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

Marju Orho-Melander,1 Olle Melander,1 Candace Guiducci,2 Pablo Perez-Martinez,3,4,5 Dolores Corella,5 Charlotte Roos,1 Ryan Tewhey,2 Mark J. Rieder,6 Jennifer Hall,7 Goncalo Abecasis,8 E. Shyong Tai,9 Cullan Welch,7 Donna K. Arnett,10 Valeriya Lyssenko,1 Eero Lindholm,1 Richa Saxena,2 Paul I.W. de Bakker,2 Noel Burtt,2 Benjamin F. Voight,2 Joel N. Hirschhorn,2 Katherine L. Tucker,11 Thomas Hedner,12 Tiinamaija Tuomi,3,11 Bo Isomaa,14 Karl-Fredrik Eriksson,1 Marja-Riitta Taskinen,13 Bjorn Wahlstrand,12 Thomas E. Hughes,15 Laurence D. Parnell,3 Chao-Qiang Lai,4 Göran Berglund,16 Leena Peltonen,17 Erkki Vartiainen,18 Pekka Jousilahti,18 Aki S. Havulinna,18 Veikko Salomaa,18 Peter Nilsson,1 Leif Groop,1,13 David Altshuler,2,19,20 E. Shyong Tai,9 Cullan Welch,7 Donna K. Arnett,10 Valeriya Lyssenko,1 Eero Lindholm,1 Richa Saxena,2 Paul I.W. de Bakker,2 Noel Burtt,2 Benjamin F. Voight,2 Joel N. Hirschhorn,2 Katherine L. Tucker,11 Thomas Hedner,12 Tiinamaija Tuomi,3,11 Bo Isomaa,14 Karl-Fredrik Eriksson,1 Marja-Riitta Taskinen,13 Bjorn Wahlstrand,12 Thomas E. Hughes,15 Laurence D. Parnell,3 Chao-Qiang Lai,4 Göran Berglund,16 Leena Peltonen,17 Erkki Vartiainen,18 Pekka Jousilahti,18 Aki S. Havulinna,18 Veikko Salomaa,18 Peter Nilsson,1 Leif Groop,1,13 David Altshuler,2,19,20 Jose M. Ordovas,4 and Sekar Kathiresan2,21

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 phe-

notypes, 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_meta = 3 × 10−56) and glucose (P_meta = 1 × 10−15) concentrations. In addition, we confirmed recent reports that the same SNP is associated with C-reactive protein (CRP) level (P = 5 × 10−12). 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 intronic 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, adenosinergic-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 x 10^{-8} and 8.7 x 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 (1,11,12). 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 of the epidemiology of carotid artery disease from a large, community-based prospective epidemiological cohort of 28,449 people recruited for a baseline examination between 1991 and 1996 (13). Of the MDC-CC participants, 597 did not provide a baseline plasma sample or did not have triglyceride data and were excluded from the analyses in MDC-CC. Of the invited subjects, 17,284 people participated in the rescreening, and of these, 17,037 individuals had DNA, 16,908 individuals had fasting triglyceride data, and 16,976 individuals had fasting glucose data available for the study. The mean follow-up time was 23.4 ± 4.6 years. Of the 17,037 individuals, 5,946 were also participants of the MDC-CC, bringing the number of unique individuals in the MPP, (i.e., not included in MDC-CC) to 11,091. Because of the overlap between MDC-CC and MPP materials, all of the phenotype association analyses included in the MPP analyses were performed in the unique cohort of 11,091 participants, whereas the prospective analyses were performed in the whole MPP cohort with DNA and available phenotypes.

Individuals on lipid-lowering medication (n = 3,510) were excluded from the triglyceride analyses, and individuals with diabetes either at baseline or follow-up (n = 3,029) were excluded from the analyses of fasting blood glucose. For the analysis of progression to type 2 diabetes in MPP, 16,061 incident cases were included. Diagnosis of diabetes was confirmed from patient records or based on a fasting plasma glucose concentration >7.0 mmol/L.

During the follow-up visit, a subgroup from the MPP study participated in more extensive metabolic studies, including a hyperglycemic-hyperinsulinemic clamp combined with indirect calorimetry to measure hepatic glucose output. All individuals underwent a physical examination, and a subgroup of 199 men (Malmö men, MPP-MM) with impaired glucose tolerance (IGT) underwent more extensive metabolic studies, including a euglycemic-hyperinsulinemic clamp combined with infusion of [3-H]glucose to measure hepatic glucose output (17). After the follow-up time, 66 of the 199 men with IGT at baseline had normal glucose tolerance, 52 had impaired fasting glucose and/or IGT, and 81 had type 2 diabetes. Type 2 diabetic patients were treated either with diet alone (42%) or with oral hypoglycemic agents, which were withheld the day before the clamp.

The Nordic DIItiazem (NORDIL) Study is an intervention study in 10,881 patients with hypertension from Sweden and Norway (diastolic blood pressure ≥100 mmHg at least twice, mean age 60 ± 7 years) of whom 5,152 Swedish patients provided DNA and were included in the present study (18). The study participants had been randomized to antihypertensive treatment with either the calcium-antagonist diltiazem or β-blocker/thiazide diuretic to compare efficacy of the two drug therapies to prevent cardiovascular endpoints (18). Lipoprotein and lipid measurements at the baseline examination (before the initiation of antihypertensive therapy) were studied in this report.

The Diabetes Registry sample consists of 2,777 type 2 diabetic patients from the Skania Diabetes 2000 Registry, a local diabetes registry in southern Sweden (19). The majority of registered patients came from the city of Malmö in southern Sweden. Type 2 diabetes was classified according to 1997 World Health Organization (WHO) criteria (20).

The Botnia-Prevalence, Prediction, and Prevention of Diabetes (Botnia-PPP) Study is a population-based study from the Botnia region of western Finland. The current study was initiated in 2004 in a population comprising ~135,000 individuals. Using a population registry, a random sample of subjects aged 18–75 years was selected: In age-groups 18–29 and 60–74 years, 1 of 10 individuals was randomly selected; and in age-group 30–59 years, 1 of 15 individuals was randomly selected. Altogether, 6,075 individuals were invited to participate in the study, and 3,621 took part. Of these individuals, 3,405 have data and DNA available for the current study.

The FINRISK97 study is a population-based cross-sectional survey which consists of 8,191 people aged 25–74 years from five geographical areas of Finland (the Helsinki, southwestern Finland, North Karelia, Oulu, and Kuopio regions) (21). The study followed the protocol of the WHO Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) Project, an international study undertaken under the auspices of the WHO.

The Valencia study population comprised 1,608 individuals randomly selected from the Valencia region on the eastern Mediterranean coast of Spain and examined between 1999 and 2003 (22). This sample comprised randomly
selected workers, using a continuously updated computerized population register, and subjects randomly selected from the general population.

The Dallas Heart Study is a multietnic, 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 (NHLBI) Family Heart Study (FHS) field centers (Minneapolis, MN, and Salt Lake City, UT) (24). The NHLBI 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 Singapore 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.

### Table 1

**Clinical characteristics of the study cohorts**

<table>
<thead>
<tr>
<th></th>
<th>Finland and Sweden</th>
<th>Sweden</th>
<th>Skandinavia Diabetes Registry 2000</th>
<th>Botnia-PPP</th>
<th>FINRISK97</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>n (men/women)</strong></td>
<td>2,930</td>
<td>5,506</td>
<td>17,037</td>
<td>5,152</td>
<td>2,777</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>28 ± 4</td>
<td>26 ± 4</td>
<td>—</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td><strong>Triglycerides (mg/dl)</strong></td>
<td>144 ± 101</td>
<td>122 ± 71</td>
<td>117 ± 71</td>
<td>159 ± 108</td>
<td>231 ± 27</td>
</tr>
<tr>
<td><strong>HDL (mg/dl)</strong></td>
<td>49 ± 15</td>
<td>53 ± 14</td>
<td>—</td>
<td>53 ± 21</td>
<td>44 ± 13</td>
</tr>
<tr>
<td><strong>LDL (mg/dl)</strong></td>
<td>145 ± 51</td>
<td>161 ± 38</td>
<td>—</td>
<td>160 ± 43</td>
<td>137 ± 40</td>
</tr>
<tr>
<td><strong>fB-glucose (mg/dl)</strong></td>
<td>121 ± 49</td>
<td>93 ± 25</td>
<td>88 ± 13</td>
<td>96 ± 28</td>
<td>189 ± 69</td>
</tr>
<tr>
<td><strong>HOMA (μmol × μU)</strong></td>
<td>1.7 ± 1.4</td>
<td>2.0 ± 2.7</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Type 2 diabetes (%)</strong></td>
<td>49.9</td>
<td>8.4</td>
<td>0.0</td>
<td>8.7</td>
<td>100.0</td>
</tr>
</tbody>
</table>

*Continued on facing page*

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 FINRISK97, 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, Neus, 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 radioimmunoassy in DGI, MDC-CC, MPP-MM, GOLDN, and Singaporean NHS-98 samples (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 7000 instrument (Applied Biosystems, Foster City, CA). The studied SNPs were in Hardy-Weinberg equilibrium in all studied 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, GTF3C2, EIF2B2, SNX17, ZNF513, PPP11G, NRBP1, KRTAP3, IFIT2, FNDC9, GCKR, C2orf16, ZNF512, CDC121, XAB1, SUPT7L, and SELC1AP. 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 > 0.8; 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 study.

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 (www.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 v.2 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
Study. RNA was extracted using the Qiagen RNeasy mini kit on pulverized tissue according to the manufacturer’s protocols (Qiagen). RNA was reverse transcribed to cDNA using the Superscript III first-strand synthesis from Invitrogen. RT-PCR was performed on an ABI 7900 using ABI TaqMan primer probe sets (Hs01564551_G1, GCK; and HS01100274_M1, GCKR) and normalized to cyclophilin. Data are expressed as ΔCT (cycle threshold) values.

**Statistical analyses.** SNP-phenotype association analyses were performed by multivariate linear regression using an additive genetic model. Because of deviation from normal distribution, fasting triglyceride, blood glucose, and CRP concentrations, and carotid IMT measurements were log transformed before analyses. For each participant, residual values were created for log-triglycerides, HDL cholesterol, and LDL cholesterol after adjustment for age, sex, and diabetes status. Residuals were created for BMI after adjustment for age and sex. For analyses of lipid traits, individuals on lipid-lowering medication were excluded (except in NORDIL and Diabetes Registry, where this information was not available). Log-fasting glucose, HOMA insulin resistance index, and insulinogenic index were studied only in nondiabetic individuals, and residuals were created after adjustment for age and sex. To limit the undue influence of outliers in the regression analysis, for each trait we excluded the bottom and top 0.5% of the trait-level distribution in each study sample. We tested the null hypothesis that the trait residuals do not differ by the analyzed genotypes.

To summarize the statistical evidence across the multiple cohorts, we conducted a fixed-effects variance-weighted meta-analysis. We computed a weighted average of the β-coefficient estimates and SEs (from the linear regression models) using the inverse of the variance in each cohort as weights. Heterogeneity between the studies was tested using the Cochran test. One-way ANOVA was used to compare expression levels of GCK and GCKR in the human liver samples according to genotype. Survival analyses were performed using Cox regression analysis with either age and sex or age, sex, LDL cholesterol, HDL cholesterol, triglycerides, BMI, systolic blood pressure (sBP), diastolic blood pressure (dBP), smoking, family history of myocardial infarction, lipid-lowering medication, antihypertensive medication, and log-CRP as independent predictors.

Analysis of association between log-carotid IMT and GCKR was performed by linear regression analysis in individuals without prevalent myocardial infarction or type 2 diabetes after adjustment for age and sex. Because our sample included a modest number of CVD events (321 incident case subjects and 4,781 event-free control subjects), we assessed power to detect an association between GCKR SNP genotype and CVD. We approximated hazard ratios using case-control design for discrete traits (31). At 5% significance level, we had a power of 0.58 for a SNP genotype with an odds ratio (OR) of 1.2 per risk allele (that is, OR 1.0, 1.20, and 1.44 for carriers of 0, 1, and 2 risk alleles, respectively), power 0.88 for an OR of 1.30, and power 0.98 for an OR of 1.4 per risk allele, assuming a risk allele frequency of 34% (the Leu allele of SNP rs1260326 has an allele frequency of 34%). For the association of Pro446Leu with carotid IMT (n = 4,850 individuals analyzed), at 5% significance level, we had a power of 0.88 to detect an association for a SNP genotype that explained 0.2% of the variance in carotid IMT.

In the MPP prospective study, the ORs for risk of developing type 2 diabetes were calculated using logistic regression analyses adjusted for age at inclusion and time to last follow-up, BMI, and sex. Differences in change of fasting triglyceride by genotype during the follow-up time were tested by linear regression analysis adjusted for age at inclusion, sex, time to last follow-up, and diabetes status. After excluding individuals with diabetes at baseline and on follow-up, differences in change of fasting glucose were tested using linear regression analyses with adjustment for age at inclusion, sex, and time to last follow-up. All statistical analyses were conducted using either SPSS version 14.0 or PLINK (32). All nominal P values of <0.05 were considered significant. All reported P values are two sided.

### 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 × 10⁻¹⁰); for example, in the MDC-CC, each copy of the T allele was associated with higher triglycerides in population-based samples and in cohorts of patients with diabetes and hypertension. Across the studies, SNP rs780094 explained between 0.1 and 1.2% of triglyceride variance (after accounting for age, sex, and diabetes status).

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 × 10⁻⁵⁰). The minor T-allele frequency varied 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 ~6.2 mg/dl in the MPP (Table 2). Mean effect size was ~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 triglycerin...
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/10.2377/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 \((P = 7.5 \times 10^{-5})\), 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 \(TT 2.9 \pm 4.3 \text{ mg/l}, P = 4.5 \times 10^{-5}\) 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 nonobese 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 (Table 3, \(P_{\text{meta}} = 1 \times 10^{-13}\)); for example, in the MDC-CC, each copy of the T allele was associated with \(-0.5 \text{ mg/dl}\) lower fasting blood glucose. In addition, T-allele carriers were more insulin sensitive as estimated by the HOMA insulin resistance index \((P_{\text{meta}} = 5.0 \times 10^{-5})\). In DGI and Botnia PPP cohorts, we could calculate the insulinogenic index during an OGTT in 999 and 3,184 nonobese 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 3

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

<table>
<thead>
<tr>
<th>Country Study</th>
<th>Fasting plasma glucose (mg/dl)</th>
<th>HOMA (insulin resistance)</th>
<th>CC (mmol/L)</th>
<th>CT (mmol/L)</th>
<th>TT (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finland and Sweden DGI 101</td>
<td>11.0 (5.92)</td>
<td>3.0 (1.97)</td>
<td>2.6 (2.1 (1.7))</td>
<td>2.5 (2.2 (1.5))</td>
<td>2.4 (2.3 (1.2))</td>
</tr>
<tr>
<td></td>
<td>10.0 (5.92)</td>
<td>2.9 (1.97)</td>
<td>2.6 (2.1 (1.7))</td>
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### Meta-analysis

<table>
<thead>
<tr>
<th>Meta-analysis</th>
<th>Fasting plasma glucose (mg/dl)</th>
<th>HOMA (insulin resistance)</th>
<th>CC (mmol/L)</th>
<th>CT (mmol/L)</th>
<th>TT (mmol/L)</th>
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### Meta-analysis (total 33,995)

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Data are means ± SD of continuous measures of blood glucose converted to plasma glucose using a correction factor of 1.13. Association analyses were conducted within study populations and included the following adjustments: age, sex, and study population. 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.

**Note:** Table entries are truncated for readability. Full data can be found in the original study report.
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 [3-3H]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 ± 0.57, 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 tag 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 triglycerides (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. The genotype consensus rate between the imputed genotypes and genotyped genotypes was ~95.7%.
similar to that of noncarriers. Thus, the association is
nally, the T-allele carriers had insulin secretion capacity
hyperinsulinemic clamp agrees with this hypothesis. Fi-
and lower hepatic glucose output during a euglyemic-
associates with higher triglycerides, lower fasting glucose,
provide a good starting point for genotype fine-mapping.
predicted by imputation are highly accurate and may
within regions of high linkage disequilibrium, genotypes
noncarriers. In addition, our data suggest that, at least
increase the causal variant for the observed associations. We also
(Pro446Leu) as the strongest association signal, suggest-
centrations in humans and modest protection from type 2
diabetes. Both imputation and genotype fine-mapping of the
locus yielded a nonsynonymous coding SNP (Pro446Leu) as the strongest association signal, suggest-
the hypothesis that this nonsynonymous coding SNP is
upregulated. These changes increase glycogen synthesis
GCK, phosphofructokinase, and fatty acid synthase are
defined. A potential explanation is the opposite and over-
on blood glucose, triglycerides, and CRP remains to be
DISCUSSION
In line with the opposite effects of GCKR-pathway manip-
ulation on glucose and triglyceride concentrations in ro-
dent 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 concen-
centrations in humans and modest protection from type 2
diabetes. Both imputation and genotype fine-mapping of the
of the GCKR locus yielded a nonsynonymous coding SNP
variant T allele instead proposes that the risk of CVD associated with
and CVD events or carotid IMT is thus not surprising but
3133506, respectively) are associated with both increased
variants in some genes (e.g., APOB) have been associated
the human protein is importantly
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-
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 lipopro-
tein lipase (LPL) and apoA5 (APOA5) genes (rs328 and rs
3133506, 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

FIG. 2. Fasting triglycerides and blood glucose levels in MPP participants before and after a mean follow-up time of 23.4 years according to GCKR
Pro446Leu. In a large prospective study, the Pro446Leu was strongly associated with higher triglycerides and lower fasting blood glucose both
at baseline and after the follow-up. In addition, among 12,528 individuals not on lipid-lowering medication, fasting triglyceride levels increased
more during the follow-up time among Leu446 allele carriers compared with homozygous Pro446 carriers ($P = 8 \times 10^{-7}$), whereas the change in
fasting blood glucose during the follow-up time was not significant among 12,964 individuals without diabetes either at baseline or at follow-up.

<table>
<thead>
<tr>
<th>Triglycerides (mg/dl)</th>
<th>Fb-glucose (mg/dl)</th>
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<tbody>
<tr>
<td>Baseline</td>
<td>Follow-up</td>
</tr>
<tr>
<td>$P = 6 \times 10^{-22}$</td>
<td>$P = 3 \times 10^{-29}$</td>
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<td>$P = 0.0039$</td>
<td>$P = 0.00048$</td>
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<table>
<thead>
<tr>
<th>CC</th>
<th>CT</th>
<th>TT</th>
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<td>110</td>
<td>110</td>
<td>120</td>
</tr>
<tr>
<td>86.0</td>
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$FIG. 2$: Fasting triglycerides and blood glucose levels in MPP participants before and after a mean follow-up time of 23.4 years according to GCKR
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fasting blood glucose during the follow-up time was not significant among 12,964 individuals without diabetes either at baseline or at follow-up.

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 glyco-
lytic flux on liver glucose and lipid metabolism. With
increased glucose utilization and glycolytic flux, PEPCK
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 metab-
olism 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 euglyemic-
hyperinsulinemic clamp agrees with this hypothesis. Fi-
ally, 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 overex-
pression in rodent models with upregulation of glucose
utilization and VLDL-triglyceride synthesis and downregu-
lation 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-
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Our finding of no association between the GCKR variant
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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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