR* (rs10423928, A-allele) that associ-

ates with lower insulin secretion after an oral glucose tolerance test (OGTT) but with only 7% [95% confidence interval (CI) = 2–12%] increased risk of type 2 diabetes (5), and recently, this A-allele was associated with lower levels of *GIPR* mRNA in human pancreatic islets (6). The moderate association with type 2 diabetes may at least partly be explained by the A-allele being associated with lower body mass index (BMI) (6).

GIP has functions outside the pancreas and has a role in fat accumulation in adipocytes, and drugs that inactivate GIPR have been suggested as a strategy to prevent, or treat, obesity (4). When fed a high-fat diet, *gipr* knockout mice were protected against obesity and disturbances in glycaemic homeostasis (7). GIP has therefore been hypothesized to form a link between energy-rich high-fat diets and development of obesity (8). When administering a GIPR antagonist to normal high-fat-fed mice, their glucose tolerance and insulin sensitivity were improved (9, 10), whereas such an effect was not observed when same mice were fed a high-carbohydrate diet (9).

In humans, the evidence of the impact of dietary macronutrient composition on risk of type 2 diabetes is inconclusive (11). By taking the genetic predisposition into account, we may be able to clarify this topic. The aim of this study was to test the hypothesis that depending on *GIPR* genotype, the specific dietary composition of fat, carbohydrates, protein, fiber, and sucrose may show different associations with BMI and with incident type 2 diabetes.

Subjects and Methods

Study population and data collection

The Malmö Diet and Cancer (MDC) study is a Swedish population-based cohort. During 1991–1996, all women born between 1923 and 1950 and men born between 1923 and 1945 living in the city of Malmö were invited to participate (12, 13). Limited Swedish language skills and mental incapacity were the only exclusion criteria. The baseline examinations included assessment of dietary intake using a modified diet history method; lifestyle, demographic factors, *etc.* using an extensive self-administered questionnaire; and anthropometrics by direct measurements conducted by nurses. Weight (kilograms) was measured using a balance-beam scale with subjects wearing light clothing and no shoes, height (centimeters) was measured with a fixed stadiometer, and waist circumference (centimeters) was measured midway between the lowest rib margin and iliac crest. Bioelectric impedance analysis was used to estimate body composition (BIA 103; JRL Systems, Mt. Clemens, MI).

A total of 28,098 subjects completed the questionnaire, anthropometric measurements, and dietary assessment, resulting in a participation rate of approximately 40% (14). After excluding individuals with diabetes at baseline (self-reported diabetes diagnosis or medication, or if date of diagnosis from registers preceded date of baseline examination) ($n = 958$), and those without

information on the *GIPR* polymorphism ($n = 2394$), 24,840 individuals remained and constitute the study sample of this project. A random subsample of those that participated between 1991 and 1994 ($n = 6103$) were invited to be involved in additional examinations including measurements of fasting whole blood glucose, plasma insulin and hemoglobin A1c (HbA1c). Homeostasis model assessment for insulin resistance (HOMA-IR) was used as a measure of insulin resistance and was calculated with the following formula: $\text{insulin} \times \text{blood glucose} / 22.5$ (15). This subsample of the cohort was invited to participate in a reexamination starting in 2007 and still ongoing. During the reexamination, an OGTT was performed; this information was available in 1899 individuals. The ethical committee at Lund University approved the MDC study (LU 51-90), and the participants have given their written informed consent. Figure 1 shows a schematic diagram of the study design.

Diet assessment method

Dietary intakes were collected at baseline using a combination of a 7-d food registration (covering cooked lunches and dinner meals and cold beverages), and a 168-item diet questionnaire (covering foods regularly consumed during the past year). In the questionnaire, participants estimated frequencies of food intake, and usual portion sizes were assessed using a booklet of photographic aids. During a 1-h interview, the participants were asked questions about food choices, food preparation practices, and portion sizes of the foods collected in the food diary (using a more extensive book of photos). The routines for coding dietary data were slightly altered in September 1994 to shorten the interview time. A method variable (diet interview method) was created to control for undue influences. This change did not reveal any major influence on the ranking of individuals (16). Also, a variable was created to delineate the season of dietary data collection. The relative validity of the dietary method has been examined with 18 d of weight food records collected over 1 yr as reference method (17). Energy-adjusted Pearson's validity correlation coefficients were as follows: fat, 0.64 and 0.69 for men and women, respectively; carbohydrates, 0.66/0.70; protein, 0.54/0.53; fiber, 0.74/0.69; and sugar, 0.60/0.74.

Genotyping

Genetic variation in *GIPR* (rs10423928) was genotyped by TaqMan allelic discrimination on ABI 7900 (Applied Biosystems, Foster City, CA) with 98% complete genotyping and a concordant rate of 99.4%. The distribution of the variant was in Hardy-Weinberg equilibrium ($P = 0.27$).

Outcome

Type 2 diabetes cases were identified by three registers: the Swedish National Diabetes Register (18), the regional Diabetes 2000 register of the Skåne region (19), and the local HbA1c register (20). National Diabetes Register and the Diabetes 2000 register required a diagnosis by a physician at a hospital according to established diagnosis criteria. To obtain diabetes cases not diagnosed at the hospital, we used the local HbA1c register that is based on information from the Department of Clinical Chemistry, Skåne University Hospital, Malmö, which has analyzed all HbA1c samples from institutional and noninstitutional care in Malmö from 1988. Individuals with at least two HbA1c values above 6.0% with the Swedish Mono-S standardization system [corresponding to 6.9% with the U.S. National Glycohemoglo-

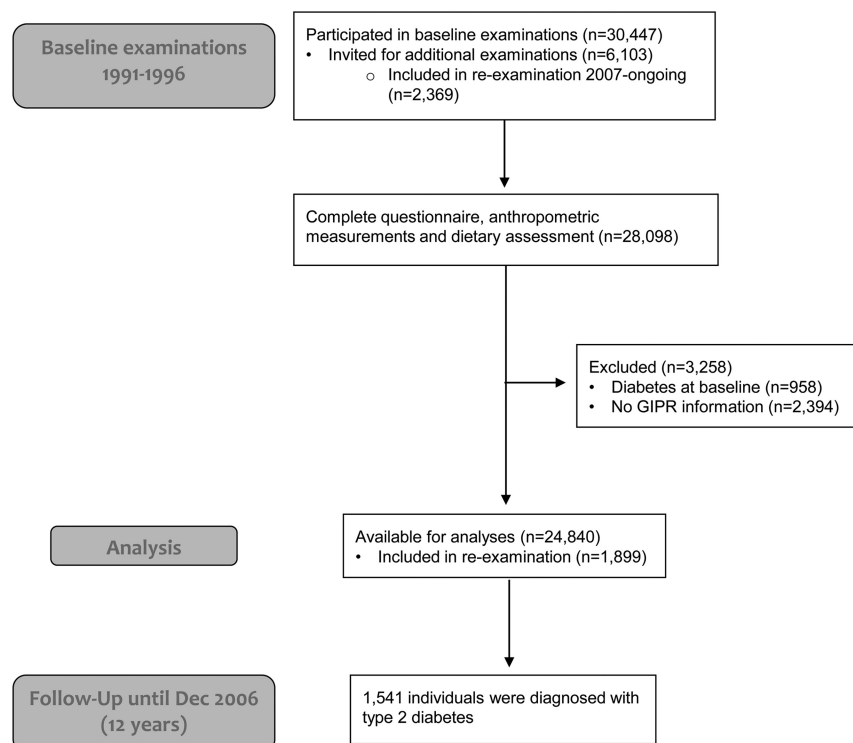


FIG. 1. Schematic diagram of the study sample.

bin Standardization Program and 52 mmol/mol with the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) units] (21, 22) were categorized as diabetes cases in this register. The subjects contributed person-time from date of enrollment until date of diabetes event, death, emigration from Sweden, or end of follow-up in December, 2006.

To validate the information on incident type 2 diabetes and to obtain a reference point when comparing results with other studies on the genetic risk of incident type 2 diabetes, we examined the strongest common type 2 diabetes susceptibility variant, the rs7903146 in *TCF7L2*, and risk of incident type 2 diabetes. Each additional risk allele of rs7903146 was associated with 43% (95% CI = 32–56%) increased risk of diabetes when adjusted for age, sex, and BMI, which is comparable to the risk estimates observed in other studies (23).

Statistical methods

PASW statistics version 18 (SPSS Inc., Chicago, IL) was used for the statistical analyses. Assuming an additive model, we used a general linear model adjusted for age and sex to test the association between the *GIPR* polymorphism and anthropometric and insulin-related measures and adjusted for age, sex, method, and season to examine the association between the polymorphism and diet variables. Carbohydrate quality is important to take into account because it may influence obesity, insulin resistance, and risk of type 2 diabetes. In the present study, we used dietary sucrose and fiber as markers of carbohydrate quality. Fat, carbohydrate, sucrose, and protein intakes were divided into tertiles based on their contribution to nonalcohol energy intake. Fiber intakes were divided into tertiles according to the dietary fiber density (grams per 1000 kcal). The association between *GIPR* genotype and BMI were examined in strata of diet tertiles adjusted for sex and age. Test for trend was calculated with diet

tertiles as a continuous variable. The interactions between diet intake and *GIPR* genotype on BMI were assessed by introducing a multiplicative factor with continuous variables in addition to the diet tertiles and genotype to the analysis. In the model, we adjusted for age, sex, diet interview method, season, and total energy intake. One major concern in cross-sectional analysis of diet and BMI is that obesity status may through social pressure influence the reported dietary intake. We therefore in sensitivity analyses excluded individuals ($n = 4569$; 18%) that potentially report nonadequate energy intake (misreporters). These individuals were identified by comparing the individually estimated physical activity level (total energy expenditure divided by basal metabolic rate), with energy intake divided by basal metabolic rate. This procedure is described in detail elsewhere (24).

Cox regression analysis was used to calculate hazard ratios (HR) of type 2 diabetes for diet variables and *GIPR* genotype. Year of follow-up was used as time-dependent variable. The interactions between diet intake and *GIPR* genotype on risk of type 2 diabetes were assessed by introducing a multiplicative factor with continuous variables

in addition to the diet variable and genotype. We first examined the interaction effects with tertiles of dietary intakes. However, to compare more extreme intakes, we also examined the risk of type 2 diabetes with quintiles of carbohydrate and fat intakes in strata of *GIPR* genotypes.

In the basic model, we adjusted for age, sex, diet interview method, season, and total energy intake. In an additional model, we also included BMI as a covariate. In the multivariate model, we also adjusted for other potential confounders: education, smoking, alcohol consumption, and leisure-time physical activity. Education was divided into elementary, primary and secondary, upper secondary, further education without a degree, and university degree. Smoking habits were categorized into current smokers (including irregular smoking), ex-smokers, and nonsmokers. Alcohol consumption was divided into six categories. Individuals with no consumption of alcohol in the 7-d food diary and who reported no alcohol consumption during the previous year in the questionnaire were categorized as zero-consumers. The other individuals were divided into gender-specific quintiles according to their reported intake in the 7-d food diary. Participants were asked to estimate the number of minutes per week, and for each of the four seasons, they spent performing 17 different physical activities. The duration of each activity was multiplied by an intensity factor, creating a leisure time physical activity score and separated into gender-specific quintiles. We used the joint effect model (25) with TT-carriers with lowest tertile intake as the reference group using the full multivariate model. We also used the heterogeneity of effect model (25) to evaluate the association of diet intake in strata of *GIPR* genotype (full multivariate model), and the association of *GIPR* genotype in strata of diet intake (adjusted for age and sex). In sensitivity analyses, we excluded individuals ($n = 5545$; 22%) answering yes to the questionnaire item, “Have you substantially changed

TABLE 1. Participant characteristics and dietary intakes according to *GIPR* genotype

	n	TT	AT	AA	Effect size (95% CI)	P trend
Number (men/women)	24,840	5,754/9,171	3,357/5,347	488/723		
Age (yr)	24,840	58.0 (7.7) ^a	58.1 (7.7)	57.9 (7.6)	−0.03 (−0.19, 0.13)	0.72
BMI (kg/m ²)	24,806	25.7 (4.0)	25.6 (3.9)	25.3 (3.7)	−0.19 (−0.27, −0.11)	<0.001
Weight (kg)	24,806	73.4 (13.5)	72.9 (13.4)	72.3 (13.1)	−0.52 (−0.77, −0.27)	<0.001
Fat weight (kg)	24,695	19.8 (6.8)	19.5 (6.7)	19.1 (6.3)	−0.29 (−0.43, −0.15)	<0.001
Lean weight (kg)	24,695	53.0 (11.0)	52.9 (10.9)	52.6 (10.8)	−0.22 (−0.36, −0.08)	0.003
Body fat (%)	24,697	26.9 (7.0)	26.7 (6.9)	26.4 (6.7)	−0.19 (−0.29, −0.09)	<0.001
Waist (cm)	24,795	83.9 (12.9)	83.5 (12.7)	83.0 (12.5)	−0.48 (−0.69, −0.26)	<0.001
Waist hip ratio	24,791	0.850 (0.09)	0.849 (0.09)	0.847 (0.09)	−0.002 (−0.004, −0.001)	<0.001
Fasting plasma insulin (pmol/liter)	4,658	47.2 (52.5)	44.9 (37.6)	46.0 (28.9)	−0.6 (−2.7, 1.5)	0.50
Fasting blood glucose (mmol/liter)	4,822	5.00 (0.74)	4.99 (0.80)	4.97 (0.59)	−0.003 (−0.04, 0.03)	0.85
HOMA-IR	4,437	1.60 (1.30)	1.57 (1.14)	1.62 (1.05)	0.01 (−0.05, 0.07)	0.31
HbA1c (%)	4,820	4.82 (0.50)	4.82 (0.52)	4.79 (0.50)	−0.003 (−0.03, 0.02)	0.80
Glucose 120 min after OGTT (mmol/liter)	1,899	7.10 (2.41)	7.22 (2.52)	7.46 (2.42)	0.17 (−0.01, 0.36)	0.06
Energy intake (kcal)	24,840	2,273 (659)	2,276 (645)	2,307 (629)	4.7 (−7.5, 16.8)	0.45
Fat (% of energy)	24,840	39.1 (6.1)	39.0 (6.1)	39.3 (6.4)	−0.02 (−0.15, 0.11)	0.73
Carbohydrate (% of energy)	24,840	45.2 (6.0)	45.2 (6.0)	45.2 (6.2)	0.03 (−0.10, 0.15)	0.70
Sucrose (% of energy)	24,840	8.64 (3.54)	8.59 (3.49)	8.67 (3.48)	−0.03 (−0.10, 0.05)	0.46
Fiber (g/1000 kcal)	24,840	9.01 (2.70)	9.06 (2.74)	8.93 (2.72)	−0.02 (−0.04, 0.07)	0.53
Protein (% of energy)	24,840	15.7 (2.5)	15.8 (2.5)	15.6 (2.6)	−0.003 (−0.06, 0.05)	0.92

Effect size refers to regression coefficient (β) and indicates the mean difference in characteristics for each additional A-allele. *P* values were based on a general linear model with the assumption of an additive genetic model. Obesity measures were adjusted for sex and age. Glucose and insulin were adjusted for sex, age, and BMI; dietary intakes were adjusted for sex, age, BMI, method, and season. *P* values for plasma insulin and HOMA-IR were based on logarithmically transformed values.

^a Crude means and sd.

your eating habits because of illness or for some other reason?" because they are suspected to have unstable food habits (26). In exploratory analyses, we examined the fat intake \times *GIPR* interaction on HbA1c, HOMA-IR and glucose 120 min after OGTT adjusted for age, sex, diet interview method, season, and total energy intake.

The importance of using an appropriate study design when investigating gene-environment interactions has been emphasized in numerous publications (27–29). Statistical power for the diet-gene interaction on incident type 2 diabetes was calculated using QUANTO (30) (<http://hydra.usc.edu/gxe>). Assuming an odds ratio (OR) of 1.10 per fat tertile (additive model) and an OR of 1.07 per *GIPR* allele (22% allele frequency, additive model), we had 80% power to detect an interaction OR of at least 1.17. In this study, BMI and incident type 2 diabetes are the main outcomes, and we have four dietary variables. Because of the highly correlated dietary variables and that we test a biological hypothesis, we have not adjusted for multiple testing correction.

Results

GIPR genotype and obesity measures

Each additional *GIPR* A-allele was associated with 0.52 kg lower weight, divided into 0.29 kg lower fat mass and 0.22 kg lower lean body mass (Table 1). The *GIPR* genotype was not associated with fasting blood glucose, plasma insulin, HbA1c, or insulin resistance measured as HOMA-IR. A borderline significance was observed for

measured glucose 120 min after OGTT (*P* = 0.06). We observed no significant difference in energy intake or dietary composition of macronutrients between carriers of different *GIPR* genotypes.

Overall, the *GIPR* genotype was associated with 0.19 kg/m² lower BMI for each additional A-allele (Table 1). We observed no significant interaction between diet intake and *GIPR* variation on BMI (Table 2). However, the different effect sizes for sucrose and fiber in the different *GIPR* genotype carriers suggested a possible interaction, although the *P* values were not statistically significant (*P* interaction = 0.11 and 0.08 for sucrose and fiber, respectively). The largest difference in BMI between the different *GIPR* genotype carriers was observed among individuals reporting low intake of sucrose or high intake of dietary fiber where each A-allele was associated with a BMI difference of 0.28 kg/m² (sucrose) and 0.29 kg/m² (fiber). These interactions were clearly attenuated after excluding misreporters, *i.e.* the 22% of the study sample with non-adequate reporting of energy intake (*P* interaction = 0.50 and 0.38 for sucrose and fiber, respectively).

GIPR genotype and incident type 2 diabetes

During an average of 11.8 yr of follow-up (292,792 person-years), 1541 individuals (5.3 cases/1000 person-years) were diagnosed with incident type 2 diabetes. Over-

TABLE 2. Mean BMI by tertiles of dietary intakes and *GIPR* genotype

	Mean intakes	TT (n = 14,925)	AT (n = 8,704)	AA (n = 1,211)	Effect size	P trend ^a	P interaction ^b
Carbohydrates (% of energy)							0.79
Low	38.7	25.8	25.5	25.5	−0.19	0.01	
Medium	45.2	25.8	25.6	25.2	−0.23	0.002	
High	51.7	25.6	25.5	25.2	−0.15	0.04	
Sucrose (% of energy)							0.11
Low	5.2	26.0	25.7	25.5	−0.28	<0.001	
Medium	8.2	25.7	25.6	25.3	−0.18	0.01	
High	12.5	25.5	25.5	25.2	−0.12	0.12	
Fiber, (g/1000 kcal)							0.08
Low	6.4	25.6	25.5	25.3	−0.11	0.12	
Medium	8.7	25.8	25.7	25.5	−0.17	0.02	
High	12.0	25.8	25.5	25.1	−0.29	<0.001	
Fat (% of energy)							0.85
Low	32.4	25.9	25.8	25.3	−0.18	0.01	
Medium	39.0	25.8	25.7	25.4	−0.19	0.01	
High	45.7	25.5	25.2	25.3	−0.21	0.004	
Protein (% of energy)							0.61
Low	13.2	25.2	25.0	24.9	−0.17	0.02	
Medium	15.6	25.6	25.4	25.5	−0.16	0.03	
High	18.5	26.3	26.2	25.6	−0.23	0.002	

Effect size refers to regression coefficient (β) and indicates the mean difference in BMI for each additional A-allele, adjusted for sex and age.

^a Adjusted for sex and age.

^b Adjusted for sex, age, season, method, and energy intake.

all, the *GIPR* A-allele was not significantly associated with incident type 2 diabetes (HR = 0.97; 95% CI = 0.89–1.06; *P* trend = 0.48 for each additional A-allele). Adjustments for BMI only slightly influenced the risk estimate (HR = 1.01; 95% CI = 0.93–1.10; *P* trend = 0.76). However, we observed significant interactions between *GIPR* genotype and intake of carbohydrate and fat on risk of incident type 2 diabetes (*P* < 0.01 for both). We observed no major differences in risk estimates after adjusting for BMI or after adjusting for potential confounders (Table 3). Each additional *GIPR* A-allele was associated with a decreased risk of type 2 diabetes among subjects with low carbohydrate intake or high fat intake but increased risk among subjects with high carbohydrate intake or low fat intake (Table 3). The results were similar in men and women (*P* interaction = 0.03 and 0.04 with fat intake for men and women, respectively), with high fat intake associated with decreased risk among AA carriers (HR for the lowest to the highest fat intake tertile were 1.00, 0.75, and 0.59 and 1.00, 0.51, and 0.41 for men and women, respectively) (Fig. 2). It also remained similar when excluding those 22% of the subjects with reported dietary change in the past, individuals that potentially have unstable food habits.

To compare more extreme intakes, we examined the risk of type 2 diabetes with quintiles of carbohydrate and fat intake in strata of *GIPR* genotypes (Fig. 3). The interactions were even more pronounced with quintiles of these diet variables (*P* = 0.0005 and 0.0006 for carbohydrates

and fat, respectively). The inverse correlation between fat and carbohydrate quintiles was high (Pearson correlation coefficient = −0.87). Among individuals homozygous for the T-allele, the highest quintile of carbohydrate intake (mean 54% of energy intake) was associated with a moderate 23% (95% CI = 5–38%; *P* trend = 0.005) decreased risk of type 2 diabetes compared with the lowest quintile (mean 37% of energy intake). Among those homozygous for the A-allele (5% of the study sample), those with the highest quintile of fat intake (mean 48% of energy intake) had more than three times decreased risk (HR = 0.31; 95% CI = 0.14–0.71) compared with those with the lowest intake of fat (mean 31% of energy intake). In addition, high intake of carbohydrates was associated with higher risk of type 2 diabetes among AA-subjects (*P* trend = 0.04).

In exploratory analyses, we also examined the fat intake \times *GIPR* interaction on HbA1c, HOMA-IR, and glucose 120 min after OGTT. However, we observed no significant interaction on any of the measures (*P* interaction = 0.42, 0.69, and 0.83 for HbA1c, HOMA-IR, and glucose 120 min after OGTT, respectively).

Discussion

In this study, we show that genetic variation in *GIPR* modifies the association between the dietary composition of fat and carbohydrates and risk of type 2 diabetes. The

TABLE 3. Interaction between *GIPR* genotype and tertiles of dietary intakes on incident type 2 diabetes

	Cases (TT/AT/AA)	HR (95% CI)			Additive model ^a	P trend ^a	P interaction ^b
		TT	AT	AA			
Carbohydrates							0.001
Low	339/178/17	1.00 (reference) ^c	0.93 (0.78–1.12)	0.53 (0.32–0.86)	0.84 (0.72–0.97)	0.02	
Medium	326/179/25	0.97 (0.83–1.13)	1.00 (0.83–1.19)	1.15 (0.76–1.73)	0.97 (0.83–1.12)	0.66	
High	267/185/25	0.82 (0.69–0.97)	1.03 (0.86–1.24)	1.15 (0.77–1.73)	1.14 (0.98–1.32)	0.09	
P trend ^b		0.01	0.27	0.01			
Sucrose							0.25
Low	333/186/24	1.00 (reference)	0.97 (0.81–1.16)	0.96 (0.63–1.45)	0.93 (0.80–1.07)	0.32	
Medium	318/182/22	1.04 (0.89–1.21)	1.11 (0.93–1.34)	0.86 (0.56–1.33)	0.96 (0.83–1.12)	0.61	
High	281/174/21	0.90 (0.77–1.06)	1.03 (0.86–1.25)	0.98 (0.63–1.52)	1.02 (0.88–1.19)	0.75	
P trend ^b		0.21	0.49	0.78			
Fiber							0.22
Low	334/188/20	1.00 (reference)	1.06 (0.88–1.28)	0.73 (0.47–1.15)	0.93 (0.80–1.07)	0.30	
Medium	313/179/25	1.00 (0.85–1.17)	1.00 (0.83–1.20)	0.99 (0.66–1.50)	0.98 (0.84–1.13)	0.75	
High	285/175/22	0.95 (0.80–1.12)	1.06 (0.88–1.28)	1.14 (0.74–1.77)	1.01 (0.87–1.18)	0.88	
P trend ^b		0.45	0.93	0.08			
Fat							0.002
Low	286/192/27	1.00 (reference)	1.21 (1.00–1.45)	1.49 (1.00–2.21)	1.12 (0.97–1.29)	0.13	
Medium	313/190/20	1.07 (0.91–1.25)	1.13 (0.94–1.36)	1.00 (0.64–1.58)	0.98 (0.85–1.14)	0.83	
High	333/160/20	1.14 (0.97–1.34)	1.05 (0.86–1.28)	0.72 (0.46–1.13)	0.82 (0.71–0.96)	0.01	
P trend ^b		0.09	0.19	0.01			
Protein							0.35
Low	285/157/18	1.00 (reference)	1.06 (0.87–1.29)	0.78 (0.48–1.25)	0.91 (0.77–1.06)	0.22	
Medium	287/171/21	1.06 (0.90–1.25)	1.13 (0.94–1.37)	0.88 (0.57–1.38)	0.99 (0.85–1.15)	0.85	
High	360/214/28	1.23 (1.05–1.45)	1.28 (1.06–1.53)	1.62 (1.10–2.40)	1.02 (0.88–1.17)	0.83	
P trend ^b		0.01	0.16	0.008			

^a The additive model refers to the risk of type 2 diabetes for each additional A-allele, adjusted for age and sex.

^b Adjusted for sex, age, season, method, energy intake, BMI, smoking habits, alcohol consumption, education, and leisure-time physical activity.

^c Joint effect model was adjusted for sex, age, season, method, energy intake, BMI, smoking habits, alcohol consumption, education, and leisure-time physical activity.

GIPR A-allele induces a deficiency of GIPR-mediated actions, and A-allele carriers are characterized by a decreased incretin effect and impaired insulin secretion (5, 6). We showed that among individuals homozygous for the A-allele, high fat/low carbohydrate intakes were associated with decreased risk of type 2 diabetes. This suggests that with high carbohydrate loads, and consequently high demands on sufficient insulin secretion from pancreatic β -cells, adequate insulin secretion is particularly important. The association between *GIPR* variant and BMI was similar with different fat and carbohydrate intakes in our study, and the interaction between diet and *GIPR* genotype on diabetes risk were similar when adjusting for BMI, which indicates that the impaired insulin secretion may be more important for the diet associations with type 2 diabetes than the decrease in BMI associated with the *GIPR* variant. Recently, another example where a discrepancy between the fat intake and genotype interaction effects on BMI and type 2 diabetes was reported is for the Pro12Ala peroxisome proliferator-activated receptor gamma polymorphism (31).

Two nearby variants are in high linkage disequilibrium with rs10423928, and of these, rs2287019 associated with lower BMI in the GIANT meta-analysis (32), whereas the other, a nonsynonymous rs1800437, in exon 10, was reported to lead to decreased basal GIP signaling

and reduced GIPR cell surface expression (33). Numerous studies in mice show that GIPR inhibition has beneficial effects on high-fat-induced obesity and related traits (9, 10, 34). For example, mice fed a high-fat diet experienced decrease in body weight, without decrease in food intake, when a GIPR antagonist was administered to them. In addition, plasma glucose and pancreatic insulin levels of these mice were restored to those of chow-fed mice, the deposition of fat in liver and muscles was decreased, and insulin sensitivity and glucose tolerance were normalized (10). Similarly, in another study, the administration of a GIPR antagonist to mice fed a high-fat diet reduced their body weight and plasma glucose concentrations, whereas the GIPR antagonist had no effect on body weight or glucose levels in mice fed a high-carbohydrate diet (9). Experiments with *gipr* knockout mice have shown that GIPR inhibition could be used not only as a treatment strategy of obesity and disturbances in metabolic-related traits but also for prevention, because the *gipr*^{-/-} mice when fed a high-fat diet did not gain weight and exhibited normal insulin sensitivity compared with control mice fed the same high-fat diet (7).

Among subjects homozygous for the T-allele (60% of the population), opposite associations were observed; diets high in carbohydrate and low in fat were associated with a lower risk of developing type 2 diabetes. These

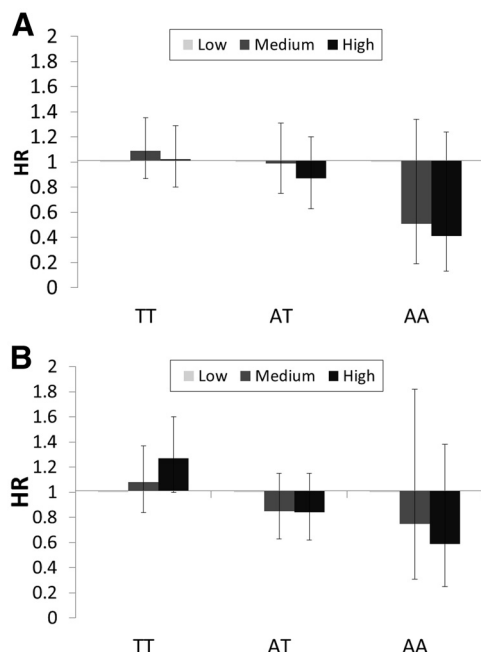


FIG. 2. Association between tertiles of fat intake and type 2 diabetes in strata of *GIPR* genotype in women (A) and men (B). Associations (HR and 95% CI) were adjusted for age, season, method, energy intake, BMI, smoking habits, alcohol consumption, education, and leisure-time physical activity. The lowest intake category was used as the reference group.

results suggest that if the β -cells are able to deliver enough insulin after food consumption, high carbohydrate intakes are not associated with increased risk of type 2 diabetes.

The interview-based dietary assessment method used in the MDC study combining a diary of cooked meals, and a questionnaire was designed to especially measure fat and fiber intakes in an elderly population (35). However, misclassification of dietary exposures in nutritional epidemiology is a challenge, and the true diet-diabetes association may be even larger because nondifferential misclassification usually attenuates the associations.

We observed stronger interactions for type 2 diabetes with total carbohydrates than with fiber or sucrose that were used as markers of carbohydrate quality. Carbohydrate-rich foods give rise to large variations in the blood glucose concentrations depending on the specific type of food, which is indicated by the glycemic index (GI). The dietary content of fiber or sucrose does not necessarily influence GI (36), which may contribute to why we do not observe significant interactions with these dietary factors. The dietary assessment methods used in epidemiological studies are commonly not developed for estimating GI as an exposure, and GI values for many foods, especially foods consumed in Europe, are not available. It is therefore difficult to estimate the GI exposure specifically for each individual in epidemiological studies (37). The influence of GI on health would preferentially be tested in an ex-

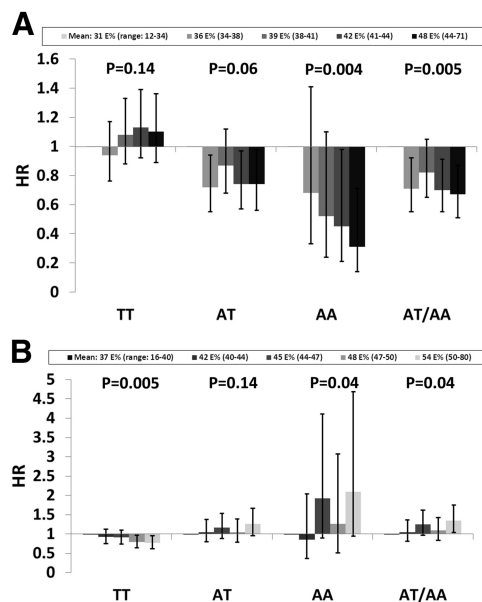


FIG. 3. Association between quintiles of fat (A) or carbohydrate (B) intake and type 2 diabetes in strata of *GIPR* genotype. Associations (HR and 95% CI) were adjusted for sex, age, season, method, energy intake, BMI, smoking habits, alcohol consumption, education, and leisure-time physical activity. The lowest intake category was used as the reference group.

perimental setting. Both wild-type and *gipr* knockout mice gained weight similarly when on high *vs.* low GI foods with identical high-carbohydrate diets (38). There were weak indications in our study that the levels of dietary intake influenced the association between *GIPR* genotype and BMI. However, it is important to note that diet and BMI were measured at the same time point, and we would need longitudinal studies to examine weight change.

Although the number of individuals homozygous for the A-allele was low ($n = 1211$; 67 incident cases), we were able to show significant relationships with fat and carbohydrate intakes in this subgroup. The more than three times lower risk of developing type 2 diabetes among those within the highest quintile of fat intake compared with the lowest quintile needs to be investigated further in even larger studies with dietary data of high quality. Ideally, dietary intervention studies for the prevention of type 2 diabetes should be conducted to test whether individuals with and without the A-allele respond differently to high-fat *vs.* high-carbohydrate diets by evaluating both the acute insulin response and long-term health effects on these individuals.

The prevalence of diabetes in this cohort was approximately 6% (which would correspond to an incidence of about 0.5–0.6%/yr). Although there are no very recent figures on diabetes prevalence and incidence from Sweden, these figures are very much in line with a recent report from Swedish-speaking Western Finland showing a similar prevalence (6.3%) in the same age group (39). The

epidemiological evidence for the risk of diabetes with different diet composition of macronutrients is inconclusive (40, 41). Our results indicate that these inconsistent results can at least partly be due to not taking the genetic predisposition into account and suggest that the influence of the dietary composition regarding fat and carbohydrates very importantly depend on individual genotype. The Swedish dietary recommendations suggest that fat should contribute to 25–35% of the energy intake. However, our results suggest that in a subsample of the population (the 40% A-allele carriers, especially the 5% homozygous for the A-allele), a higher relative fat intake and lower carbohydrate intake may be preferable to prevent diabetes development. Our study also exemplifies hidden heritability problem of multifactorial diseases; genetic risk (*GIPR* genotype) and environmental risk (diet) may need to be considered together to define the risk of disease.

In conclusion, our results indicate that the risk of type 2 diabetes by dietary intake of carbohydrate and fat is dependent on individual *GIPR* genotype. In line with the results in *gipr* knockout mice, A-allele carriers and in particular AA-genotype carriers, were at reduced risk of type 2 diabetes when consuming a diet high in fat and low in carbohydrates, whereas for the 60% of the population homozygous for the T-allele, a diet high in carbohydrates and low in fat associated with protection.

Acknowledgments

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The authors' contributions were as follows: E.S., V.L., and L.G. planned the project; E.S. and M.O.M. designed the research; V.L. and M.O.M. provided genotype data; E.S. analyzed data and performed statistical analysis; B.G. assisted with statistical analyses; E.S. drafted the paper; and all authors contributed to the data interpretation and read and approved the final content.

Of the 28,098 participants in the MDC cohort, 1758 incident diabetes cases and 1758 controls are included in the EPIC InterAct Consortium for the study of genetic factors and gene-lifestyle interactions in regard to incident diabetes. Being a large cohort study, the MDC represents a different study design, compared with the case-control study design of EPIC InterAct. The dietary data used within EPIC InterAct is harmonized between several

study centers, and many details found in the MDC dietary data are lacking in these harmonized data. That is, different study designs, different study sizes, and unequal dietary data ensure the uniqueness of the present study *vs.* the pooled analyses performed within the EPIC InterAct.

Disclosure Summary: The authors have nothing to disclose.

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