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 rs179884 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 effect 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 rs179884 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

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
Several studies have reported the reciprocal effect of the minor A-allele of rs780094, we observed consistent and significant association of the minor T-allele with increased fasting triglycerides and FPG in the combined cohort (Fig. 1A). There was a highly significant interaction between the two SNPs on age, sex, BMI, 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 = 0.005), after adjusting for age, sex, and BMI (where appropriate) assuming an additive model. In the combined analysis, calculated P values were calculated from linear regression adjusted for sex, age, and BMI (where appropriate).
Combined European and Asian data, strongly support the hypothesis that polymorphisms at \( GCKR \) and triglycerides 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.
RESEARCH DESIGN AND METHODS

The study design, ascertainment 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 immunoradiometric assay (IMRSA, Linco Research, Missouri, USA). Total cholesterol was measured colorimetrically using a Roche Modular System while triglycerides and HDL cholesterol were measured enzymatically. Fatty acids composition were measured utilizing gas chromatography. Anthropometric parameters including body weight, height, and blood pressure were measured.

Genotyping. Based on the existing evidence of association of GCKR rs780094 and GCK rs179884 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 TaqMan SNP genotyping assays (Sequenom, San Diego, CA). Genotyping call rates were 0.9 and 0.98 for rs780094 and rs179884, respectively. GCKR rs1260326 polymorphism was also genotyped in the 600-adult cohort in order to assess its LD with the 15q25.1 region.

Meta-analyses of the associations of GCKR rs780094 with triglycerides and GCK rs179884 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 rs780094 was used as proxy for the T-allele of rs1260326 in the French study (8) was used as proxy for the T-allele of rs780094 in the Chinese study (7). We used an additive model with the observed frequencies of 0.46 for the T-allele of rs1260326 in the French study (8) was used as proxy for the T-allele of rs780094 in the Chinese study (7). The P value for each study was calculated as the difference between the two means divided by the pooled standard deviation across studies (Cochran’s Q statistic). For the association of GCKR rs179884 on FPG, the primary P value was calculated as the difference between the mean FPG levels of subjects with the T-allele of rs179884 and those with the C-allele of rs179884 after adjustment for sex, age, BMI, and study cohorts (adult or adolescent). For the meta-analysis, the P value of rs179884 under the random-effects model, in which both random variations within and between different studies were incorporated (24), was reported.

We estimated study power under 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 rs179884 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 rs179884 (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.

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REFERENCES

1. Matschinsky FM: Glucokinase, glucose homeostasis, and diabetes mellitus. Curr Diab Rep 5:171–176, 2005
2. van Schaftingen E, Vandercammen A, Detheux M, Davies DR: The regulatory protein of liver glucokinase. Adv Enzyme Regul 32:133–148, 1992
3. Gloyn AL: Glucokinase (GCK) mutations in hyper- and hypoglycemia.
maturity-onset diabetes of the young, permanent neonatal diabetes, and hyperinsulinemia of infancy. *Hum Mutat* 22:353–362, 2003

4. Weedon MN, Clark VJ, Qian Y, Ben-Shlomo Y, Timpson N, Ebrahim S, Lawlor DA, Pembrey ME, Ring S, Wilkin TJ, Voss LD, Jeffery AJ, Metcalf B, Ferrucci L, Corsi AM, Murray A, Melzer D, Knight B, Shields B, Smith GD, Hattersley AT, Di Rienzo A, Frayling TM. A common haplotype of the glucokinase gene alters fasting glucose and birth weight: association in six studies and population-genetics analyses. *Am J Hum Genet* 79:901–1001, 2006

5. Weedon MN, Frayling TM, Shields B, Knight B, Turner T, Metcalf BS, Voss LD, Wilkin TJ, McCarthy A, Ben-Shlomo Y, Smith GD, Ring S, Jones R, Golden J, ALSPAC Study Team, Byberg L, Mann V, Axelson T, Syvanen AC, Leon D, Hattersley AT. Genetic regulation of birth weight and fasting glucose by a common polymorphism in the islet cell promoter of the glucokinase gene. *Diabetes* 54:576–581, 2005

6. Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PL, Chen H, Roix JJ, Kathiresan S, Hirschhorn JN, Daly MJ, Hughes GE, Groop L, Altshuler D, Almgren P, Florez JC, Meyer J, Ardlie K, Bengtsson Boström K, Isomaa B, Lettre G, Lindblad U, Lyon HN, Melander O, Newton-Cheh C, Nilsson P, Orho-Melander M, Rastam L, Speliotes EK, Taskinen MR, Tuomilehto J, Tuomilehto T, Eriksson KF, Jorgensen T, Hattersley AT, Di Rienzo A, Frayling TM: A common haplotype of the glucokinase regulatory protein gene is associated with increased plasma triglyceride and C-reactive protein but lower fasting glucose concentrations. *Diabetes* 57:2226–2233, 2008

7. Sparso T, Andersen G, Nielsen TS, Burgdorf KS, Gjesing AP, Nielsen AL, Albrechtsen A, Rasmussen SS, Jorgensen T, Borch-Johnsen K, Sandbaek A, Lauritzen T, Madsbad S, Hansen T, Pedersen O. The GCKR rs780094 polymorphism is associated with elevated fasting serum triacylglycerol, reduced fasting and OGTT-related insulinemia, and reduced risk of type 2 diabetes. *Diabetologia* 51:70–75, 2008

8. Vaxillaire M, Cavalcanti-Proenca C, Dechaume A, Tichet J, Marre M, Balkau B, Froguel P, the DESIR Study Group: The common P446L polymorphism in GCKR inversely modulates fasting glucose and triglyceride levels, and reduces type 2 diabetes risk in a prospective general French population. *Diabetologia* 51:2523–2537, 2008

9. Orho-Melander M, Melander O, Guiducci C, Perez-Martinez P, Corella D, Roos C, Tewhey R, Rieder MJ, Hall J, Abecasis G, Tai ES, Welch C, Arnett DK, Lyssenko V, Lindholm E, Saxena R, de Bakker PI, Burtt NP, Voight BF, Hirschhorn JN, Tucker KL, Hedner T, Tuomilehto J, Isomaa B, Eriksson KF, Taskinen MR, Wahlström B, Hughes GE, Pannell LD, Lai CQ, Bergheld G, Peltonen L, Vartiainen E, Jouhkimainen P, Havulinna AS, Salomaa V, Nilsson P, Groop L, Altshuler D, Ordovas JM, Kathiresan S. Common missense variant in the glucokinase regulatory protein gene is associated with increased plasma triglyceride and C-reactive protein but lower fasting glucose concentrations. *Diabetes* 57:3112–3121, 2008

10. Randle PJ, Garland PB, Hales CN, Newsholme EA: The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* 1:785–789, 1963

11. Holmkvist J, Almgren P, Lyssenko V, Lindgård CM, Eriksson KK, Isomaa B, Tuomi T, Nilsson P, Groop L. Common variants in maturity-onset diabetes of the young genes and future risk of type 2 diabetes. *Diabetes* 57:1738–1744, 2008

12. Rose CS, Ek J, Urhammer SA, Glumer C, Borch-Johnsen K, Jorgensen T, Pedersen O, Hansen T. A −30G>A polymorphism of the β-cell-specific glucokinase promoter associates with hyperglycemia in the general population of whites. *Diabetes* 54:3026–3031, 2005

13. Wiljer C, Sanna S, Jackson AC, Scuteri A, Bonnycastle LL, Clarke R, Heath SC, Timpson NJ, Najjar SS, Stringham HM, Strait J, Duren WL, Maschio A, Busonero F, Mulas A, Albai G, Swift AJ, Morken MA, Narisu N, Bennett D, Parish S, Shen H, Galan P, Meneton P, Hercberg S, Zelenika D, Chen WM, Li Y, Scott LJ, Scheet PA, Sundvall J, Watanabe RM, Nagaraja R, Ebrahim S, Lawlor DA, Ben-Shlomo Y, Davey-Smith G, Shuldiner AR, Collins R, Bergman RN, Uda M, Tuomilehto J, Cao A, Collins FS, Lakatta E, Lathrop GM, Boehnke M, Schlessinger D, Mohlke KL, Abecasis GR: New: identified loci that influence lipid concentrations and risk of coronary artery disease. *Nat Genet* 40:161–169, 2008

14. Horikawa Y, Miyake K, Yasuda K, Enya M, Hirotta Y, Yamagata H, Oka Y, Iwasaki N, IWamoto Y, Yamada Y, Seino Y, Maegawa H, Kashiwagi A, Yamamoto K, Tokunaga K, Takeda J, Kasuga M: Replication of genome-wide association studies of type 2 diabetes susceptibility in Japan. *J Clin Endocrinol Metab* 93:3136–3141, 2008

15. Ng MC, Park KS, Oh B, Tan CH, Cho YM, Shin HD, Lam VK, Ma RC, So WY, Cho YS, Kim HL, Lee HK, Chan JC, Cho NH: Implication of genetic variants near TCF7L2, SLC30A8, HHEX, CDKAL1, CDKN2A/B, IGF2BP2 and FTO in type 2 diabetes and obesity in 6,719 Asians. *Diabetes* 57:2226–2233, 2008

16. Ng MC, Wang Y, So WY, Cheng S, Vissikis S, Zee RY, Fernandez-Cruz A, Lindpaintner K, Chan JC: Ethnic differences in the linkage disequilibrium and distribution of single-nucleotide polymorphisms in 35 candidate genes for cardiovascular diseases. *Genomics* 83:559–565, 2004

17. Ozaki R, Qiao Q, Wong GW, Chan MH, Ko GT, Wang Y, Visvikis S, Fernandez-Cruz A, Chan JC, Cho NH: Implication of genetic variants near TCF7L2, SLC30A8, HHEX, CDKAL1, CDKN2A/B, IGF2BP2 and FTO in type 2 diabetes and obesity in 6,719 Asians. *Diabetes* 57:2226–2233, 2008

18. Liu KH, Chan YL, Chan WB, Chan JC, Chu CW: Mesenteric fat thickness is an independent determinant of metabolic syndrome and identifies subjects with increased carotid intima-media thickness. *Diabetes Care* 29:370–384, 2006

19. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC: PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 81:559–575, 2007

20. Dixon WJ: Simplified estimation from censored normal samples. *The Annals of Mathematical Statistics* 31:385–391, 1960

21. Tukey JW: The future of data analysis. *The Annals of Mathematical Statistics* 33:18, 1962

22. Benjamini Y, Yekutieli D, movie, Yekutieli D, movie. Controlling the false discovery rate in behavior genetics research. *Behav Brain Res* 125:279–284, 2001

23. van den Oord EJ, Sullivan PF: False discoveries and models for gene discovery. *Trends Genet* 19:557–542, 2003

24. DerSimonian R, Laird N: Meta-analysis in clinical trials. *Control Clin Trials* 7:177–188, 1986

25. Purcell S, Cherry SS, Sham PC: Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* 19:149–150, 2003