Common Missense Variant in the Glucokinase Regulatory Protein Gene Is Associated With Increased Plasma Triglyceride and C-Reactive Protein but Lower Fasting Glucose Concentrations

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OBJECTIVE—Using the genome-wide association approach, we recently identified the glucokinase regulatory protein gene (GCKR, rs780094) region as a novel quantitative trait locus for plasma triglyceride concentration in Europeans. Here, we sought to study the association of GCKR variants with metabolic phenotype, including measures of glucose homeostasis, to evaluate the GCKR locus in samples of non-European ancestry and to fine-map across the associated genomic interval.

RESEARCH DESIGN AND METHODS—We performed association studies in 12 independent cohorts comprising >45,000 individuals representing several ancestral groups (whites from Northern and Southern Europe, whites from the U.S., African Americans from the U.S., Hispanics of Caribbean origin, and Chinese, Malays, and Asian Indians from Singapore). We conducted genetic fine-mapping across the −417-kb region of linkage disequilibrium spanning GCKR and 16 other genes on chromosome 2p23 by imputing untyped HapMap single nucleotide polymorphisms (SNPs) and genotyping 104 SNPs across the associated genomic interval.

RESULTS—We provide comprehensive evidence that GCKR rs780094 is associated with opposite effects on fasting plasma triglyceride (P\textsubscript{meta} = 3 × 10\textsuperscript{-56}) and glucose (P\textsubscript{meta} = 1 × 10\textsuperscript{-19}) concentrations. In addition, we confirmed recent reports that the same SNP is associated with C-reactive protein (CRP) level (P = 5 × 10\textsuperscript{-10}). Both fine-mapping approaches revealed a common missense GCKR variant (rs1260326, Pro446Leu, 34% frequency, \(r^2 = 0.93\) with rs780094) as the strongest association signal in the region.

CONCLUSIONS—These findings point to a molecular mechanism in humans by which higher triglycerides and CRP can be coupled with lower plasma glucose concentrations and position GCKR in central pathways regulating both hepatic triglyceride and glucose metabolism. *Diabetes* 57:3112–3121, 2008

Recently, in the genome-wide association Diabetes Genetics Initiative (DGI) Study for 19 traits, including plasma lipids, we provided evidence that the glucokinase (GCK) regulatory protein gene (GCKR) region was a novel quantitative trait locus associated with plasma triglyceride concentration (1). Of all single nucleotide polymorphisms (SNPs) tested, an intrinsic SNP at GCKR (rs780094) explained the greatest proportion of interindividual variability in plasma triglycerides (1).

GCKR regulates GCK, which functions as a glucose sensor responsible for glucose phosphorylation in the first step of glycolysis. The discoveries that inactivating muta-
tions in GCK cause maturity onset diabetes of the young type 2 (2) and activating GCK mutations lead to permanent hyperinsulinemic hypoglycemia (3) emphasize that GCK plays a major role in glucose metabolism. GCK-deficient mice have reduced GCK expression but maintain nearly normal GCK activity and show impaired glucose clearance (4). Furthermore, adeno HViral-mediated overexpression of GCKR in mouse liver increased GCK activity and lowered fasting blood glucose (5) and overexpression of GCK in liver led to lowered blood glucose and increased triglyceride concentrations (6,7). Thus, experimental evidence suggests that perturbation of the GCKR pathway has opposing effects of triglyceride and glucose metabolism.

In our original report, SNP rs780094 in GCKR was associated with fasting triglyceride levels in two independent samples, each of Northern European ancestry (P = 3.7 × 10⁻⁸ and 8.7 × 10⁻⁸, respectively) (1). After initial identification and replication of a chromosomal region associated with a trait, key next steps include extension of the association finding to related phenotypes, validation of the association finding in different ethnicities, and fine-mapping to identify the putative causal variant. Recently, our initial finding was replicated in a Danish study in which a strong association was found between the rs780094 T allele and elevated fasting triglyceride levels but also lower insulin levels, better insulin sensitivity, and a moderately decreased risk of type 2 diabetes (8). In addition, recent genome-wide association studies identified an association between the same GCKR intronic SNP and C-reactive protein (CRP) levels (9,10).

Hereby, we sought to examine the effect of SNP rs780094 on triglycerides and related metabolic traits, including fasting glucose concentrations, in 12 samples representing a range of ancestral groups and including a large prospective study with a mean follow-up time of 23 years. In addition, we performed fine-mapping in one of these samples to identify the strongest association signal in the region.

RESEARCH DESIGN AND METHODS

The genetic association studies were performed in 12 study samples as shown in Table 1. For all studies, informed consent was obtained from the study subjects, and the study protocols were approved by local ethics committees. All study cohorts genotyped for GCKR as part of this experiment are included in this report.

The DGI Study material consisting of 2,931 individuals from Finland and Sweden (1,464 patients with type 2 diabetes and 1,467 nondiabetic control subjects) was ascertained as previously described (11,12). DGI samples were genotyped on the Affymetrix 500K chip (1) and were used in the present study for the in silico fine-mapping. In addition, the DGI samples were used for the genotype fine-mapping of the GCKR locus and for the analyses of apolipoprotein B (apoB) and free fatty acids (FFAs) according to rs780094.

The Malmo Diet and Cancer Study–Cardiovascular Cohort (MDC-CC) represents 6,103 people that were randomly selected to participate in a study to identify the strongest association signal in the region.
selected workers, using a continuously updated computerized population register, and subjects randomly selected from the general population.

The Dallas Heart Study is a multiethnic, probability-based sample of Dallas County, weighted such that 50% of the study population was black (23). Ethnicity was self-reported and consisted of non-Hispanic blacks, non-Hispanic whites, and Hispanics. The study population included 3,469 individuals from one of these three ethnic groups with fasting venous blood samples.

The Genetics of Lipid-Lowering Drugs and Diet Network (GOLDN) Study sample consists of 1,062 individuals and is part of the Program for Genetic Interactions Network (24). The majority of participants in the GOLDN Study were re-recruited from three-generational pedigrees from two National Heart, Lung, and Blood Institute (NHBLI) Family Heart Study (FHS) field centers (Minneapolis, MN, and Salt Lake City, UT) (24). The NHBLI FHS is a multicenter, population-based study of genetic and environmental determinants of cardiovascular disease (CVD) and associated risk factors. Nearly all subjects were of European ancestry.

The longitudinal Boston Puerto Rican Health Study includes 837 Puerto Rican (Hispanics of Caribbean origin) men and women aged 45–75 years in the greater Boston area (25,26). As one of eight nationally funded National Institutes of Health (NIH) Centers on Population Health and Health Disparities, the study is investigating health disparities in the Puerto Rican population. Participants were recruited from the Boston area through door-to-door enumeration, following a sampling scheme based on identification of the 2000 U.S. Census blocks containing Hispanics, and in partnership with community organizations.

The Singaporean National Health Survey 98 (Singaporean NHS-98) study was an initiative to determine the risk factors for the major noncommunicable diseases in Singapore (27,28). A total of 3,973 subjects who participated in the Singaporean NHS-98 and had the data needed for the current study were included in this study. In brief, 11,200 individuals from addresses representing the house-type (a proxy for socioeconomic status) distribution of the entire Singaporean housing population were selected from the National Database on Dwellings. From these individuals, a random sample was selected by disproportionate stratified and systematic sampling. The Malays and Indians were oversampled to ensure that prevalence estimates for these minority groups were reliable.

**Lipid and glucose phenotypes.** Plasma total cholesterol, HDL cholesterol, and triglycerides in each study were measured using standard enzymatic methods from fasting blood samples, with the exception of FINRISK7, in which the lipid measurements were performed from blood samples collected in a “semifasting” state; i.e., the participants were instructed to fast for 4 h and to avoid fatty meals earlier during the day. ApoB in the DGI was measured using an immunochemical assay (Orion Diagnostica, Espoo, Finland), and FFA was measured by an enzymatic colorimetric ACS-ACOD-MEHA method (Wako Chemicals, Neuss, Germany).

Fasting blood or plasma glucose was measured by glucose oxidase methods as previously described (1,16–18, 22–27), and blood glucose was converted to plasma glucose using a correction factor of 1.13. Fasting serum insulin was measured using radiolimmonoassy in DGI, MDC-CC, MPP-MM, GOLDN, and Singaporean NHS-98 samples (1,11–13,21,24,25). Homeostasis model assessment (HOMA) insulin resistance index was calculated using the following formula: fasting plasma glucose × fasting insulin/22.5. The insulinogenic index was calculated as ([insulin 30 – fasting insulin]/[glucose 30 – fasting glucose]).

**Genotyping.** Genotyping was performed either by matrix-assisted laser desorption ionization-time of flight mass spectrometry on the Sequenom MassARRAY platform (San Diego, CA) or by allelic discrimination method on the ABI 7900 instrument (Applied Biosystems, Foster City, CA). The studied SNPs were in Hardy-Weinberg equilibrium in all studies populations (P > 0.01) except rs780094 in the Singaporean Chinese population (P = 0.000063). Because of deviation from Hardy-Weinberg equilibrium, a random sample of 12% of the Chinese samples was reanalyzed in a separate assay, and the genotyping error rate was 0.3%.

**Genotype fine-mapping.** GCKR rs780094 lies in a large region of linkage disequilibrium on chromosome 2. We defined the associated interval to be ~417 kb based on linkage disequilibrium between the index SNP (rs780094) and SNPs upstream and downstream of the index SNP. rs1049817 was furthest upstream with an r² > 0.25 with the index SNP, and rs13023194 was the furthest downstream with an r² > 0.25 with the index SNP. The interval between rs1049817 and rs13023194 spans 416,543 bases (National Center for Biotechnology Information human genome sequence Build 35). In this interval, there are 17 annotated genes: MPV17, GTXF32C, EIF2BA, SNX17, ZNF513, PPM1G, NRBP1, KRTCAP3, IFT172, FNDCN, GCKR, C20orf16, ZNF512, CCD121X1, BAX1, SUPT7L, and ZNF513. To fine-map the association signal across this interval, we selected 120 SNPs for genotyping based on the following criteria: 1) tag SNPs (n = 33) that captured all common alleles (minor allele frequency >0.05) across the ~417 kb at an r² >0.25, 2) all coding SNPs (n = 83) present in HapMap CEU for these genes; and 3) a set of SNPs predicted to be microRNA binding sites (n = 4). Of these 120 SNPs, 104 were successfully designed for genotyping assays and were genotyped in the DGI sample.

**In silico fine-mapping.** We also conducted fine-mapping using a second approach: imputation of untyped SNPs. We imputed untyped SNPs across the region using a recently developed Markov Chain Haplotyping algorithm (MACH 1.0) (29). This method predicts genotypes for untyped SNPs in a given study using two inputs: genotypes at typed SNPs in the study sample and the entire set of genotypes in HapMap (http://hapmap.org) for a given reference sample. Here, the inputs were the following: genotypes from the Affymetrix 500K array in the DGI study and ~2.2 million SNPs in the HapMap CEU samples (a reference sample of European ancestry).

**Expression studies.** To evaluate whether the transcript level of GCKR or GCK varied by genotype, we studied 101 human liver samples with both measured transcript levels and genotypes. Transcript levels for 60 samples were assessed by the Human ReF8 v2 Illumina chip, and genotypes were measured by the Illumina 550K array (30). For 41 additional samples, GCKR and GCK transcript levels were measured in human liver tissue obtained from the University of Minnesota Tissue Procurement Center (Minneapolis, MN) following institutional review board guidelines. DNA was extracted using a Qiagen extraction protocol according to the manufacturer’s directions. Samples were genotyped by primer extension with detection by matrix-assisted

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**TABLE 1**

Clinical characteristics of the study cohorts

|                        | Finland (ref. 1) | DGI | MDC-CC (ref. 13) | MPP (ref. 16) | NORDIL (ref. 18) | Registry (ref. 19) | Botnia-PPP | FINRISK97 |
|------------------------|------------------|-----|------------------|---------------|-----------------|-------------------|-------------|-----------|
| n (men/women)          | 1,448/1,482      | 2,930 | 5,506            | 10,927/6,110  | 2,567/2,585      | 1,583/1,075       | 1,663/1,841 | 3,495     | 8,191     |
| Age (years)            | 62 ± 11          | 58 ± 6 | 46 ± 7           | 60 ± 7        | 63 ± 11          | 49 ± 16          | 49 ± 16     | 48 ± 13   |
| BMI (kg/m²)            | 28 ± 4           | 26 ± 4 | 24 ± 3           | 28 ± 4        | 30 ± 5           | 26 ± 4           | 27 ± 5      |
| Triglycerides (mg/dl)  | 144 ± 101        | 159 ± 108 | 231 ± 275        | 116 ± 71      | 133 ± 92         |                   |             |           |
| HDL (mg/dl)            | 49 ± 15          | 53 ± 14 | —                | 53 ± 21       | 44 ± 13          | —                | 54 ± 14     |
| LDL (mg/dl)            | 145 ± 51         | 160 ± 43 | 137 ± 40         | —            | 134 ± 36         |                   |             |           |
| fB-glucose (mg/dl)     | 121 ± 49         | 96 ± 28          | 189 ± 69          | 84 ± 15       | —                | —                | —          |
| HOMA (mmol × mU)       | 1.7 ± 1.4        | 2.0 ± 2.7        | —                | —                | —                | —                | —          |
| Type 2 diabetes (%)    | 49.9             | 8.4             | 0.0              | 8.7           | 100.0            | 3.8             | 5.4        |

Continued on facing page
RESULTS

Association with plasma triglyceride concentrations.

The initial association of rs780094 with triglyceride concentrations was studied in each of 12 study samples, representing a range of ancestral groups (Table 2). In all but one sample, the T allele was associated with higher triglycerides (Table 2; \( P \) for association ranging from 0.29 to \( 6 \times 10^{-10} \)); for example, in the MDC-CC, each copy of the T allele was associated with \( \sim 5.5 \) mg/dl higher triglycerides. Across the studies, SNP rs780094 explained between 0.1 and 1.2% of the trait-level variance in each of the studied 46,549 individuals.

Combining the data from the studied 46,549 individuals provided robust evidence for association between the minor T allele at rs780094 and higher triglyceride levels (meta-analysis \( P = 3 \times 10^{-56} \), Table 2). The minor T-allele frequency ranged from 16.1% in U.S. blacks to 47.6% in the Spanish from Valencia, and the effect size per T allele ranged from 0.6 mg/dl in Dallas Heart Study Hispanics to \( \sim 6.2 \) mg/dl in the MPP (Table 2). Mean effect size was \( \sim 4.2 \) mg/dl, and we did not observe significant heterogeneity between the studies (\( P = 0.15 \)). We found that the T allele was associated with higher triglycerides in population-based samples and in cohorts of patients with diabetes and hypertension. Furthermore, the T allele was associated with higher triglycerides regardless of the mean triglycer-
ide level in the sample (e.g., the mean triglyceride concentration is considerably lower in a population-based sample, such as MDC-CC [at 122 mg/dl] compared with the Diabetes Registry cohort [at 231 mg/dl] comprising entirely individuals with type 2 diabetes).

**Association with plasma triglyceride-related metabolic traits.** We next explored the relationship between SNP rs780094 and related metabolic traits, including plasma LDL cholesterol, HDL cholesterol, apolipoprotein concentrations, FFA concentrations, and BMI. SNP rs780094 was not associated with LDL cholesterol or HDL cholesterol in any of the samples (supplementary Table 1, which is available in an online appendix at http://dx.doi.org/10.2337/db08-0516). As expected, given the correlation between triglyceride and apoB concentration \( (r = 0.42 \ [P < 0.0001] \) and 0.57 \([P < 0.0001]\) in DGI and FINRISK97, respectively), rs780094 was associated with apoB concentration in a meta-analysis of the DGI and FINRISK97 cohorts \( (P = 7.5 \times 10^{-8}) \), with the T-allele carriers having the highest apoB concentration. Fasting FFA and triglyceride concentrations are weakly correlated \( (r = 0.25 \) and 0.20 in DGI and MPP-MM, respectively), and we did not observe any association between rs780094 and fasting FFA \( (P = 0.39 \) and 0.90 in DGI and MPP-MM cohorts, respectively) or suppression of FFA levels at 2 h in an OGTT \( (P = 0.70 \) and 0.98 in DGI and MPP-MM cohorts, respectively). GCKR rs780094 was nominally associated with BMI in the Singaporean NHS-98 study \( (P = 0.04) \) but not in any of the other samples (supplementary Table 2).

Because two genome-wide association studies recently reported association between CRP levels and rs780094 and rs1260326 (9,10), we tested for association between GCKR rs1260326 and rs780094 and CRP in MDC-CC. Both SNPs were strongly associated with CRP levels with the T-allele carriers having significantly higher levels \( (CC 2.5 \pm 4.7, CT 2.6 \pm 4.2, \) and TT 2.9 \pm 4.3 mg/l, \( P = 4.5 \times 10^{-5} \) in linear regression analysis of rs1260326 adjusted for age and sex).

**Association with measures of glucose metabolism.** We studied the association of SNP rs780094 with fasting glucose concentrations in 33,995 nondiabetic individuals and HOMA estimates of insulin resistance in 11,084 non-diabetic individuals. Despite the fact that the T allele was consistently associated with higher triglycerides, T-allele carriers had significantly lower fasting plasma glucose levels in six of the studied populations, and a similar trend was observed in the other populations \( (P_{meta} = 1 \times 10^{-12}) \); for example, in the MDC-CC, each copy of the T allele was associated with \(-0.5 \) mg/dl lower fasting blood glucose. In addition, T-allele carriers were more insulin sensitive as estimated by the HOMA insulin resistance index \( (P_{meta} = 5.0 \times 10^{-8}) \). In DGI and Botnia PPP cohorts, we could calculate the insulinogenic index during an OGTT in 999 and 3,184 nondiabetic individuals, respectively. Insulin secretion capacity calculated as the insulinogenic index did not differ significantly between the different GCKR genotype carriers \( (P = 0.72 \) and 0.27, respectively). Encouraged by these results for intermediate traits, we also tested association of rs780094 with type 2 diabetes in our Nordic cohorts with similar minor allele frequency of the SNP (DGI, MDC-CC, NORDIL, Diabetes Registry, and FINRISK97). Of 24,034 individuals, 5,578 had type 2 diabetes.

### Table 2

| Country      | Study                  | CC (mg/dl) | CT (mg/dl) | TT (mg/dl) | Minor allele frequency | Z score | \( P \) value |
|--------------|------------------------|------------|------------|------------|------------------------|---------|--------------|
| Finland      | DGI*                   | 136 ± 89 (1,142) | 144 ± 103 (1,194) | 164 ± 114 (300) | 0.34 | -5.76 | 3.7 \times 10^{-8} |
| Sweden       | MDC-CC*                | 117 ± 70 (2,207) | 123 ± 71 (2,457) | 128 ± 74 (639) | 0.35 | -5.45 | 1.7 \times 10^{-7} |
| Sweden       | MPP                    | 107 ± 70 (4,059) | 113 ± 77 (4,616) | 117 ± 72 (1,425) | 0.37 | -6.17 | 1.3 \times 10^{-9} |
| Sweden       | NORDIL                 | 151 ± 78 (2,223) | 157 ± 79 (2,220) | 172 ± 87 (572) | 0.34 | -6.14 | 7.4 \times 10^{-9} |
| Sweden       | Skania Diabetes 2000 Registry | 205 ± 148 (1,076) | 224 ± 97 (1,069) | 253 ± 104 (259) | 0.33 | -4.87 | 1.8 \times 10^{-6} |
| Finland      | Botnia-PPP             | 106 ± 58 (1,273) | 115 ± 69 (1,429) | 125 ± 69 (409) | 0.36 | -5.62 | 4.8 \times 10^{-8} |
| Finland      | FINRISK                | 128 ± 86 (3,009) | 130 ± 89 (3,398) | 142 ± 105 (931) | 0.36 | -5.00 | 8.0 \times 10^{-7} |
| Spain        | Valencia               | 108 ± 66 (446) | 112 ± 64 (792) | 118 ± 71 (370) | 0.48 | -2.26 | 0.02 |
| U.S.         | Dallas Heart Study blacks | 103 ± 90 (1,163) | 108 ± 78 (444) | 110 ± 59 (44) | 0.16 | -2.53 | 0.01 |
| U.S.         | Dallas Heart Study Hispanics | 149 ± 138 (244) | 151 ± 126 (263) | 161 ± 155 (58) | 0.34 | -0.56 | 0.29 |
| U.S.         | Dallas Heart Study whites | 131 ± 115 (342) | 135 ± 97 (455) | 164 ± 124 (145) | 0.40 | -4.04 | 4.4 \times 10^{-5} |
| U.S.         | GOLDN                  | 109 ± 75 (378) | 143 ± 90 (538) | 133 ± 87 (146) | 0.39 | -2.24 | 0.03 |
| U.S.         | BPRHS                  | 153 ± 104 (423) | 165 ± 92 (330) | 174 ± 118 (84) | 0.30 | -2.96 | 0.003 |
| Singapore    | Singapore NHS-98 Chinese | 112 ± 64 (842) | 120 ± 71 (1233) | 131 ± 82 (616) | 0.46 | -5.41 | 3.0 \times 10^{-5} |
| Singapore    | Singapore NHS-98 Malays | 132 ± 75 (268) | 150 ± 91 (332) | 153 ± 97 (134) | 0.41 | 0.02 |
| Singapore    | Singapore NHS-98 Asian Indians | 140 ± 79 (332) | 151 ± 89 (188) | 163 ± 109 (28) | 0.22 | 0.08 |

Total \( n = 46,549 \), 19,427, 20,958, 6,164, Meta-analysis \( P \) value \( 3 \times 10^{-56} \)

Data means ± SD (n) (continuous raw measures). Association analyses were conducted with an outcome variable of residual log-triglyceride concentration after adjustment for age, sex, and diabetes status. For ease of interpretation, unadjusted triglyceride concentrations are presented in the table. To convert the values to millimoles per liter, multiply triglycerides by 0.01129. All MDC-CC results have been reported in ref. 1. BPRHS, Boston Puerto Rican Health Study.
TABLE 3

Measures of glucose tolerance according to genotype at GCKR rs780094 among nondiabetic individuals within the study populations.

| Country Study | Fasting plasma glucose (mg/dl) | HOMA (insulin resistance) |
|---------------|-------------------------------|---------------------------|
|               | CC                            | CT                        | TT                        |
|               | 0.04                          | 0.06                      | 0.08                      |
|               | 0.04                          | 0.06                      | 0.08                      |
|               | 0.04                          | 0.06                      | 0.08                      |
|               | 0.04                          | 0.06                      | 0.08                      |
|               | 0.04                          | 0.06                      | 0.08                      |
|               | 0.04                          | 0.06                      | 0.08                      |
|               | 0.04                          | 0.06                      | 0.08                      |
|               | 0.04                          | 0.06                      | 0.08                      |
|               | 0.04                          | 0.06                      | 0.08                      |
|               | 0.04                          | 0.06                      | 0.08                      |
|               | 0.04                          | 0.06                      | 0.08                      |

| Country Study | HOMA (insulin resistance) |
|---------------|---------------------------|
|               | CC                        | CT                        | TT                        |
|               | 0.04                      | 0.06                      | 0.08                      |
|               | 0.04                      | 0.06                      | 0.08                      |
|               | 0.04                      | 0.06                      | 0.08                      |
|               | 0.04                      | 0.06                      | 0.08                      |
|               | 0.04                      | 0.06                      | 0.08                      |
|               | 0.04                      | 0.06                      | 0.08                      |
|               | 0.04                      | 0.06                      | 0.08                      |
|               | 0.04                      | 0.06                      | 0.08                      |
|               | 0.04                      | 0.06                      | 0.08                      |
|               | 0.04                      | 0.06                      | 0.08                      |
|               | 0.04                      | 0.06                      | 0.08                      |

Data are means ± SD (n) or continuous raw measures of blood glucose converted to plasma glucose using a correction factor of 1.13. Association analyses were conducted with an outcome variable of residual log-glucose concentration after adjustment for age and sex. For ease of interpretation, unadjusted glucose concentrations are presented in the table. HOMA insulin resistance index was calculated using the following formula: fasting plasma glucose × fasting insulin/22.5. Two-sided P values are shown for all cohorts. To convert the fasting plasma glucose values to millimoles per liter, multiply by 0.055.
te, and the frequency of the rs780094 T-allele was 35.2% among nondiabetic individuals compared with 33.4% in type 2 diabetic patients (OR 0.93 [95% CI 0.88–0.97], P = 0.0006). Of the nondiabetic individuals, 2.3% were homozygous for the T allele compared with 10.9% of type 2 diabetic patients, and the TT genotype was associated with a protection from type 2 diabetes when compared with CC-genotype carriers (OR 0.84 [0.76–0.93], P = 0.0008).

We next evaluated whether the rs780094 genotype was associated with hepatic glucose output in 125 men who had undergone a hyperinsulinemic-euglycemic clamp with infusion of [3-H3]glucose and assessment of basal and clamp hepatic glucose production. *GCKR* rs780094 T-allele carriers did not have a significantly lower basal rate of hepatic glucose production (CC 2.26 ± 0.27, CT 2.18 ± 0.20, and TT 2.16 ± 0.09 mg · kg⁻¹ · min⁻¹, P = 0.10), but their hepatic glucose output during the hyperinsulinemic state was slightly lower compared with that of C-allele carriers (CC 0.26 ± 0.59, CT 0.20 ± 0.40, and TT 0.09 ± 0.22 mg · kg⁻¹ · min⁻¹, P = 0.01).

**Fine-mapping of the *GCKR* locus.** We fine-mapped the *GCKR* region with two different approaches, by genotyping and coding SNPs across the region and by imputing untyped SNPs (or so-called in silico fine-mapping) (Fig. 1). We genotyped 104 SNPs across the ~417 kb region and studied the association of these SNPs with triglyceride concentrations (supplementary Table 3). A common *GCKR* coding SNP rs1260326 (Pro446Leu) gave the strongest signal for association with triglyceride levels (P = 9.4 × 10⁻¹⁰).

Fine-mapping by imputation also revealed that *GCKR* coding SNP rs1260326 (Pro446Leu) gave the strongest signal for triglyceride concentrations (P = 1.5 × 10⁻⁹) in the associated interval on chromosome 2p23. In HapMap CEU, *GCKR* coding SNP rs1260326 shows strong linkage disequilibrium to the intronic SNP rs780094 (r² = 0.93). We performed regression analysis, including both rs1260326 and rs780094 as predictors of triglyceride levels in MDC-CC, but because of the strong correlation between the SNPs, none of the two were significant in this analysis (P = 0.18 and 0.80 for rs1260326 and rs780094, respectively).

Figure 1 summarizes the results of both fine-mapping approaches. Both the genotyping and the in silico fine-mapping methods indicated the Pro446Leu as the variant with the strongest association with triglyceride levels. To evaluate the fidelity of the MACH imputation algorithm (24), we compared the genotypes generated by Sequenom genotyping for 57 SNPs with that predicted by imputation. The genotype consensus rate was 95.7%.

**Longitudinal changes in fasting triglyceride and glucose stratified by *GCKR* Pro446Leu genotype.** In the MPP cohort, the Pro446Leu was strongly associated with higher triglycerides and lower fasting blood glucose both at baseline (P = 6 × 10⁻²² and 0.0005, respectively) and after the mean follow-up period of 23.4 years (P = 3 × 10⁻³⁰ and 0.004, respectively) (Fig. 2). In addition, the triglyceride levels of the Leu446 carriers increased more over time compared with those of homozygous Pro446 carriers (P = 8 × 10⁻⁵), whereas change in fasting glucose over time did not differ by genotype status (Fig. 2).

In the MPP study, among 17,037 individuals free of type 2 diabetes at baseline, 2,063 (12.1%) individuals developed type 2 diabetes during the follow-up period. Carriage of the Leu allele tended to protect from development of type 2 diabetes (OR 0.96 [95% CI 0.91–1.02], P = 0.27).

**Association of *GCKR* variation with CVD and carotid IMT.** In MDC-CC, 321 individuals experienced the first CVD end point during the mean follow-up time of 10.5 ± 1.8 years. Neither rs780094 nor rs1260326 predicted CVD (P = 0.85 and 0.45, respectively). The results were similar when age, sex, LDL cholesterol, HDL cholesterol, triglycerides, BMI, sBP, dBP, smoking, family history of myocardial infarction, lipid-lowering medication, antihypertensive medication, and CRP were included as covariates. *GCKR* variants were also not associated with carotid IMT in MDC-CC. No association was detected between *GCKR* variants and common carotid artery IMT (P = 0.94 and 0.63 for rs780094 and rs1260326, respectively).

**Hepatic expression of *GCK* and *GCKR* according to *GCKR* genotypes.** We next examined whether rs780094 or Pro446Leu was associated with transcript levels of *GCKr* and/or *GCK* in human liver. In a modest number of liver samples (n = 60), neither rs780094 nor Pro446Leu genotype was associated with transcript levels of *GCK* or *GCKR* (supplementary Table 4).
DISCUSSION

In line with the opposite effects of GCKR-pathway manipulation on glucose and triglyceride concentrations in rodent models and a recent association study in Danes (8), our study provides compelling evidence that common DNA sequence variants in GCKR are associated with opposite effects on fasting triglyceride and glucose concentrations in humans and modest protection from type 2 diabetes. Both imputation and genotype fine-mapping of the GCKR locus yielded a nonsynonymous coding SNP (Pro446Leu) as the strongest association signal, suggesting the hypothesis that this nonsynonymous coding SNP is the causal variant for the observed associations. We also provide evidence that the Leu446 allele carriers increase their triglyceride levels more over time compared with noncarriers. In addition, our data suggest that, at least within regions of high linkage disequilibrium, genotypes predicted by imputation are highly accurate and may provide a good starting point for genotype fine-mapping.

The exact mechanism for the effect of the GCKR variant on blood glucose, triglycerides, and CRP remains to be defined. A potential explanation is the opposite and over-riding effects of increased glucose utilization and glycolytic flux on liver glucose and lipid metabolism. With increased glucose utilization and glycolytic flux, PEPCK and glucose-6-phosphatase are downregulated, whereas GCK, phosphofructokinase, and fatty acid synthase are upregulated. These changes increase glycogen synthesis and malonyl CoA concentration and direct fatty-acyl-CoA into de novo lipogenesis and VLDL triglyceride production (33). However, the consequence of in vivo glucose metabolism is enhanced suppression of hepatic glucose output (33). Our observation that the GCKR variant T allele associates with higher triglycerides, lower fasting glucose, and lower hepatic glucose output during a euglycemic-hyperinsulinemic clamp agrees with this hypothesis. Finally, the T-allele carriers had insulin secretion capacity similar to that of noncarriers. Thus, the association is similar to that of GCK-30G/A, which affects the glucose levels needed to induce insulin secretion.

Our human studies propose the hypothesis that GCKR Pro446Leu may mimic the consequences of GCK overexpression in rodent models with upregulation of glucose utilization and VLDL-triglyceride synthesis and downregulation of gluconeogenesis. The Pro446Leu variant has been introduced into rat cDNA but was not found to affect the functional properties of the rat protein when prepared by overexpression in Escherichia coli (34). Unfortunately, the human protein could not be produced using that expression system (32). Although human and rat GCKR share 88% identity, the human protein is importantly different from rat GCKR: human GCKR is a more potent inhibitor of GCK than rat GCKR in the absence of fructose-6-phosphate, and human GCKR has higher affinity for fructose-6-phosphate (35). Thus, the potential impact of the amino acid difference on overall structure and function of human GCKR remains to be defined.

There are conflicting data on the association between circulating triglyceride concentrations and risk of CVD (36,37). Our data combined with data for other common variants suggest a potential explanation for the varying risk associated with high triglycerides. DNA sequence variants in some genes (e.g., APOB) have been associated with both increased triglycerides and markers of increased atherosclerosis risk, such as elevated LDL cholesterol (38). Similarly, common genetic variations in both lipoprotein lipase (LPL) and apoA5 (APOA5) genes (rs328 and rs3133506, respectively) are associated with both increased triglycerides and markers of increased atherosclerosis risk, such as decreased HDL cholesterol (39,40). However, at GCKR Pro446Leu, the variant allele is associated with higher triglycerides and higher CRP levels but also with a favorable metabolic marker, namely decreased glucose. Our finding of no association between the GCKR variant and CVD events or carotid IMT is thus not surprising but instead proposes that the risk of CVD associated with
higher triglycerides may vary based on the specific profile of genetic variants in different genes contributing to an increased triglyceride concentration. However, given the limited number of CVD events in our study, this result needs further confirmation in other studies.

We provide convincing evidence that common variation in GCKR is associated with opposite effects on fasting plasma triglyceride and glucose concentrations in multiple human populations and demonstrate that the strongest association signal resides at coding SNP rs1260326 (Pro446Leu) in GCKR. Taken together, the data position GCKR in central pathways regulating both hepatic triglyceride and glucose metabolism in humans.

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