A −30G>A Polymorphism of the β-Cell–Specific Glucokinase Promoter Associates With Hyperglycemia in the General Population of Whites

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A graded relationship has been reported between fasting and postprandial plasma glucose levels and the subsequent risk of cardiovascular morbidity and mortality. We hypothesized that the *GCK* −30G>A promoter polymorphism is associated with elevated glycemia in the middle-aged general population of whites, as well as with features of the World Health Organization (WHO)-defined metabolic syndrome. The *GCK* −30G>A polymorphism was genotyped in the population-based Inter99 study cohort (5,965 subjects) and in 332 nondiabetic subjects and 1,063 patients with type 2 diabetes. In the Inter99 cohort, the *GCK* −30A allele was associated with increased fasting ($P < 0.001$) and post–oral glucose tolerance test (OGTT) plasma glucose levels ($P < 0.001$), and in the same cohort, the *GCK* −30A allele was more frequent among 1,325 subjects with the metabolic syndrome than among 1,679 subjects without any components of the metabolic syndrome ($P = 0.002$). Moreover, the *GCK* −30A allele frequency was higher among 2,587 subjects with impaired glucose regulation (IGR) than among 4,773 glucose-tolerant subjects (17.3% [95% CI 16.2–18.3] vs. 15.0% [14.3–15.7], $P < 0.001$, odds ratio GG vs. GA 1.21 [1.08–1.36], GG vs. AA 1.62 [1.17–2.24]). In conclusion, the *GCK* −30G>A polymorphism associates with elevated fasting and post-OGTT glycemia in the middle-aged general population of whites, as well as with IGR and other features of the WHO-defined metabolic syndrome. *Diabetes* 54:3026–3031, 2005

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CAD, coronary artery disease; GCK, glucokinase; IFG, impaired fasting glycemia; IGR, impaired glucose regulation; IGT, impaired glucose tolerance; MAF, minor allele frequency; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; WHO, World Health Organization.

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Several studies have demonstrated a graded relationship between fasting and postprandial plasma glucose levels and the subsequent occurrence of cardiovascular morbidity and mortality (1), a relationship that is apparent at plasma glucose levels below the diabetic threshold. Furthermore, it is likely that a high 2-h plasma glucose concentration following an oral glucose load is associated with an increased risk of death, independent of the concentration of fasting plasma glucose (2). Although glycemic levels (even in the normal range) and the occurrence of impaired glucose tolerance (IGT) and type 2 diabetes are evidently influenced by genetic factors (3–5), the specific molecular mechanisms underlying the progression to hyperglycemic states remain uncertain. The glucose sensor of the pancreatic β-cell, glucokinase (GCK), plays a crucial role in determining the threshold for glucose-stimulated insulin secretion, and studies of maturity-onset diabetes of the young caused by mutations in *GCK* have shown that decreased function of the enzyme causes hyperglycemia due to reduced glucose sensing (6,7). Also, common variations in *GCK* have been suggested to be involved in common forms of type 2 diabetes, and a particular focus has been given to a −30G>A polymorphism (rs1799884) in the pancreatic β-cell–specific promoter of *GCK*. This variant, with a minor allele frequency (MAF) in Danish whites of 17–18% (8), has been investigated in different ethnic populations for association with type 2 diabetes and diabetes-related quantitative traits, yielding inconsistent results (8–15). However, in recent studies, the −30G>A polymorphism was associated with elevated levels of glycohemoglobin and fasting plasma glucose (16,17), thus strengthening previous associations between this variant and impaired glucose regulation (IGR). Intriguingly, the variant was also associated with increased risk of coronary artery disease (CAD) and, among patients with CAD, an augmented prevalence of type 2 diabetes (16). The objectives of the present study were, in a relatively large-scale investigation, to evaluate whether the −30G>A polymorphism of *GCK* at the population level of middle-aged whites associates with elevated glycemia and features of the World Health Organization (WHO)-defined metabolic syndrome.

RESEARCH DESIGN AND METHODS

Details of the study populations are given in Table 1. For studies of genotype-quantitative traits, we excluded all patients with treated type 2 diabetes (n = 97). Thus, 5,868 middle-aged white Danish subjects from the Inter99 cohort, established at the Research Centre for Prevention and Health (4,441 subjects with normal glucose tolerance [NGT], 495 subjects with impaired fasting glycemia [IFG], 684 subjects with IGT, and 248 patients with screen-detected and untreated type 2 diabetes), were investigated for associations between genotype and fasting and post–oral glucose tolerance test (OGTT) glycemia and other quantitative traits (Table 2). All participants from the Inter99 cohort (including patients with treated type 2 diabetes; in total 5,965 middle-aged white Danish subjects) were evaluated in a case-control study examining the association between genotype and the metabolic syndrome according to 1999 WHO standards (18) (Table 3). In the latter study, subjects having no components of the metabolic syndrome were considered control subjects. The case-control studies of IGR included subjects with IFG, IGT, type 2 diabetes, and NGT sampled at the Steno Diabetes Center and from the Inter99 cohort (Table 4). All nondiabetic subjects underwent a standard 75-g OGTT, and only subjects with NGT were included as control subjects in studies of IGR. All participants were Danish whites by self-report and recruited from the same area of Denmark. Informed written consent was obtained from all subjects before participation. The study was approved by the Ethical Committee of Copenhagen and was in accordance with the principles of the Helsinki Declaration II.

Biochemical and anthropometric measures. All subjects were examined after a 12-h overnight fast. Plasma glucose concentration, serum specific insulin, HbA_{1c}, serum triglycerides, and levels of serum cholesterol were measured as previously described (19,20). All subjects were measured for body height, weight, waist circumference, and hip circumference in the supine position and in light indoor clothes and without shoes.

Genotyping. Genomic DNA was isolated from human leukocytes using standard methods. Genotyping of the *GCK* −30G>A polymorphism (rs1799884) was performed using chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (DNA MassARRAY) analyzing PCR-generated primer extension products as reported (21). Genotype data were obtained in 98% of the DNA samples, and 91 samples were genotyped in duplicates with no discrepancies in the genotype scores.

Statistical analysis. Binary logistic regression analyses and Fisher's exact test were applied to test for differences in genotype distribution and to examine for differences in allele frequencies, respectively. A general linear model was applied to test anthropometrical and biochemical variables (or transformed variables) for differences between genotype groups. Genotype and sex were considered as fixed factors and age and BMI as covariates. The analyses using the binary logistic regression and general linear model were performed using SPSS (version 12.0; SPSS, Chicago, IL). $P < 0.05$ was considered significant.

RESULTS

Applying a codominant model, the *GCK* −30G>A polymorphism was investigated for its possible influence on quantitative traits of circulating plasma glucose and serum insulin levels in the population-based Inter99 sample, which involved 5,868 middle-aged white Danish subjects (4,441 subjects with NGT, 495 subjects with IFG, 684 subjects with IGT, and 248 patients with screen-detected and untreated type 2 diabetes), as shown in Table 2. At the population level, carriers of the A allele of the −30G>A polymorphism had increased concentrations of fasting plasma glucose ($P < 0.001$), as well as 30- and 120-min post-OGTT plasma glucose ($P = 0.03$ and $P < 0.001$, respectively) (Table 2). Also, the A allele was associated with an increase in the insulin resistance index (defined as homeostasis model assessment of insulin resistance, P = 0.006). We also examined the polymorphism with respect to fasting and post-OGTT stimulated serum levels of insulin, insulinogenic $index_{insulin}$, serum lipids, and blood pressure; however, no statistically significant associations with the investigated quantitative traits and genotype were detected (Table 2).

Potential associations of the −30G>A polymorphism

TABLE 1
Clinical characteristics of the examined Danish white individuals

	Glucose-tolerant subjects from the Inter99 cohort	Subjects with IFG from the Inter99 cohort	Subjects with IGT from the Inter99 cohort	Type 2 diabetic patients from the Inter99 cohort	Glucose-tolerant subjects from the Steno Diabetes Center	Type 2 diabetic patients from the Steno Diabetes Center
n (men/women)	4,441 (2,067/2,374)	495 (365/130)	684 (335/349)	345 (213/132)	332 (157/175)	1,063 (638/417)
Age at examination (years)	45 ± 8	49 ± 7	48 ± 8	51 ± 7	62 ± 5	59 ± 11
BMI (kg/m²)	25.5 ± 4.0	27.9 ± 4.6	28.1 ± 5.2	30.4 ± 5.7	26.1 ± 3.6	29.5 ± 5.2
Fasting plasma glucose (mmol/l)	5.3 ± 0.4	6.3 ± 0.2	5.7 ± 0.5	8.1 ± 3.0	5.1 ± 0.4	9.8 ± 3.5
HbA_{1c} (%)	—	—	—	7.0 ± 1.6	—	8.1 ± 1.6
Mean age at diagnosis (years)	—	—	—	45 ± 10	—	53 ± 10

Data are means ± SD. The type 2 diabetic patients were recruited from the outpatient clinic at the Steno Diabetes Center (n = 1,063) and from the population-based Inter99 cohort established at the Research Centre for Prevention and Health (n = 345, of whom 248 were screen detected and untreated) (refs. 19,20). Subjects with NGT were ascertained from the Steno Diabetes Center and randomly recruited through the central person register or through the Inter99 cohort, and subjects with IFG and IGT were recruited from the Inter99 cohort. In the present study, type 2 diabetic patients with diabetes due to known chronic pancreatitis, hemochromatosis, severe insulin resistance, maturity-onset diabetes of the young, or maternally inherited diabetes and deafness; patients with a family history of first-degree relatives with type 1 diabetes; patients with an insulin requirement within the 1st year after diabetes diagnosis; or patients with a fasting serum C-peptide level ≤150 pmol/l at the time of recruitment were excluded from the category of clinically defined type 2 diabetes. IFG, IGT, and type 2 diabetes were diagnosed in accordance with WHO criteria (ref. 18). IFG: fasting plasma glucose concentration between 6.1 and 6.9 mmol/l and a 2-h post–glucose load plasma glucose concentration <7.8 mmol/l; IGT: fasting plasma glucose <7.0 mmol/l and 2-h plasma glucose ≥7.8 mmol/l and <11.1 mmol/l; type 2 diabetes: fasting plasma glucose ≥7.0 mmol/l and/or 2-h plasma glucose ≥11.1 mmol/l.

TABLE 2
Anthropometric and metabolic characteristics of the population-based Inter99 sample involving 5,868 middle-aged Danish white subjects (4,441 subjects with NGT, 495 subjects with IFG, 684 subjects with IGT, and 248 patients with screen-detected and untreated type 2 diabetes) stratified according to the −30G>A genotype of *GCK*

−30G>A	G/G	G/A	A/A	*P*
n (men/women)	4,161 (2,064/2,097)	1,575 (792/783)	132 (69/63)	
Age (years)	46 ± 8	46 ± 8	45 ± 8	
BMI (kg/m^2)	26.2 ± 4.5	26.2 ± 4.6	26.1 ± 4.4	0.6
Plasma glucose				
Fasting (mmol/l)	5.5 ± 0.7	5.6 ± 1.0	5.7 ± 1.2	<0.001
30-min post-OGTT (mmol/l)	8.7 ± 1.9	8.8 ± 1.9	8.9 ± 2.2	0.03
120-min post-OGTT (mmol/l)	6.1 ± 2.1	6.3 ± 2.2	6.7 ± 2.6	<0.001
Serum insulin				
Fasting (pmol/l)	42 ± 27	43 ± 28	45 ± 34	0.08
30-min post-OGTT (pmol/l)	293 ± 184	287 ± 189	283 ± 156	0.6
120-min post-OGTT (pmol/l)	216 ± 213	221 ± 219	207 ± 173	0.5
HOMA-IR (pmol/l × mmol/l)	10.4 ± 7.7	10.9 ± 8.3	12.0 ± 12.5	0.006
Insulinogenic index$_{insulin}$	29.4 ± 19.6	29.5 ± 20.4	25.4 ± 13.3	0.08
Waist circumference (cm)	86 ± 13	87 ± 13	86 ± 14	0.4
Waist-to-hip ratio	0.855 ± 0.086	0.859 ± 0.088	0.853 ± 0.093	0.2
Systolic blood pressure (mmHg)	130 ± 17	131 ± 18	132 ± 17	0.2
Diastolic blood pressure (mmHg)	82 ± 11	83 ± 12	83 ± 11	0.3
Fasting serum triglycerides (mmol/l)	1.3 ± 1.4	1.4 ± 1.4	1.3 ± 0.6	0.4
Fasting serum cholesterol (mmol/l)	5.5 ± 1.1	5.5 ± 1.1	5.5 ± 1.0	1.0
Fasting serum HDL cholesterol (mmol/l)	1.4 ± 0.4	1.4 ± 0.4	1.4 ± 0.4	0.6

Data are means ± SD. Values of BMI, plasma glucose, fasting serum triglycerides, waist circumference, and waist-to-hip ratio were logarithmically transformed, and serum insulin, homeostasis model assessment of insulin resistance (HOMA-IR), and fasting serum HDL cholesterol values were transformed using cube root before statistical analysis. Calculated *P* values were adjusted for age, sex, and BMI (and potential significant interactions among these factors) and calculated assuming a codominant model. The insulinogenic index$_{insulin}$ was calculated as fasting serum insulin (pmol/l) subtracted from 30-min post-OGTT serum insulin (pmol/l) divided by 30-min post-OGTT plasma glucose (mmol/l). HOMA-IR was calculated as fasting plasma glucose (mmol/l) multiplied by fasting serum insulin (pmol/l) divided by 22.5.

with components of the WHO-defined metabolic syndrome were evaluated in a case-control study involving all participants from the Inter99 cohort (Table 3). The A allele associated with the metabolic syndrome, showing a significant discrepancy in both genotype distribution (P = 0.004) and MAF (P = 0.002) between subjects classified as having the metabolic syndrome and control subjects with no features of the metabolic syndrome. Furthermore, the A allele strongly associated with IGR ($P < 0.001$), and both the genotype distribution and MAF differed significantly between subjects with insulin resistance and control subjects (P = 0.03 and P = 0.008, respectively). Also, a borderline nonsignificant divergence in genotype distribution and MAF between subjects with hypertension or indexes of obesity and control subjects was observed (Table 3).

In addition, the −30G>A polymorphism of *GCK* was genotyped in a case-control study involving 2,587 subjects with IGR (1,408 type 2 diabetic patients recruited from the Steno Diabetes Center [n = 1,063] and the population-based Inter99 cohort [n = 345], 684 IGT subjects from the Inter99 cohort, and 495 IFG subjects from the Inter99 cohort) and 4,773 subjects with NGT (recruited from the Steno Diabetes Center [n = 332] and the population-based Inter99 cohort [n = 4,441]) (Table 4). We found that the −30A allele was significantly associated with IGR, with an odds ratio for GG vs. GA of 1.21 (95% CI 1.08–1.36) and GG vs. AA of 1.62 (1.17–2.24) (Table 4). When analyzing the IFG, IGT, and type 2 diabetic subgroups separately, we observed a tendency toward an association of the A allele with elevated glycemia within all subgroups (Table 4). Comparing subjects with IGT and glucose-tolerant control subjects, the −30G>A polymorphism was associated with a difference in genotype distribution (P = 0.001) and with a higher frequency of the A allele among IGT subjects (18.9% [16.9–21.0]) than among control subjects (15.0% [14.3–15.7]) ($P < 0.001$). Interestingly, the risk of having IGT increased in an allele-dependent manner, with an odds ratio of 1.30 (1.09–1.55) for heterozygous and 1.87 (1.18–2.96) for homozygous carriers compared with carriers of the wild-type allele.

DISCUSSION

At the population level of middle-aged white people, in the present relatively large-scale epidemiological study, we found a significant association of the −30G>A *GCK* polymorphism with both increased fasting and post-OGTT levels of circulating glucose, extending previous evidence (16,17). A few other diabetogenic variants have been reported to influence plasma glucose levels in the general population, including *HNF4A* rs1884614C>T (22) and *HNF4A* rs2144908A>G (23). However, none of these genetic variants have an impact similar to the order of magnitude observed for the −30G>A *GCK* polymorphism.

The present study also provides evidence that the −30G>A *GCK* polymorphism influences susceptibility to phenotypes of IGR and the WHO-defined metabolic syndrome. While our analyses revealed a borderline association of the minor allele of the −30G>A polymorphism with type 2 diabetes per se, a more marked impact of the

TABLE 3
Genotype frequency of the *GCK* −30G>A polymorphism according to the 1999 WHO-defined metabolic syndrome components among middle-aged white subjects of the Inter99 cohort

	Control subjects	IGR	Insulin resistance	Hypertension	Dyslipidemia	Indexes of obesity	Micro-albuminuria	One or more metabolic syndrome component	Metabolic syndrome according to 1999 WHO criteria
n (men/women)	1,679 (502/1177)	1,544 (920/624)	1,539 (859/680)	2,446 (1455/991)	1,570 (1013/557)	2,589 (1757/832)	165 (73/92)	4,419 (2540/1879)	1,325 (895/430)
G/G	1,222 (73)	1,024 (66)	1,055 (69)	1,709 (70)	1,095 (70)	1,801 (70)	112 (68)	3,099 (70)	890 (67)
A/G	423 (25)	469 (30)	443 (29)	679 (28)	436 (28)	725 (28)	52 (32)	1,215 (27)	402 (30)
A/A	34 (2)	51 (3)	41 (3)	58 (2)	39 (2)	63 (2)	1 (1)	105 (2)	33 (2)
MAF [% (95% CI)]	14.6 (13.4–15.8)	18.5 (17.1–19.9)	17.1 (15.7–18.4)	16.3 (15.2–17.3)	16.4 (15.1–17.7)	16.4 (15.4–17.4)	16.4 (12.4–20.4)	16.1 (15.4–16.9)	17.7 (16.2–19.1)
P_{GD}	—	<0.001	0.03	0.07	0.2	0.06	0.2	0.06	0.004
P_{MAF}	—	<0.001	0.008	0.05	0.05	0.03	0.4	0.04	0.002

Data are n (%) unless otherwise indicated. P values compare genotype distribution (P_{GD}) and MAFs (P_{MAF}) between cases for each component and control subjects having no components of the metabolic syndrome. P values of P_{GD} were calculated assuming a dominant model (GG vs. GA + AA) and were adjusted for sex and age. All genotype groups obeyed Hardy-Weinberg equilibrium. Each component of the metabolic syndrome was defined according to 1999 WHO criteria (ref. 18). Metabolic syndrome was defined as having IGR or insulin resistance together with two or more of the following: hypertension, dyslipidemia, indexes of obesity, or microalbuminuria. Components of the metabolic syndrome: IGR: IFG (fasting plasma glucose ≥6.1 mmol/l and 2-h plasma glucose <7.8 mmol/l), IGT (fasting plasma glucose <7.0 mmol/l and 2-h plasma glucose ≥7.8 mmol/l and <11.1 mmol/l), or type 2 diabetes (fasting plasma glucose ≥7.0 mmol/l and/or 2-h plasma glucose ≥11.1 mmol/l); insulin resistance: subjects belonging to the highest quartile of fasting serum insulin (≥51 pmol/l in the Inter99 cohort); hypertension: subjects in treatment for hypertension and/or having an arterial blood pressure ≥140/90 mmHg; dyslipidemia: subjects having fasting serum triglyceride levels ≥1.7 mmol/l and/or fasting HDL cholesterol levels <0.9 mmol/l for men and 1.0 mmol/l for women; indexes of obesity: subjects having a waist-to-hip ratio >0.9 for men and 0.85 for women and/or a BMI >30 kg/m^2; microalbuminuria: subjects having a albumin-to-creatinine ratio ≥30 mg/g (ref. 18).

TABLE 4
Genotype distribution and MAFs of the −30G>A polymorphism of *GCK* among 1,408 patients with type 2 diabetes, 684 subjects with IGT, 495 subjects with IFG, and 4,773 NGT control subjects

	Glucose-tolerant subjects	Subjects with IGT	Subjects with IFG	Type 2 diabetic patients	Subjects with IGR (type 2 diabetes, IGT, and IFG)
−30G>A (n)	4,773	684	495	1,408	2,587
G/G	3,442 (72)	449 (66)	338 (68)	980 (70)	1,767 (68)
G/A	1,231 (26)	211 (31)	144 (29)	391 (28)	746 (29)
A/A	100 (2)	24 (4)	13 (3)	37 (3)	74 (3)
MAF [% (95% CI)]	15.0 (14.3–15.7)	18.9 (16.9–21.0)	17.2 (14.8–19.5)	16.5 (15.1–17.9)	17.3 (16.2–18.3)
P_{GD}		0.001	0.07	0.05	<0.001
P_{MAF}		<0.001	0.07	0.05	<0.001
Odds ratio (95% CI)					
GG vs. GA		1.30 (1.09–1.55)	1.19 (0.96–1.47)	1.14 (0.98–1.33)	1.21 (1.08–1.36)
GG vs. AA		1.87 (1.18–2.96)	1.44 (0.79–2.62)	1.50 (0.93–2.20)	1.62 (1.17–2.24)
GG vs. GA + AA		1.34 (1.13–1.59)	1.21 (0.98–1.48)	1.16 (1.00–1.35)	1.24 (1.11–1.39)
GG + GA vs. AA		1.73 (1.10–2.73)	1.37 (0.75–2.49)	1.38 (0.90–2.12)	1.53 (1.11–2.12)

Data are n (%) unless otherwise indicated. P values compare genotype distribution (P_{GD}) and MAFs (P_{MAF}) between cases (subjects with IGT, subjects with IFG, patients with type 2 diabetes, and a merged group consisting of all subjects with IGT, IFG, and type 2 diabetes) and glucose-tolerant subjects. Patients having type 2 diabetes were recruited at the Steno Diabetes Center (n = 1,063) and from the population-based Inter99 cohort established at the Research Centre for Prevention and Health (n = 345) (refs. 19,20). Subjects with IFG (n = 495) and IGT (n = 684) were recruited from the population-based Inter99 cohort. Glucose-tolerant subjects were recruited from population-based studies at the Steno Diabetes Center (n = 332) and the Inter99 cohort (n = 4,441). P values of P_{GD} were calculated assuming a dominant model (GG vs. GA + AA) and adjusted for sex and age. All genotype groups obeyed Hardy-Weinberg equilibrium.

variant was detected on susceptibility to IGT. In terms of genetic versus environmental influences on type 2 diabetes susceptibility, this finding is of particular interest and supports previous heritability studies, including a Danish twin study, generating considerably higher heritability estimates for the IGT state compared with manifest type 2 diabetes (3).

GCK is a key regulatory enzyme in the pancreatic β-cell, and it plays a crucial role in determining the threshold for glucose-stimulated insulin secretion. Heterozygous inactivating mutations in GCK cause maturity-onset diabetes of the young subtype 2, in which hyperglycemia is present from birth. The decreased expression (haploinsufficiency) of functional GCK seems to be the cause of the observed hyperglycemia among maturity-onset diabetes of the young subtype 2 patients. The mechanism by which the −30G>A polymorphism causes hyperglycemia is uncertain. The present findings may indicate that the minor A allele or a genetic variant with which it is in linkage disequilibrium alters the expression of *GCK*. However, this hypothesis remains to be tested.

A progressive relationship between plasma glucose levels and cardiovascular risk that extends below the threshold chosen for diabetes has been demonstrated (1). We therefore tested whether risk factors for cardiovascular disease were associated with the −30G>A polymorphism and found that the A allele, which associates with elevated plasma glucose levels, also associates with the metabolic syndrome. This finding is in line with a recent study showing that the A allele increases the risk of angiographically verified CAD and associates with type 2 diabetes among CAD patients (16). Thus, available data, including the present findings of the −30G>A polymorphism, may point to a direct effect of hyperglycemia and the risk of development of the metabolic syndrome and CAD. However, the exact mechanisms leading from slightly elevated glycemic levels to CAD remain undefined. From epidemiological studies, it is evident that an early and aggressive regulation of glycemic levels seems crucial for the prevention of CAD. In this respect, GCK may be considered an attractive drug target.

The present study provides evidence that the −30G>A promoter polymorphism of *GCK* associates with elevated fasting and post-OGTT glycemia in a middle-aged general population of whites. The same variant confers an increased risk of IGT and the metabolic syndrome according to 1999 WHO criteria.

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