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(Article begins on next page)
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 Marja-Riitta Taskinen,13 Bjo¨rn Wahlstrand,12 Thomas E. Hughes,15 Laurence D. Parnell,3 Chao-Qiang Lai,10 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 phenotypes, 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 (Pmeta = 3 × 10−50) and glucose (Pmeta = 1 × 10−15) concentrations. In addition, we confirmed recent reports that the same SNP is associated with C-reactive protein (CRP) level (P = 5 × 10−7). Both fine-mapping approaches revealed a common missense GCKR variant (rs1260326, Pro446Leu, 34% frequency, r2 = 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-

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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. GCKr-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 × 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 intrinsic 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 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 1999 and 2003 (22). This sample comprised 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 from the Valencia region on the eastern Mediterranean coast of Spain and examined between 1999 and 2003 (22).

The Nordic Diltiazem (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 end points (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 Malmo 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-PP) 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 3,021 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 initiated 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 from the Valencia region on the eastern Mediterranean coast of Spain and examined between 1999 and 2003 (22)
TABLE 1
Clinical characteristics of the study cohorts

<table>
<thead>
<tr>
<th></th>
<th>Finland and Sweden</th>
<th>Sweden</th>
<th>Skania Diabetes 2000</th>
<th>Botnia-PPP</th>
<th>FINRISK97</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(ref. 1)</td>
<td>(ref. 13)</td>
<td>(ref. 16)</td>
<td>(ref. 19)</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>2,930</td>
<td>5,506</td>
<td>17,037</td>
<td>5,152</td>
<td>2,777</td>
</tr>
<tr>
<td>n (men/women)</td>
<td>1,448/1,482</td>
<td>2,282/3,224</td>
<td>10,927/6,110</td>
<td>2,567/2,585</td>
<td>1,583/1,075</td>
</tr>
<tr>
<td>Age (years)</td>
<td>62 ± 11</td>
<td>58 ± 6</td>
<td>46 ± 7</td>
<td>60 ± 7</td>
<td>63 ± 11</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28 ± 4</td>
<td>26 ± 4</td>
<td>24 ± 3</td>
<td>28 ± 4</td>
<td>30 ± 5</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>144 ± 101</td>
<td>122 ± 71</td>
<td>117 ± 71</td>
<td>159 ± 108</td>
<td>231 ± 275</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>49 ± 15</td>
<td>53 ± 14</td>
<td>—</td>
<td>53 ± 21</td>
<td>44 ± 13</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>145 ± 51</td>
<td>161 ± 38</td>
<td>—</td>
<td>160 ± 43</td>
<td>137 ± 40</td>
</tr>
<tr>
<td>fβ-glucose (mg/dl)</td>
<td>121 ± 49</td>
<td>93 ± 25</td>
<td>88 ± 13</td>
<td>96 ± 28</td>
<td>189 ± 69</td>
</tr>
<tr>
<td>HOMA (mmol × mU)</td>
<td>1.7 ± 1.4</td>
<td>2.0 ± 2.7</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Type 2 diabetes (%)</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
Laser desorption ionization-time of flight mass spectroscopy using a Sequenom platform. RNA was extracted using the Qiagen RNaseasy 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

**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 SNP-phenotype association analyses were performed to 6

**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 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 $P = 3 \times 10^{-56}$, Table 2). 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 $\sim 6.2$ 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 × 10⁻²), 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 ± 4.7, CT 2.6 ± 4.2, and TT 2.9 ± 4.3 mg/l, P = 4.5 × 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 non-diabetic 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_meta = 1 × 10⁻¹⁵; 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 × 10⁻⁸). In DGI and Botnia PPP cohorts, we could calculate the insulinogenic index during an OGTT in 999 and 3,184 non-diabetic 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 dia-

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**Table 2**

Triglyceride concentrations according to genotype at *GCKR* rs780094 in 12 studies comprising 46,549 individuals

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

Total n = 46,549

19,427

20,958

6,164

Meta-analysis P value 3 × 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 P values are two sided. *DGI and 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

<table>
<thead>
<tr>
<th>Country Study</th>
<th>Fasting plasma glucose (mg/dl)</th>
<th>HOMA (insulin resistance)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC (mmol/L)</td>
<td>CT (mmol/L)</td>
</tr>
<tr>
<td>Finland and Sweden DGI 101</td>
<td>31 (609)</td>
<td>99</td>
</tr>
<tr>
<td>Sweden MDC-CC 99</td>
<td>9 (2,043)</td>
<td>99</td>
</tr>
<tr>
<td>Sweden MPP 87</td>
<td>9 (3,893)</td>
<td>87</td>
</tr>
<tr>
<td>Sweden NORDIL 102</td>
<td>16 (2,024)</td>
<td>102</td>
</tr>
<tr>
<td>Finland Botnia-PPP 83</td>
<td>10 (1,383)</td>
<td>82</td>
</tr>
<tr>
<td>Spain Valencia 94</td>
<td>17 (433)</td>
<td>92</td>
</tr>
<tr>
<td>U.S. Dallas Heart Study blacks</td>
<td>91</td>
<td>13 (1,102)</td>
</tr>
<tr>
<td>U.S. Dallas Heart Study Hispanics</td>
<td>94</td>
<td>10 (222)</td>
</tr>
<tr>
<td>U.S. Dallas Heart Study whites</td>
<td>92</td>
<td>12 (347)</td>
</tr>
<tr>
<td>U.S. GOLDN 99</td>
<td>13 (353)</td>
<td>100</td>
</tr>
<tr>
<td>U.S. BPRHS 104</td>
<td>31 (262)</td>
<td>103</td>
</tr>
<tr>
<td>Singapore Singapore NHS-98 Chinese</td>
<td>19 (823)</td>
<td>99</td>
</tr>
<tr>
<td>Singapore Singapore NHS-98 Malays</td>
<td>25 (256)</td>
<td>105</td>
</tr>
<tr>
<td>Singapore Singapore NHS-98 Asian Indians</td>
<td>33 (298)</td>
<td>102</td>
</tr>
<tr>
<td>Total: 33,995</td>
<td>14,048</td>
<td>15,258</td>
</tr>
</tbody>
</table>

Data are means ± SD (n) or continuous raw measures of blood glucose converted to plasma glucose or 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/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.
GCKR concentrations (supplementary Table 3). A common
studied the association of these SNPs with triglyceride
10 untyped SNPs (or so-called in silico fine-mapping) (Fig. 1).
ing tag and coding SNPs across the region and by imputing
GCKR
region with two different approaches, by genotyp-
GCKR
0.20, and TT 2.16
carriers (CC 0.26
carriers did not have a significantly lower basal rate of
hepatic glucose production during the hyperinsulinemic
clamp hepatic glucose production.

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
dial infarction, lipid-lowering medication, antihypertensive
erides, BMI, sBP, dBP, smoking, family history of myocar-
disease, and CRP were included as covariates.

We fine-mapped 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 \times 10^{-10}).

Fine-mapping by imputation also revealed that GCKR
coding SNP rs1260326 (Pro446Leu) gave the strongest
signal for triglyceride concentrations (P = 1.5 \times 10^{-10}) 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 be-
tween 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-

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 \times 10^{-22} and 0.0005, respectively) and after the mean follow-up period of
23.4 years (P = 3 \times 10^{-20} 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 \times 10^{-15}), 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 trended 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, triglyc-
erides, BMI, sBP, dBP, smoking, family history of myocar-
dial 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).

FIG. 1. In silico and genotype fine-mapping of the GCKR locus. To define the strongest signal for the association on chromosome 2p23 for
triglycerides, a region spanning ~417 kb and containing 17 annotated genes was fine-mapped by two different approaches, imputation of untyped
SNPs (29) (or so-called in silico fine-mapping) (A) and genotyping tagging SNPs across the region (B). Both in silico and genotype 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%.
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 overriding 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 an euglycemic-hyperinsulinemnic 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 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
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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REFERENCES


