Interaction Effect of Genetic Polymorphisms in Glucokinase (*GCK*) and Glucokinase Regulatory Protein (*GCKR*) on Metabolic Traits in Healthy Chinese Adults and Adolescents

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OBJECTIVE—Recent studies in European populations have reported a reciprocal association of glucokinase regulatory protein (*GCKR*) gene with triglyceride versus fasting plasma glucose (FPG) levels and type 2 diabetes risk. GCKR is a rate-limiting factor of glucokinase (GCK), which functions as a key glycolytic enzyme for maintaining glucose homeostasis. We examined the associations of two common genetic polymorphisms of *GCKR* and *GCK* with metabolic traits in healthy Chinese adults and adolescents.

RESEARCH DESIGN AND METHODS—Two single nucleotide polymorphisms (SNPs), rs780094 at *GCKR* and rs1799884 at *GCK*, were genotyped in 600 healthy adults and 986 healthy adolescents. The associations of these SNPs with metabolic traits were assessed by linear regression adjusted for age, sex, and/or BMI. We also tested for the epistasis between these two SNPs and performed a meta-analysis among European and Asian populations.

RESULTS—The T-allele of *GCKR* rs780094 was associated with increased triglycerides ($P = 5.4 \times 10^{-7}$), while the A-allele of *GCK* rs1799884 was associated with higher FPG ($P = 3.1 \times 10^{-7}$). A novel interaction effect between the two SNPs on FPG was also observed ($P = 0.0025$). Meta-analyses strongly supported the additive effects of the two SNPs on FPG and triglycerides, respectively.

CONCLUSIONS—In support of the intimate relationship between glucose and lipid metabolisms, *GCKR* and *GCK* genetic polymorphisms interact to increase FPG in healthy adults and adolescents. These risk alleles may contribute to increased diabetes risk in subjects who harbor other genetic or environmental/lifestyle risk factors. ***Diabetes* 58:765–769, 2009**

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Received 16 September 2008 and accepted 5 December 2008.

Published ahead of print at http://diabetes.diabetesjournals.org on 10 December 2008. DOI: 10.2337/db08-1277.

The glycolytic enzyme glucokinase (GCK) is a glucose sensor that plays a key role in maintaining blood glucose homeostasis. In the pancreatic β-cells, GCK controls insulin secretion and biosynthesis (1). In the liver, GCK regulates glycogen synthesis and gluconeogenesis, and its activity is competitively inhibited by glucokinase regulatory protein (GCKR) (1,2).

In support of these functions, rare mutations in the *GCK* gene have been found to be associated with maturity-onset diabetes of the young (MODY), permanent neonatal diabetes, and hyperinsulinemia of infancy (3). Moreover, a common promoter variant (−30A) (rs1799884) of *GCK* has been found to be associated with increased fasting plasma glucose (FPG) and lowered birth weight in general Caucasian populations (4,5). Recently, a genome-wide association (GWA) study conducted by the Diabetes Genetics Initiative identified a common intronic polymorphism at *GCKR* (rs780094) associated with plasma triglyceride level (6). Subsequent independent studies in Danish (7) and French (8) populations, as well as meta-analysis and fine-mapping (9) studies, confirmed that the minor alleles of rs780094 and rs1260326 (Pro446Leu), which are in strong linkage disequilibrium (LD), were associated with higher levels of triglyceride and C-reactive protein but lower fasting glucose, insulin, and/or insulin resistance.

In this study, we examined the association of the *GCKR* rs780094 and *GCK* rs1799884 polymorphisms with type 2 diabetes–related quantitative traits in two independent samples of 600 adult and 986 adolescent Chinese residents in Hong Kong. Due to the intimate relationship between glucose and lipid metabolisms (10) and the functional interaction between GCKR and GCK, we also hypothesized the presence of epistasis effects between these two single nucleotide polymorphisms (SNPs) on FPG and fasting triglyceride levels.

We genotyped two SNPs, rs1799884 in *GCK* and rs780094 in *GCKR*, in a total of 1,586 healthy subjects including adults and adolescents. The frequencies of the A-allele of rs1799884 were similar between our adult (0.16) and adolescent (0.19) cohorts, as well as the HapMap CHB data (0.20). Although the T-allele frequencies of rs780094 in our data (0.46 for both cohorts) were lower than in the HapMap CHB (0.60), they were similar to the frequency reported in a group of Singapore Chinese (0.46) from a meta-analysis study (9).

The clinical characteristics of the adult and adolescent cohorts are summarized in Table 1. When comparing the

TABLE 1
Clinical and metabolic characteristics of 1,586 healthy Chinese adults and adolescents

	Adults	Adolescents
n (male/female)	600 (270/330)	986 (463/523)
Age (years)	41.4 ± 10.5	15.3 ± 1.9
BMI (kg/m^2)	22.9 ± 3.3	19.9 ± 3.5
Systolic blood pressure (mmHg)	115.4 ± 16.3	118.0 ± 12.5
Diastolic blood pressure (mmHg)	72.3 ± 11.2	72.6 ± 9.6
Total cholesterol (mmol/l)	5.0 ± 0.9	4.2 ± 0.7
Triglycerides (mmol/l)	0.9 (0.6–1.3)	0.8 (0.6–1.0)
HDL cholesterol (mmol/l)	1.6 ± 0.4	1.6 ± 0.3
LDL cholesterol (mmol/l)	3.0 ± 0.8	2.3 ± 0.6
FPG (mmol/l)	4.8 ± 0.4	4.7 ± 0.3
Fasting plasma insulin (pmol/l)	41.2 (26.5–58.7)	45.1 (35.3–60.4)

Data are means ± SD or median (interquartile range).

distributions of metabolic traits among different genotype carriers of *GCKR* rs780094, we observed consistent and strong association of the minor T-allele with increased triglycerides in both adult and adolescent cohorts ($4.0 \times 10^{-6} < P < 0.005$), after adjusting for age, sex, and BMI (Table 2). We also observed a strong association of the minor A-allele of *GCK* rs1799884 with increased FPG in both cohorts ($P < 6.4 \times 10^{-5}$) after adjusting for covariates (Table 3). The associations became more significant in the combined cohort ($P = 5.4 \times 10^{-7}$ for triglycerides with rs780094; $P = 3.1 \times 10^{-7}$ for FPG with rs1799884) (Tables 2 and 3) and remained significant after controlling for false discovery rate ($P < 0.0056$ for triglycerides with rs780094; $P < 0.0028$ for FPG with rs1799884). These findings are in agreement with reports from Caucasian populations (4,6–9,11–13), as well as a recent Japanese study (mean triglyceride levels of 1.07, 1.13, and 1.18 mmol/l, respectively, for CC, CT, and TT genotypes of rs780094, $P = 0.097$) (14).

We further examined possible gene-gene interaction effects of *GCKR* rs780094 and *GCK* rs1799884 on fasting triglycerides and FPG in the combined cohort using additive genetic models (Fig. 1*A*). There was a highly significant interaction effect between the two SNPs on FPG ($P = 0.0025$ for the interaction after adjusting for age, sex, BMI, and study cohort). Each copy of the rs1799884 A-allele was associated with a 0.18 ($P < 0.0001$), 0.09 ($P = 0.0002$), and 0.02 mmol/l ($P = 0.53$) change in FPG levels for rs780094 TT, CT, and CC carriers, respectively. On the other hand, the T-allele of rs780094 showed a trend of increased FPG in rs1799884 AA carriers (0.14 mmol/l per allele, $P = 0.13$) but decreased FPG (−0.05 mmol/l per allele) in GG carriers ($P = 0.0045$). However, no interaction was observed for fasting triglycerides (Fig. 1*B*).

Several studies have reported the reciprocal effect of the C-allele of rs7800094 with decreased triglycerides but increased FPG, as well as the joint additive effects of rs7800094 and rs1799884 on FPG levels (7–9). However, only a Danish study examined nonadditive epistasis effects, and no interaction between these two SNPs was found (8). Our results suggest a more complex scenario. The risk effect of the A-allele of GCK rs1799884 on FPG was strongest in TT carriers of rs780094, followed by CT and CC carriers. Similar to other studies, we observed that the C-allele of rs780094 was associated with increased

TABLE 2
Clinical and metabolic characteristics of Chinese adults and adolescents stratified according to genotypes of GCKR rs780094

	Hong Kong adults				Hong Kong adolescents				
	CC	CT	TT	*P*	CC	CT	TT	*P*	Combined *P*
n	177	284	130		280	453	200		
BMI (kg/m^2)	23.0 ± 3.2	22.9 ± 3.2	22.7 ± 3.3	0.396	19.9 ± 3.5	19.9 ± 3.5	20.0 ± 3.8	0.843	0.778
Systolic blood pressure (mmHg)	114.8 ± 16.9	115.6 ± 16.2	115.7 ± 16.2	0.468	118.8 ± 13.5	117.9 ± 12.0	117.2 ± 12.6	0.083	0.465
Diastolic blood pressure (mmHg)	72.1 ± 10.8	72.8 ± 11.6	71.5 ± 11.1	0.975	73.1 ± 9.8	72.2 ± 9.4	72.8 ± 9.9	0.592	0.665
Total cholesterol (mmol/l)	5.0 ± 0.9	5.0 ± 1.0	5.2 ± 0.9	0.109	4.2 ± 0.7	4.2 ± 0.7	4.3 ± 0.7	0.295	0.062
Triglycerides (mmol/l)	0.8 (0.6–1.2)	0.9 (0.6–1.3)	1.0 (0.6–1.6)	0.005	0.7 (0.6–0.9)	0.8 (0.6–1.0)	0.8 (0.6–1.1)	4.0×10^{-6}	5.4×10^{-7}
HDL cholesterol (mmol/l)	1.6 ± 0.4	1.5 ± 0.4	1.5 ± 0.4	0.587	1.6 ± 0.3	1.5 ± 0.3	1.6 ± 0.3	0.603	0.461
LDL cholesterol (mmol/l)	3.0 ± 0.8	3.0 ± 0.9	3.1 ± 0.8	0.226	2.3 ± 0.6	2.2 ± 0.6	2.3 ± 0.6	0.933	0.349
FPG (mmol/l)	4.8 ± 0.4	4.8 ± 0.4	4.8 ± 0.4	0.724	4.8 ± 0.3	4.7 ± 0.3	4.7 ± 0.4	0.173	0.211
Fasting plasma insulin (pmol/l)	43.8 (30.3–61.4)	40.2 (25.2–55.9)	42.3 (28.4–60.2)	0.449	47.1 (36.1–63.6)	44.2 (35.2–59.9)	44.8 (34.3–59.5)	0.172	0.162

Data are means ± SD or median (interquartile range). *P* values were calculated from linear regression adjusted for sex, age, and BMI (where appropriate) assuming an additive model. In the combined analysis, calculated *P* values were also adjusted for study cohorts (adult or adolescent).

FPG, but only in carriers of the more common GG variant of rs1799884 ($P = 0.0045$), whereas a nominal association in the opposite direction was observed for AA carriers ($P = 0.13$). This interaction explains the lack of overall association of rs7800094 with FPG and fasting insulin levels in our population.

The interaction effect is possibly due to the perturbation of the upstream GCKR/GCK system, in which failure of the receptor (GCKR) may influence the ability of GCK polymorphisms to confer effect on FPG levels. Despite smaller sample sizes in separate cohorts, these interaction effects showed a nominal significance in both adult ($P = 0.058$) and adolescent ($P = 0.022$) cohorts, suggesting that our finding is unlikely to be due to chance alone (supplementary Fig. 1*A* and *B*, available in an online appendix at http://diabetes.diabetesjournals.org/cgi/content/full/db08-1277/DC1). Due to the rarity of the rs1799884 AA genotype in both Europeans and Asians (4 and 3%, respectively), as well as the lower TT genotype frequency of rs7800094 in Europeans versus Asians (14 vs. 23%, respectively) (7), the lack of interaction seen in Europeans may be partly due to the rarity of the AA/TT high-risk carriers and the corresponding low power of detection. Alternatively, different risk-conferring alleles for the same gene are now increasingly recognized, which may confound the association (15,16). Additional interaction analyses in other studies are warranted to clarify our findings.

Meta-analysis of the association between *GCKR* (rs780094) and fasting triglycerides in combined European and Asian cohorts showed increases of 0.10 (95% CI 0.07–0.13) and 0.22 (0.18–0.26) in standardized mean difference (SMD) for the CT and TT genotypes, respectively, when compared with the CC reference genotype (supplementary Fig. 2). Likewise, an additive trend of increases of 0.14 (0.10–0.18) and 0.31 (0.12–0.49) in SMD of FPG for AG and AA genotypes, respectively, when compared with the GG reference genotype, was observed for *GCK* (rs1799884) (supplementary Fig. 3). Due to significant heterogeneity among the study cohorts ($P < 0.1$), the combined SMDs were calculated based on a random-effects model.

Our meta-analyses of the association between *GCKR* and triglycerides and between *GCK* and FPG, using combined European and Asian data, strongly support the additive effects of the risk alleles of the two SNPs on triglycerides and FPG. Nevertheless, there are notable differences in the effect size between the European and Asian studies. Given interethnic differences in risk-allele frequency, genetic effect size, and environmental exposure, one might expect considerable variation in the population-attributable risk of these genotypes on diabetes in different populations.

In conclusion, we have confirmed the risk associations of two common genetic polymorphisms of *GCK* and *GCKR* on type 2 diabetes–related metabolic traits as well as the significant interactive effects of these polymorphisms on FPG in healthy Chinese adults and adolescents. These risk alleles may add to the overall risk of type 2 diabetes in subjects who harbor other genetic or environmental/lifestyle factors. Finally, the interactions of these two genetic polymorphisms have provided a hypothesis to improve our understanding of dysregulation of intermediary metabolism. This hypothesis, that polymorphisms at *GCKR* may perturb the GCKR/GCK system and thus modify the effect of *GCK* polymorphisms and lead to altered glucose metabolism, warrants further functional studies.

TABLE 3
Clinical and metabolic characteristics of Chinese adults and adolescents stratified according to genotypes of GCK rs1799884

	Hong Kong adults				Hong Kong adolescents				
	GG	AG	AA	*P*	GG	AG	AA	*P*	Combined *P*
n	332	132	12		652	296	36		
BMI (kg/m^2)	22.8 ± 3.4	22.7 ± 2.9	22.1 ± 2.6	0.399	20.0 ± 3.6	19.7 ± 3.4	20.5 ± 3.9	0.559	0.388
Systolic blood pressure (mmHg)	115.5 ± 16.5	113.8 ± 14.4	114.7 ± 22.2	0.201	117.8 ± 12.6	118.1 ± 12.0	119.1 ± 14.2	0.579	0.596
Diastolic blood pressure (mmHg)	71.8 ± 11.7	71.0 ± 10.1	71.4 ± 13.3	0.319	72.6 ± 9.9	72.6 ± 9.0	71.4 ± 9.4	0.729	0.334
Total cholesterol (mmol/l)	5.0 ± 0.9	5.0 ± 1.0	4.8 ± 1.2	0.567	4.2 ± 0.7	4.2 ± 0.7	4.0 ± 0.6	0.592	0.888
Triglycerides (mmol/l)	0.8 (0.6–1.2)	0.9 (0.6–1.4)	0.9 (0.6–1.2)	0.138	0.8 (0.6–1.0)	0.8 (0.6–1.0)	0.7 (0.6–0.8)	0.894	0.796
HDL cholesterol (mmol/l)	1.6 ± 0.4	1.5 ± 0.4	1.5 ± 0.4	0.011	1.6 ± 0.3	1.5 ± 0.3	1.6 ± 0.2	0.777	0.084
LDL cholesterol (mmol/l)	2.9 ± 0.8	3.0 ± 0.7	2.8 ± 1.1	0.887	2.2 ± 0.6	2.3 ± 0.6	2.1 ± 0.5	0.504	0.647
FPG (mmol/l)	4.8 ± 0.4	4.9 ± 0.4	5.0 ± 0.4	6.4×10^{-5}	4.7 ± 0.3	4.8 ± 0.3	4.8 ± 0.5	3.0×10^{-5}	3.1×10^{-7}
Fasting plasma insulin (pmol/l)	40.3 (25.1–58.0)	43.2 (27.0–55.9)	44.9 (30.9–70.9)	0.681	45.8 (36.1–61.6)	44.2 (33.9–59.0)	45.8 (36.2–58.7)	0.978	0.878

Data are expressed as means ± SD or median (interquartile range). *P* values were calculated from linear regression adjusted for gender, age, and BMI (where appropriate) assuming an additive model. In the combined analysis, calculated *P* values were also adjusted for study cohorts (adult or adolescent).

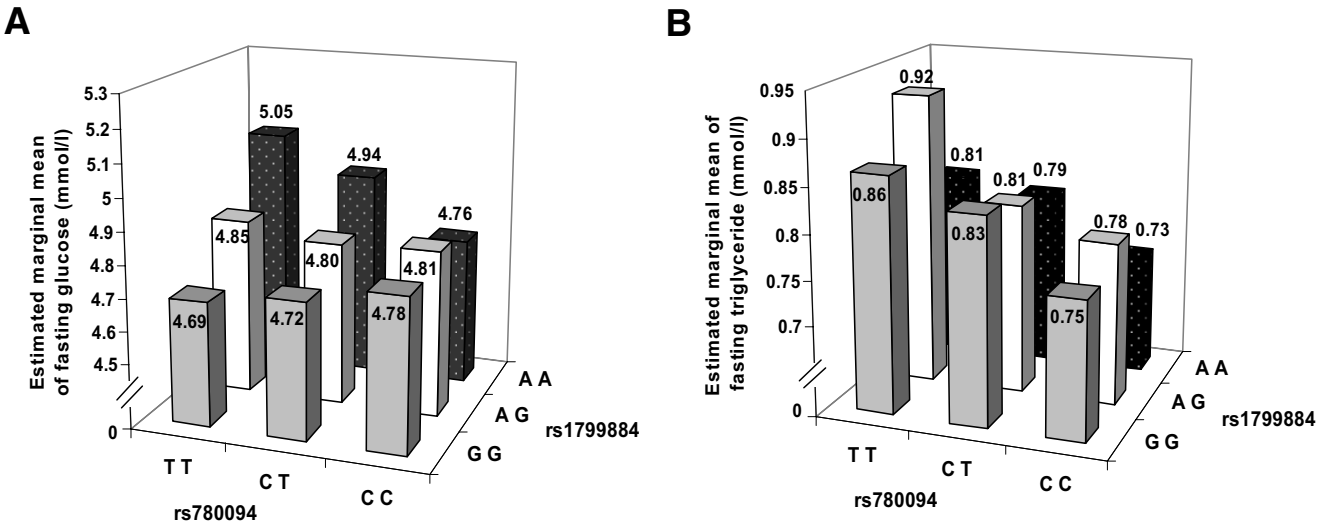

FIG. 1. Interaction effect of *GCKR* rs780094 and *GCK* rs1799884 on FPG (*A*) and fasting triglyceride (*B*) assuming additive models for both SNPs with adjustment for sex, age, BMI, and study cohorts (adult or adolescent).

RESEARCH DESIGN AND METHODS

The study design, ascertainment, inclusion criteria, and phenotyping of the study subjects have been described previously (15,17,18). All subjects were of southern Han Chinese ancestry and residing in Hong Kong. The adult cohort (mean age 41.4 ± 10.5 years, 45% male) consists of 600 participants of a community-based health screening program or hospital staff. The adolescent cohort consists of 986 healthy subjects (mean age 15.3 ± 1.9 years, 47% male) recruited from a population-based school survey for risk factor assessment. Subjects with FPG ≥6.1 mmol/l were excluded. This study was approved by the Clinical Research Ethics Committee of the Chinese University of Hong Kong. Written informed consent was obtained from all participants or parents of adolescents as appropriate.

All study subjects were examined in the morning after an overnight fast. Anthropometric parameters including body weight, height, and blood pressure were measured. Fasting blood samples were collected for measurement of FPG, insulin, and lipids. FPG, total cholesterol, triglycerides, and HDL cholesterol were measured enzymatically by the Roche Modular Analytics system (Roche Diagnostics, Mannheim, Germany) with precision of the assays within that specified by the manufacturer. Plasma insulin was measured by an enzyme-linked immunosorbent assay (DAKOCytomation, Cambridgeshire, U.K.).

Genotyping. Based on the existing evidence of association of *GCKR* rs780094 and *GCK* rs1799884 polymorphisms with triglycerides, FPG, and type 2 diabetes (4,5,7–9,11,12,14), these two SNPs were genotyped in all study subjects using genomic DNA. We did not attempt to test for association of all tagging SNPs of the respective genes. The *GCKR* rs1260326 polymorphism was also genotyped in the 600-adult cohort in order to assess its LD with rs780094. Due to their strong LD ($r^2 = 0.9$ in both HapMap CEU data and the present Chinese study), subsequent analyses were performed for rs780094 only. Genotyping was performed at the McGill University and Genome Quebec Innovation Centre using primer extension of multiplex products with detection by MALDI-TOF mass spectroscopy on a Sequenom MassARRAY platform (Sequenom, San Diego, CA). Both SNPs were in Hardy-Weinberg equilibrium ($P > 0.05$) in adult and adolescent cohorts separately, as assessed by the exact test of PLINK (19). The genotype call rates were >92% for both SNPs. Genotyping accuracy was demonstrated by a 100% concordance rate in 26 blinded duplicate samples.

Statistical analysis. Continuous data are presented as mean ± SD or median (interquartile range). Plasma triglycerides and fasting plasma insulin were logarithmically transformed due to skewed distributions. Each trait was Winsorized separately in adult and adolescent cohorts by replacing extreme values with four SDs from the mean (20,21). Less than 0.3% of data were replaced.

Within each cohort, associations between genotypes and metabolic traits were tested by multiple linear regression, with age, sex, and BMI as covariates (where appropriate). In the combined analysis, an additional dummy variable "study cohort" coded as 0 for adults and 1 for adolescents was included in the regression model. To assess gene-gene interaction effect on FPG and triglyceride levels, linear regression including the main and interaction effects of *GCKR* rs780094 and *GCK* rs1799884 under additive models was applied. Multiple testing of phenotypic traits and SNPs was corrected by controlling the false discovery rate using the Benjamini-Hochberg approach (22,23).

Meta-analyses of the associations of *GCKR* rs780094 with triglycerides and *GCK* rs1799884 with FPG were assessed by MedCalc for Windows, version 9.2.0.0 (MedCalc Software, Mariakerke, Belgium). For the association of *GCKR* rs780094 on triglyceride, the T-allele of rs1260326 in the French study (8) was used as proxy for the T-allele of rs780094 ($r^2 = 0.9$ in the HapMap CEU data). Hedges *G* statistic was used to calculate the SMD (the difference between the two means divided by the pooled standard deviation) across studies under the fixed effects model. To address heterogeneity of SMDs across studies (Cochran's *Q* statistic, $P < 0.1$), the overall effect size (SMD) under the random-effects model, in which both random variations within and between different studies were incorporated (24), was reported.

We estimated study power using a genetic power calculator (25). Assuming an additive model with the observed frequencies of 0.46 for the T-allele of *GCKR* rs780094 and 0.17 for the A-allele of *GCK* rs1799884 in our Chinese population, our sample size has 87% power to detect a per-allele effect of increasing triglycerides by >0.16 mmol/l for rs780094 (7) and 70% power of increasing FPG by >0.1 mmol/l for rs1799884 (12), at an α level of 0.05. All statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC) unless specified otherwise. Two-tailed *P* values <0.05 were considered statistically significant.

ACKNOWLEDGMENTS

This study was funded by the Hong Kong Government Research Grant Committee Central Allocation Scheme (CUHK 1/04C) and the CUHK Direct Grant (2006.1.041).

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

We are grateful to all study subjects who took part in these studies. We thank Cherry Chiu and Dr. Gary Wong for recruitment of study subjects and Lunan Chow, Alex Ng, Patty Tse, and Janice Ho for technical assistance. We thank the Chinese University of Hong Kong Clinical Trials and Information Technology Services Centre for support of computing resources. Special thanks are extended to all nursing and medical staff at the Prince of Wales Hospital Diabetes and Endocrine Centre for their dedication and professionalism.

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