Low-Frequency Genetic Variant in the Hepatic Glucokinase Gene Is Associated With Type 2 Diabetes and Insulin Resistance in Chinese Population

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Glucokinase (GCK) regulates insulin secretion and hepatic glucose metabolism, and its inactivating variants could cause diabetes. We aimed to evaluate the association of a low-frequency variant of GCK (rs13306393) with type 2 diabetes (T2D), prediabetes, or both (impaired glucose regulation [IGR]) in a Chinese population. An association study was first conducted in a random cluster sampling population (sample 1: 537 T2D, 768 prediabetes, and 1,912 control), and then another independent population (sample 2: 3,896 T2D, 2,301 prediabetes, and 868 control) was used to confirm the findings in sample 1. The A allele of rs13306393 was associated with T2D (odds ratio 3.08 [95% CI 1.77–5.36], *P* = 0.00007) in sample 1; rs13306393 was also associated with prediabetes (1.67 [1.05–2.65], *P* = 0.03) in sample 2. In a pooled analysis of the two samples, the A allele increased the risk of T2D (1.57 [1.15–2.15], *P* = 0.005), prediabetes (1.83 [1.33–2.54], *P* = 0.0003) or IGR (1.68 [1.26–2.25], *P* = 0.0004), insulin resistance estimated by HOMA (β = 0.043, *P* = 0.001), HbA_{1c} (β = 0.029, *P* = 0.029), and urinary albumin excretion (β = 0.033, *P* = 0.025), irrespective of age, sex, and BMI. Thus, the Chinese-specific low-frequency variant increased the risk of T2D through reducing insulin sensitivity rather than islet β-cell function, which should be considered in the clinical use of GCK activators in the future.

Glucokinase (GCK) is a hexokinase isozyme that catalyzes glucose to glucose-6-phosphate (G6P), the first step of both glycolysis and glycogen synthesis. GCK is predominantly expressed in hepatocytes and pancreatic β-cells with different isoforms. The liver isoform plays a central role in regulating glucose uptake and controlling glycogen synthesis, breakdown, and storage (1).

In our previous study (2), we observed that the minor allele frequency (MAF) (0.026) of single nucleotide polymorphism (SNP) rs13306393 in patients with type 2 diabetes (T2D) had an increasing trend in a Chinese population, which had not been evaluated in genome-wide association studies (3–5). Moreover, this SNP is located at the intron near exon 1b, which is exclusively expressed in liver. It would give us a good human genetic model to study the effects of abnormal liver GCK on glucose metabolism.

Thus, we hypothesized that this low-frequency variant of GCK could increase susceptibility for T2D in the Chinese population by specifically affecting the transcription of hepatic GCK. We also evaluated the association of rs13306393 with T2D and prediabetes in a Chinese population.

RESEARCH DESIGN AND METHODS

Discovery Cohort (Sample 1)

All subjects aged 25–75 years were from a population-based study of T2D and metabolic syndrome in a rural region of Beijing (Pinggu), China. As previously described (6), this study was performed from March 2012 to May 2013 using two-stage stratified random cluster sampling. All patients with diabetes in sample 1 were screened for the variants of GCK exons, and seven subjects with

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GCK-maturity-onset diabetes of the young were excluded (2). A 75-g oral glucose tolerance test (OGTT) was performed for the subjects without a history of diabetes, and hemoglobin A_{1c} (HbA_{1c}) (reference range 4.0–6.0% [20–42 mmol/mol]) was determined for each subject. A total of 537 subjects with diabetes (fasting plasma glucose [FPG] ≥7.0 mmol/L, 2-h postload plasma glucose [2hPG] ≥11.1 mmol/L, HbA_{1c} ≥6.5% [48 mmol/mol], or previously diagnosed diabetes), 768 subjects with prediabetes (6.1 mmol/L ≤ FPG < 7.0 mmol/L, 7.8 mmol/L ≤ 2hPG < 11.1 mmol/L, or 6.0% [42 mmol/mol] ≤ HbA_{1c} < 6.5% [48 mmol/mol]), and 1,912 control subjects were included in the study (Table 1).

Validation Cohort (Sample 2)

All the subjects were recruited from Peking University People's Hospital in Beijing or nearby communities from October 2006 to December 2018 as the validation cohort. A total of 3,896 subjects with diabetes (FPG ≥7.0 mmol/L, 2hPG ≥11.1 mmol/L, HbA_{1c} ≥6.5% [48 mmol/mol], or previously diagnosed diabetes), 2,301 subjects with prediabetes (6.1 mmol/L ≤ FPG < 7.0 mmol/L, 7.8 mmol/L ≤ 2hPG < 11.1 mmol/L, or 6.0% [42 mmol/mol] ≤ HbA_{1c} < 6.5% [48 mmol/mol]), and 868 control subjects were included in sample 2 (Table 1). Of these, 599 control subjects and 464 subjects with prediabetes had no HbA_{1c} results and were classified only according to FPG or 2hPG.

This study was performed in accordance with the tenets of the Declaration of Helsinki and was approved by the institutional review board at Peking University People's Hospital. Written informed consent was obtained from all subjects.

Biochemical Measurements and Clinical Information

OGTT was performed in the morning after >8 h of fasting. Creatinine; FPG; total, HDL, and LDL cholesterol; triglycerides (TGs); and uric acid were examined using an automated biochemical instrument. Insulin was measured by electrochemiluminescence immunoassay. HbA_{1c} was quantified by high-performance liquid chromatography. HOMA was used to evaluate β-cell function and insulin sensitivity (7). The urinary albumin-to-creatinine ratio (ACR) was determined by C311 analyzer.

Genotyping for SNP rs13306393

In total, 2,468 subjects in sample 1, as described in our previous study (2), underwent Sanger sequencing analysis. The remaining of sample 1 and all sample 2 subjects were genotyped by a TaqMan allelic discrimination assay (primers shown in Supplementary Table 1). Ninety-eight samples with the A allele and 246 samples without the A allele were verified by Sanger sequence; the coincidence rate was 100%. The genotype frequencies in both samples were in accordance with Hardy-Weinberg equilibrium.

Human Liver GCK Promoter Plasmid Construct and Luciferase Reporter Assay

Two reporters, including the SNP and the hepatic GCK (NM_033507) promoter (8) ($GCK_{Liver\text{-}promoter\text{-}Luc\text{-}rs13306393T}$ and $GCK_{Liver\text{-}promoter\text{-}Luc\text{-}rs13306393A}$), were constructed (shown in Supplementary Fig. 1). Because the transfection efficiency of the human hepatocellular carcinoma HepG2 cell line and normal liver L-02 cell line was too low to be used for luciferase reporter assay in our study, HEK-293T cells had to be used. The transfected HEK-293T cells with $GCK_{Liver\text{-}promoter\text{-}Luc\text{-}rs13306393T}$ or $GCK_{Liver\text{-}promoter\text{-}Luc\text{-}rs13306393A}$ construct were harvested and assayed for luciferase activity. Each transfection experiment was carried out in triplicate on at least three independent occasions.

Statistics

Statistical analysis was performed using SPSS version 23.0 software. Differences in allele and genotype frequencies were analyzed using Pearson χ^2 test. Logistic regression analysis was performed to calculate MAF-specific odds ratios (ORs) with 95% CIs. Quantitative trait association analyses were performed using multiple linear regressions. The Genetic Power Calculator (https://zzz.bwh.harvard.edu/gpc) from Harvard University was used for power calculations.

Data and Resource Availability

All data sets of this article are available from the corresponding authors upon request.

RESULTS

Association of rs13306393 With T2D and Impaired Glucose Regulation in Sample 1

As shown in Table 2, the allele and genotype distributions between the subjects with T2D or impaired glucose regulation (IGR) and control subjects were significantly different. Logistic analysis showed that the A allele of rs13306393 was associated with T2D (OR 3.076 [95% CI 1.766–5.358], $P = 0.000072$) or IGR (1.836 [1.132–2.976], $P = 0.014$), but there was no association with prediabetes (1.131 [0.602–2.125], $P = 0.702$).

Since the subjects with an HbA_{1c} level of 5.7–6.0% (39–42 mmol/mol) carried the same risk of developing diabetes over 5 years of follow-up as those with impaired fasting glucose (9), we reclassified 281 subjects with 5.7% (39 mmol/mol) ≤ HbA_{1c} < 6.0% (42 mmol/mol) in 1,912 control subjects as prediabetes and reanalyzed the data. The ORs were 2.655 (95% CI 1.489–4.734) for T2D ($P = 0.001$), 0.726 (0.390–1.350) for prediabetes ($P = 0.312$), and 1.265 (0.777–2.059) for IGR ($P = 0.345$).

Confirming the Association in Sample 2 and the Pooled Samples

As shown in Table 2, the carrier of the A allele had a higher risk for prediabetes irrespective of age, sex, and BMI (OR 1.668 [95% CI 1.048–2.654], $P = 0.031$) in sample 2. In a pooled analysis of two case-control samples, the A allele carrier was also associated with T2D (1.570 [1.148–2.145], $P = 0.005$), prediabetes (1.833 [1.325–2.537], $P = 0.000255$), and IGR (1.681 [1.258–2.245], $P = 0.000439$).

Table 1—Subject characteristics

	Sample 1			Sample 2		
	T2D (n = 537)	Prediabetes (n = 768)	Control (n = 1,912)	T2D (n = 3,896)	Prediabetes (n = 2,301)	Control (n = 868)
Age (years)	54.2 ± 10.2	51.8 ± 11.5†	45.6 ± 11.8¶¶¶	54.1 ± 14.4	53.0 ± 10.2	54.5 ± 8.2
Age at diagnosis (years)	51.2 ± 10.2***	—	—	47.0 ± 13.2	—	—
Male/female sex (n)	292/245	422/346	839/1,073	2,034/1,862	890/1,411	250/618
BMI (kg/m^2)	27.3 ± 3.7***	27.0 ± 3.7†††	25.4 ± 3.7	26.0 ± 3.9	26.4 ± 3.2	25.4 ± 3.5
Waist circumference (cm)						
Male	94.4 ± 9.7	92.1 ± 9.8	89.3 ± 9.6	93.3 ± 10.7	92.7 ± 8.3	88.5 ± 9.1
Female	91.9 ± 9.6***	90.5 ± 9.0†††	83.2 ± 9.8	87.9 ± 10.5	86.2 ± 9.2	83.0 ± 9.6
SBP (mmHg)	138.6 ± 17.5***	136.3 ± 17.1†††	126.6 ± 17.3¶	129.6 ± 17.1	124.5 ± 16.2	125.0 ± 18.8
DBP (mmHg)	90.6 ± 13.1***	89.1 ± 11.5†††	83.5 ± 11.9¶¶¶	78.8 ± 10.5	78.0 ± 9.5	79.3 ± 11.5
FPG (mmol/L)	7.57 (6.67, 9.34)***	6.02 (5.51, 6.28)	5.29 (5.01, 5.54)¶¶¶	7.19 (6.08, 8.97)	5.94 (5.42, 6.32)	5.15 (4.88, 5.45)
2hPG (mmol/L)	13.12 (11.26, 17.44)***	8.24 (7.13, 8.99)†††	5.80 (4.95, 6.63)	12.47 (9.67, 15.63)	8.70 (7.95, 9.63)	5.77 (4.89, 6.54)
Fasting insulin (μU/mL)	8.98 (5.27, 13.70)***	8.20 (5.44, 12.72)†††	6.74 (4.39, 9.92)¶	10.09 (6.69, 15.66)	9.11 (6.21, 12.95)	7.01 (5.10, 9.57)
2-h postload insulin (μU/mL)	52.05 (26.26, 87.68)**	57.25 (33.44, 100.23)	33.67 (20.09, 53.37)	41.82 (25.60, 67.05)	59.38 (34.83, 101.56)	31.45 (18.64, 54.11)
HbA_{1c} (mmol/mol)[a]	55.0 ± 17.5***	38.0 ± 4.5†††	34.0 ± 3.5	65.0 ± 25.0	39.0 ± 3.0	36.0 ± 3.5
HbA_{1c} (%)[a]	7.2 ± 1.6***	5.6 ± 0.4†††	5.3 ± 0.3	8.1 ± 2.3	5.7 ± 0.3	5.4 ± 0.3
HOMA-β	41.06 (23.35, 69.45)***	71.72 (44.37, 109.35)†††	77.98 (50.86, 112.74)¶¶¶	55.38 (31.65, 95.08)	78.24 (51.89, 114.85)	86.82 (59.91, 122.35)
HOMA-IR	3.18 (1.92, 5.16)*	2.14 (1.38, 3.31)†††	1.57 (1.02, 2.36)	3.32 (2.06, 5.39)	2.34 (1.60, 3.43)	1.61 (1.16, 2.23)
TC (mmol/L)	5.16 ± 1.10***	5.10 ± 0.96†††	4.79 ± 0.90¶¶	4.77 (4.05, 5.46)	4.85 (4.26, 5.50)	4.87 (4.18, 5.54)
LDL-C (mmol/L)	2.91 ± 0.88	2.97 ± 0.80†††	2.75 ± 0.76¶¶¶	2.97 ± 0.92	3.15 ± 0.84	3.01 ± 0.84
HDL-C (mmol/L)						
Male	1.07 ± 0.35	1.13 ± 0.36	1.10 ± 0.29¶¶¶	1.06 ± 0.31	1.12 ± 0.26	1.25 ± 0.34
Female	1.12 ± 0.24***	1.16 ± 0.27†††	1.25 ± 0.31¶¶¶	1.24 ± 0.35	1.30 ± 0.32	1.41 ± 0.38
TG (mmol/L)	1.71 (1.07, 2.55)	1.38 (0.94, 2.07)††	1.03 (0.68, 1.59)¶¶¶	1.57 (1.12, 2.28)	1.46 (1.09, 2.05)	1.14 (0.82, 1.61)
UA (μmol/L)						
Male	303.6 ± 78.0***	334.8 ± 81.0	322.2 ± 81.0	372.4 ± 96.1	—	—
Female	255.9 ± 67.3***	263.5 ± 62.8	237.9 ± 57.7	320.2 ± 93.7	—	—
CRE (μmol/L)	59.6 (49.9, 70.0)	60.0 (52.0, 70.0)	57.0 (49.0, 67.0)	62.0 (52.1, 73.0)	66.0 (57.0, 79.0)	61.8 (53.3, 74.2)
ACR (mg/g)	14.43 (6.10, 39.71)***	7.62 (2.88, 18.85)	4.50 (1.69, 10.93)	7.94 (3.52, 26.96)	7.40 (4.43, 14.58)	—
eGFR (mL/min/1.73 m^2)	131.8 ± 36.3*	130.8 ± 33.7†††	137.7 ± 32.1¶¶¶	124.9 ± 76.6	112.6 ± 31.9	113.0 ± 30.6

Data are mean ± SD or median (interquartile range). Statistical analysis was performed using SPSS version 23.0 software. International System of Units (SI)/non-SI conversion calculations: glucose, mmol/L * 18.02 = mg/dL; insulin, μU/mL * 6.945 = pmol/L; TC, mmol/L * 38.61 = mg/dL; TG, mmol/L * 88.50 = mg/dL; LDL, mmol/L * 38.61 = mg/dL; HDL, mmol/L * 38.61 = mg/dL; UA, mg/dL * 59.485 = μmol/L; and CRE, mg/dL * 88.4 = μmol/L. eGFR = 175 * CRE (mg/dL)$^{-1.234}$ * age (years)$^{-0.179}$ * 0.79 (if female). CRE, serum creatinine; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL-C, HDL cholesterol; HOMA-β, HOMA of β-cell function; LDL-C, LDL cholesterol; SBP, systolic blood pressure; TC, total cholesterol; UA, uric acid. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, sample 1 vs. sample 2 subjects with T2D. †$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$, sample 1 vs. sample 2 subjects with prediabetes. ¶$P < 0.05$, ¶¶$P < 0.01$, ¶¶¶$P < 0.001$, sample 1 vs. sample 2 control subjects. [a]HbA_{1c} indicates that there were 1,063 subjects who did not have standardized HbA_{1c} results in sample 2. All the subjects without a history of diabetes were given a 75-g OGTT. Diabetes was diagnosed according to FPG ≥7.0 mmol/L, 2hPG ≥11.1 mmol/L, or HbA_{1c} ≥48 mmol/mol (6.5%). The subjects with FPG <6.1 mmol/L, 2hPG <7.8 mmol/L, and HbA_{1c} <42 mmol/mol (6.0%) were selected for the control group. The remaining subjects were classified as having prediabetes. Subjects without an HbA_{1c} who were not previously diagnosed as having diabetes were classified according to their FPG and 2hPG levels.

Table 2—Logistic regression analysis of association of SNP rs13306393 with T2D, prediabetes, and IGR

	Gene type, TT/TA/AA			MAF (%)								
Sample	T2D	Prediabetes	Control	T2D	Prediabetes	Control	OR (95% CI)[a]	$P_{logistic}$[a]	OR (95% CI)[b]	$P_{logistic}$[b]	OR (95% CI)[c]	$P_{logistic}$[c]
1	509/28/0	752/16/0	1,875/37/0	2.61	1.04	0.97	3.076 (1.766–5.358)	0.000072	1.131 (0.602–2.125)	0.702	1.836 (1.132–2.976)	0.014
2	3,765/130/1	2,201/99/1	845/23/0	1.69	2.19	1.32	1.185 (0.750–1.872)	0.468	1.668 (1.048–2.654)	0.031	1.401 (0.904–2.171)	0.131
1 and 2	4,274/158/1	2,953/115/1	2,720/60/0	1.80	1.91	1.08	1.570 (1.148–2.145)	0.005	1.833 (1.325–2.537)	0.000255	1.681 (1.258–2.245)	0.000439

Genotypic and allelic frequencies of rs13306393 were computed and checked for deviation from Hardy-Weinberg equilibrium. SNP rs13306393 was in Hardy-Weinberg equilibrium in both samples 1 and 2 ($P > 0.05$). Differences in allele and genotype frequencies between the subjects with T2D or prediabetes and the control subjects were analyzed using Pearson χ^2 test. Logistic regression analysis was performed to calculate MAF-specific ORs with 95% CIs after adjustment for age, sex, and BMI as covariates. The additive model for minor allele was used, and ORs were calculated by logistic regression analysis. *P* values were adjusted for age, sex, and BMI. [a]Significance between T2D and control. [b]Significance of MAF between prediabetes and control. [c]Significance between IGR and control.

Table 3—Linear regression analysis of each phenotype with genotypes of rs13306393 in subjects without antidiabetic treatments in each sample and pooled samples

	Sample 1		Sample 2		Samples 1 and 2	
Phenotype	Standardized β	*P* value	Standardized β	*P* value	Standardized β	*P* value
BMI (kg/m^2)[a]	0.014	0.441	−0.011	0.504	0.001	0.939
FPG (mmol/L)	0.057	0.002**	−0.016	0.461	0.018	0.145
2hPG (mmol/L)	0.057	0.003**	0.007	0.684	0.032	0.008**
HbA_{1c} (mmol/mol) (%)	0.060	0.001**	−0.024	0.249	0.029	0.029*
Fasting insulin (μU/mL)	0.029	0.109	0.037	0.022*	0.035	0.004**
2-h postload insulin (μU/mL)	0.012	0.547	0.019	0.416	0.028	0.048*
HOMA-IR	0.050	0.016*	0.037	0.023*	0.043	0.001**
HOMA-β	−0.013	0.543	0.027	0.094	0.014	0.225
TC (mmol/L)	−0.025	0.183	0.004	0.784	−0.009	0.466
TG (mmol/L)	−0.016	0.348	0.030	0.067	0.017	0.139
LDL-C (mmol/L)	−0.023	0.230	0.010	0.557	0.003	0.784
HDL-C (mmol/L)[b]						
Male	−0.024	0.414	−0.039	0.135	−0.027	0.123
Female	−0.023	0.385	0.003	0.901	−0.001	0.930
UA (μmol/L)[b]						
Male	−0.051	0.075	—	—	−0.043	0.075
Female	0.031	0.248	—	—	0.028	0.248
CRE (μmol/L)	−0.019	0.442	0.009	0.726	0.014	0.389
ACR (mg/g)	0.032	0.085	0.022	0.347	0.033	0.025*
eGFR (mL/min/1.73 m^2)	0.016	0.445	−0.006	0.902	0.008	0.660

There were 1,912 control subjects, 768 subjects with prediabetes, and 330 subjects with newly diagnosed T2D included in sample 1. There were 868 control subjects, 2,301 subjects with prediabetes, and 410 subjects with newly diagnosed T2D included in sample 2. Quantitative trait association analyses were performed using multiple linear regressions adjusting for age, sex, and BMI. Association analyses of BMI and waist circumference were adjusted for age and sex. Because of the low frequency of minor alleles for the homozygote of rs13306393, a dominant model was used in logistic and linear regression analyses. In all hypothesis tests, two-tailed $P < 0.05$ was considered statistically significant. International System of Units (SI)/non-SI conversion calculations: glucose, mmol/L * 18.02 = mg/dL; insulin, μU/mL * 6.945 = pmol/L; TC, mmol/L * 38.61 = mg/dL; TG, mmol/L * 88.50 = mg/dL; LDL, mmol/L * 38.61 = mg/dL; HDL, mmol/L * 38.61 = mg/dL; UA, mg/dL * 59.485 = μmol/L; and CRE, mg/dL * 88.4 = μmol/L. CRE, serum creatinine; eGFR, estimated glomerular filtration rate; HDL-C, HDL cholesterol; HOMA-β, HOMA of β-cell function; LDL-C, LDL cholesterol; TC, total cholesterol; UA, uric acid. [a]Adjusted for sex and age. [b]Adjusted for BMI and age. *$P < 0.05$, **$P < 0.01$.

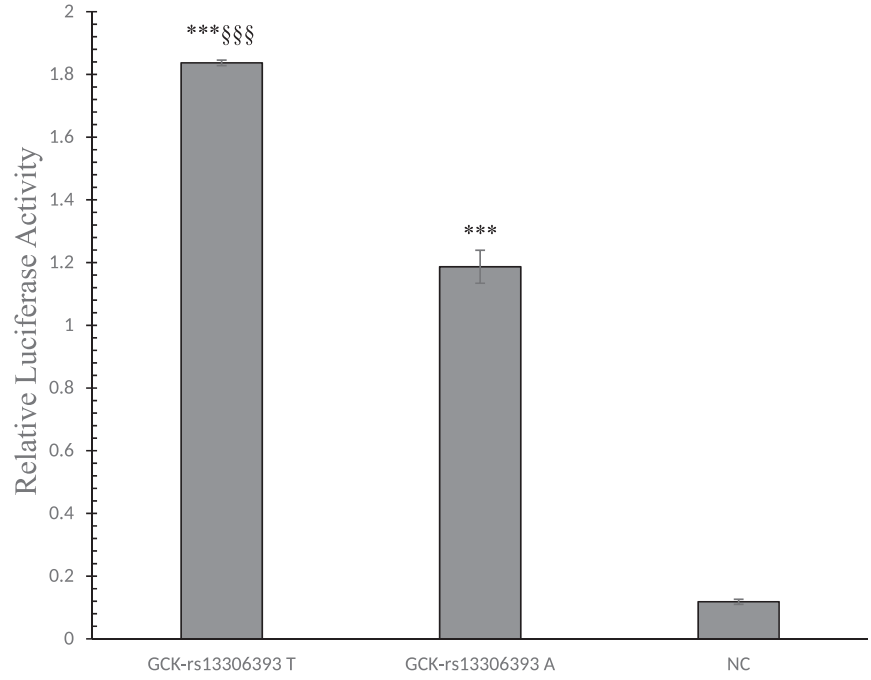

Figure 1—GCK rs13306393T>A regulates expression of the human GCK promoter. Luciferase activity declined in HEK-293T cells transfected with $GCK_{Liver\text{-}promoter\text{-}Luc\text{-}rs13306393A}$ reporter compared with $GCK_{Liver\text{-}promoter\text{-}Luc\text{-}rs13306393T}$. Error bars represent the mean ± SD of three independent experiments. ***$P < 0.001$ compared with the normal control (NC) group (empty plasmid CV236); §§§$P < 0.001$ for the $GCK_{Liver\text{-}promoter\text{-}Luc\text{-}rs13306393T}$ group compared with the $GCK_{Liver\text{-}promoter\text{-}Luc\text{-}rs13306393A}$ group. Statistical significance was determined by two-tailed Student *t* test.

When we reclassified 46 subjects with HbA_{1c} 5.7–6.0% (39–42 mmol/mol) in 868 control subjects as prediabetes in sample 2, the association of the A allele with prediabetes remained significant (OR 1.735 [95% CI 1.072–2.808], $P = 0.025$). In the pooled analysis of the two samples, OR was 1.441 (1.047–1.982) for T2D ($P = 0.025$), 1.571 (1.127–2.191) for prediabetes ($P = 0.008$), and 1.498 (1.114–2.015) for IGR ($P = 0.008$).

Genotype-Phenotype Relationship Analysis

As shown in Table 3, the subjects taking no antidiabetic drugs in sample 1 (1,912 control subjects, 768 subjects with prediabetes, and 330 subjects with T2D) were used for linear regression analysis. The A allele of rs13306393 was positively associated with FPG ($P = 0.002$), HbA_{1c} ($P = 0.001$), and HOMA of insulin resistance (HOMA-IR) ($P = 0.016$) after adjustment for age, sex, and BMI.

The association with HOMA-IR ($P = 0.023$) was confirmed in sample 2 (868 control subjects, 2,301 subject with prediabetes, and 410 subjects with T2D). In the pooled samples, associations with 2hPG ($P = 0.008$), HbA_{1c} ($P = 0.029$), HOMA-IR ($P = 0.001$), and ACR ($P = 0.025$) were also observed.

The Effect of rs13306393 on Human Hepatic GCK Transcription

As shown in Fig. 1, luciferase activity was significantly lower in cells transfected with $GCK_{Liver\text{-}promoter\text{-}Luc\text{-}rs13306393A}$ than with $GCK_{Liver\text{-}promoter\text{-}Luc\text{-}rs13306393T}$ ($P < 0.001$).

DISCUSSION

In the current study, we report that a novel, low-frequency variant located at IVS1b ds+12 (NC_000007.14: g.44159061T>A) of the liver transcript of the GCK gene is associated with T2D in a Chinese population. Its frequency is much higher in East Asian (0.02015 in The Genome Aggregation Database) than other ethnic populations (0.0002). It was positively related not only to glycemic traits (FPG, 2hPG, HbA_{1c}) but also to insulin resistance (HOMA-IR).

This study included a total of 4,433 subjects with T2D, 3,069 subject with prediabetes, and 2,780 control subjects and had nearly 100% power under a genetics dominant model at $P = 0.05$ when T2D prevalence was 11.6% (10) and OR 1.7. In our previous genome-wide association study on T2D (5) and others in Chinese subjects (3,4) wherein common variants of GCK had been evaluated, we did not observe linkage disequilibrium relationships between rs13306393 and common variants of GCK associated with T2D and related traits (Supplementary Fig. 2 and Supplementary Table 3). Thus, rs13306393 was an independent locus associated with T2D in Chinese.

In past decades, a few of population-specific genetic variants, such as p.Gly319Ser of HNF1A in the Oji-Cree population (11), p.Glu508Lys of HNF1A in the Latino population (12), and p.Arg1420His of ABCC8 in Southwest American Indians (13), had been reported to increase the risk for T2D. In fact, many T2D genetic loci initially discovered in the European descent Caucasian population

had not been replicated in the Chinese population and vice versa. The risk allele frequency at some genetic loci among ethnic populations varies greatly (14). Thus, the low-frequency variant at rs13306393 may be a unique genetic factor in East Asians.

To our knowledge, there has been no reliable study to date showing that GCK variants increase insulin resistance. In contrast to subjects with GCK-maturity-onset diabetes of the young who present with both primary reduced insulin secretion as a result of impaired GCK function of pancreatic β-cell (15), the A allele of rs13306393 might affect liver GCK function rather than islet GCK, and hyperglycemia and diabetes should be attributed to insulin resistance and secondary β-cell dysfunction.

It is also worth noting that the A allele of rs13306393 positively associates with ACR, the biomarker of early kidney disease. This finding might be due to the insulin resistance that resulted from this variant. Similarly, in liver-specific GCK knockout mice, hyperglycemia, diabetes, weight gain, insulin resistance, and renal complication were observed (16).

Interestingly, the phenotypes of rs13306393A were very similar to variants of GCK regulation protein (GCKR), which controls GCK activity specifically in liver. Many studies have demonstrated that variants of GCKR related to deceased GCK activity are associated with increased levels of FPG, 2hPG, HbA_{1c}, T2D, and HOMA-IR; low levels of TGs; and chronic kidney disease (17–19). In fact, we observed that the allele distributions of the GCKR variant (rs780094) (20), which was reported to associate with T2D, were very similar between the subjects with ($n = 81$) or without ($n = 162$) the A allele of rs13306393 (Supplementary Table 2). These results suggest that this variant is involved in the development of T2D, liver insulin resistance, and renal complications independently of GCKR variants.

The association of rs13306393 with T2D and IGR in a Chinese population has potential clinical implications. Approximately 2% of people in the Chinese population carry the A allele, and these individuals have reduced liver GCK activity and are the most appropriate population for GCK activators (GKAs). Further studies are warranted to validate the hypothesis that the patients carrying the A allele of rs13306393 could greatly benefit from GKAs.

Our study has some limitations. First, although the in vitro study showed that the A allele of rs13306393 might reduce the transcription level of the GCK gene, we could not make a firm conclusion. With the lack of appropriate animal and cell models, functional study of a variant at a nonconservative region in an intron is difficult. New advanced techniques, such as gene editing that is based on human pluripotent stem cells, are needed in the future. Second, the dates, methods, and numbers for recruiting study subjects were different between two independent case-control samples. Sample 1 subjects were from a community, and most subjects with prediabetes and diabetes had mild hyperglycemia and were diagnosed according to a single FPG, 2hPG, or HbA_{1c} measurement. However, most subjects in sample 2 were recruited from hospitals, and they usually had histories of hyperglycemia. These differences might explain the inconsistent ORs of prediabetes between samples 1 and 2. Additionally, some patients might have been misclassified into the control groups owing to a lack of HbA_{1c} results, which might increase the chance of negative results. Further studies with larger sample sizes, more homogeneous populations, and more proper criteria of classification for glucose tolerance are needed.

In conclusion, we identified a low-frequency variant in liver GCK that might be unique to East Asians. This variant was associated with an increased risk of T2D, IGR, and insulin resistance. Further studies are warranted to validate the hypothesis that patients carrying rs13306393A could greatly benefit from GKAs.

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