Triglyceride Response to an Intensive Lifestyle Intervention Is Enhanced in Carriers of the *GCKR* Pro446Leu Polymorphism

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Context: Glucokinase regulatory protein (GCKR) regulates the trafficking and enzymatic activity of hepatic glucokinase, the rate-limiting enzyme in glycogen synthesis and glycolysis. The intronic single-nucleotide polymorphism (SNP) rs780094 (intron 16) and the missense SNP rs1260326 (P446L) in the *GCKR* gene are strongly associated with increased circulating triglyceride and C-reactive protein levels and, paradoxically, reductions in diabetes incidence, fasting glucose levels, and insulin resistance.

Objective, Setting, and Patients: We sought to replicate these associations and evaluate interactions with lifestyle and metformin interventions in the multiethnic Diabetes Prevention Program (DPP).

Interventions and Main Outcome Measures: We genotyped the two *GCKR* SNP in 3346 DPP participants and evaluated association with progression to diabetes and both baseline levels and changes in triglycerides, homeostasis model assessment of insulin resistance (HOMA-IR), oral disposition index, and inflammatory markers along with their interactions with DPP interventions.

Results: *GCKR* variation did not predict development of type 2 diabetes. At baseline, the 446L allele was associated with higher triglyceride and C-reactive protein levels (both $P < 0.0001$) and lower fasting glucose ($P = 0.001$) and HOMA-IR ($P = 0.06$). The lifestyle intervention was associated with a decrease in magnitude of the effect of the 446L allele on triglyceride levels (interaction $P = 0.04$). Metformin was more effective in reducing HOMA-IR in carriers of the P446 allele (interaction $P = 0.05$).

Conclusions: Intensive lifestyle intervention appears to partially mitigate the effect of the 446L allele on higher triglycerides, whereas the P446 allele appears to enhance responsiveness to the HOMA-IR-lowering effect of metformin. (***J Clin Endocrinol Metab* 96: E1142–E1147, 2011**)

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Abbreviations: AIM, Ancestry-informative markers; CRP, C-reactive protein; CV, coefficient of variation; DIo, oral disposition index; DPP, Diabetes Prevention Program; GCK, glucokinase; GCKR, glucokinase regulatory protein; HOMA-IR, insulin sensitivity by homeostasis model assessment; SNP, single-nucleotide polymorphism; TG, triglyceride; tPA, tissue plasminogen activator.

Common variants in the glucokinase regulatory protein gene (*GCKR*), primarily rs780094 and rs1260326 (P446L), are associated with increased fasting circulating triglyceride (TG) levels (1–7), postprandial TG (7), and a greater increase in TG over time (4). Interestingly, the alleles associated with increased TG are associated with better insulin sensitivity by homeostasis model assessment (HOMA-IR) (1, 4, 8, 9) and lower risk for type 2 diabetes (4, 10). Given the usual negative correlation between insulin sensitivity and TG levels, these findings seem paradoxical. However, a change in GCKR resulting in higher glucokinase (GCK) activity could lead to both reduced glucose and increased TG levels via the stimulation of lipogenic genes by the glycolytic pathway (11). Consistent with this notion and the observed associations, the 446L isoform was recently reported *in vitro* to increase hepatic GCK activity (12).

In the Diabetes Prevention Program (DPP), an intensive lifestyle intervention markedly lowered TG (13). Thus, the DPP is a unique resource in which to clarify the nature and implications of the paradoxical *GCKR*-associated TG/glucose discordance as well as replicate and extend the association of *GCKR* with C-reactive protein (CRP) (4, 14). The multiethnic nature of the DPP also opens the possibility to study the role of *GCKR* in ethnic differences in TG. TG levels are markedly lower in African-Americans than in Caucasians (15, 16), which has recently been attributed to multiple genetic differences rather than a single or small number of major genes (17). Intriguingly, in the Jackson Heart Study, the effect size of the rs780094 intronic single-nucleotide polymorphism (SNP) was decreased in which local ancestry was assigned as African rather than European based on ancestry-informative markers (AIM) (17).

In addition to replicating the association of the T alleles at SNP rs1260326/rs780094 with higher TG/CRP levels, lower fasting glucose/HOMA-IR (but not insulin secretion) at baseline, and decreased diabetes incidence, we hypothesized that these alleles would be associated with other inflammatory markers [tissue plasminogen activator (tPA) and fibrinogen] and modify the effect of the metformin and lifestyle interventions on 1-yr changes in these quantitative traits and on diabetes incidence. We also hypothesized that the coding SNP (rs1260326/P446L) would show a stronger association in African-Americans than the intronic SNP (rs780094).

Materials and Methods

The Diabetes Prevention Program

The DPP is a multicenter trial that examined whether lifestyle modification or metformin therapy prevents the development of diabetes in individuals at risk, defined by elevated fasting glucose, impaired glucose tolerance, and overweight or obesity. Details of the DPP study design have been described elsewhere (18). After an average of 2.8 yr of follow-up, the lifestyle modification group had a 58% reduction in diabetes incidence, whereas the metformin group had a 31% reduction compared with placebo (19). Triglyceride levels were lower in all three groups after 1 yr. This decline was significantly greater in the intensive lifestyle intervention group (−25.4 mg/dl) than in the placebo (−11.9 mg/dl) and metformin (−7.4 mg/dl) groups (13). A total of 3346 participants provided consent and had DNA available for analysis. Individuals taking lipid-lowering medications at baseline (n = 166) were excluded from all analyses. Participants randomized to the prematurely terminated troglitazone arm (n = 348) were studied only for quantitative traits.

Genotyping

GCKR polymorphisms

DNA was extracted from peripheral blood leukocytes with standard methods. Genotyping was carried out by allele-specific primer extension of multiplex amplified products and detection using matrix-assisted laser desorption ionization time-of-flight mass spectrometry on a Sequenom iPLEX platform (20). Call rates were greater than 99%. Genotype frequencies were consistent with Hardy-Weinberg equilibrium expectations within each self-reported ethnic group.

Ancestry informative markers

Thirty AIM were also genotyped. In Caucasian and African-American subjects, global percent African and European ancestry was estimated using STRUCTURE trained on the HapMap CEU and YRI populations (21).

Quantitative glycemic, TG, and inflammatory marker measures

Baseline and annual fasting measures of glucose, insulin, TG, and inflammatory markers were performed on all subjects Oral glucose tolerance tests were performed at baseline and annually in subjects who had not developed diabetes before the 1-yr examination. All biochemical measurements were made at the DPP Central Biochemistry Laboratory (Northwest Lipid Metabolism and Diabetes Research Laboratories, Seattle, WA). Plasma glucose was measured by the glucokinase method [coefficient of variation (CV) 1.4%]. Insulin measurements were performed by a polyethylene glycol-accelerated double-antibody RIA (CV 5.6%). Triglyceride levels were measured using enzymatic assays (CV 1.7%) standardized to the Centers for Disease Control and Prevention reference methods (22). CRP (CV 3.5%) and fibrinogen levels (CV 4.6%) in plasma were immunochemically measured using the Nephelometer autoanalyzer (Dade Behring, Deerfield, IL). The tPA levels (CV 13.9%) were measured in citrated plasma using an ELISA (Asserachrom tPA; Diagnostica Stago, Parsippany, NJ) (16).

HOMA-IR as an inverse measure of insulin sensitivity was calculated as fasting insulin × (fasting glucose/18.01)/22.5 (8). The oral disposition index (DIo) was defined as [(insulin at 30 min) − (insulin at 0 min)]/[(glucose at 30 min) − (glucose at 0 min)] × (1/fasting insulin) (23).

We elected to analyze quantitative traits at 1 yr because the greatest effect on weight loss was observed in the lifestyle mod-

ification group at yr 1, and a substantial number of subjects did not complete the oral glucose tolerance test in the third year (because they had either developed diabetes already or because the trial ended before they reached that time point) (19).

Statistical analysis

We examined genotype (under an additive model), intervention, and genotype-by-intervention interactions as independent predictors of time to onset of diabetes using Cox regression models for the participants randomized to the placebo, metformin, and lifestyle intervention arms. Baseline glycemic variables, HOMA-IR, DIo, TG, and CRP levels were log transformed for nonnormality, and the geometric means were compared across genotypic groups (rs1260326 and rs780094 CC, CT, and TT) by ANOVA (general model, 2 *df F* test) and also modeling the genotype as an additive (1 *df*) effect. We compared the 1-yr changes in quantitative variables using analysis of covariance models with the independent variables of additive genotypic effect, treatment group, and interaction terms of genotype and treatments. All analyses were adjusted for sex, age, self-reported ethnicity, waist circumference, and body mass index. Although the correlation between waist circumference and body mass index was high (rho = 0.89; $P < 0.001$), the condition index was acceptable at 3.0. Additional analyses were stratified by ethnicity and/or adjusted for AIM-estimated proportion of African ancestry.

Results

Progression to diabetes

Neither SNP was significantly associated with progression to diabetes [rs1260326/P446L: hazard ratio 0.95 (95% confidence interval 0.84–1.07); rs780094: hazard ratio 0.86 (95% confidence interval 0.85–1.09)], and no interaction with treatment group was observed.

Linkage disequilibrium and ethnic differences

We found a low correlation between the two SNP in the African-American DPP cohort ($r^2 = 0.63$; Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). For this reason, combined with recent *in vitro* evidence (12) that P446L is much more likely to be functional than rs780094, we chose to focus our report on the P446L variant.

Both SNP were associated with TG levels in all ethnic groups (with borderline significance in American Indians) except African-Americans (Supplemental Table 2), in which the genotype geometric means were 103, 105, and 106 mg/dl for the rs780094 CC, CT, and TT genotypes, respectively ($P=0.61$). In African-Americans, the spread among genotypes for the rs1260326/P446L SNP was greater (TG of 103, 107, and 114 mg/dl) but still not significant ($P = 0.23$).

In the subset of 2072 Caucasian and African-American subjects whose ancestry was successfully inferred with AIM, adjusting for either self-reported ethnicity or marker-based ancestry (Supplemental Table 3) similarly attenuated the association between rs1260326/P446L and TG levels ($P = 9 \times 10^{-7}$ or 8×10^{-7}, respectively) in comparison with no adjustment for ethnic background (1×10^{-21}). There were no significant P446L × self-reported ethnicity ($P=0.29$) or P446L × African ancestry ($P = 0.13$) interactions.

Baseline measures of TG and glucose-related traits

The *GCKR* rs1260326-T (446L) allele was strongly associated with increased TG ($P = 1.8 \times 10^{-9}$) and moderately associated with lower fasting glucose ($P = 0.001$) and borderline associated with lower HOMA-IR ($P = 0.06$) (Supplemental Table 4). There was no association with DIo.

Inflammatory markers

The rs1260326-T allele strongly associated with increased CRP levels ($P = 6 \times 10^{-5}$) independently of TG (Supplemental Table 4) and modestly with increased fibrinogen ($P = 0.05$) but not tPA.

Changes at 1 yr

There was significant SNP × lifestyle interaction on TG ($P = 0.04$); the 446L (rs1260326-T) allele associated with enhanced TG reduction in the lifestyle arm (Supplemental Table 5). This translates into a reduced TG-raising effect of the 446L variant in the presence of intensive lifestyle change (Fig. 1). Also seen was interaction between the SNP and metformin in HOMA-IR change (Fig. 2). The 446L allele is associated with a smaller decrease in HOMA-IR in response to the metformin intervention ($P = 0.05$) than the P446 allele (Supplemental Table 5). There were no significant genotype × treatment interactions for any other glycemic (glucose and insulin in Supplemental Table 5) or inflammatory traits (data not shown). In the small number

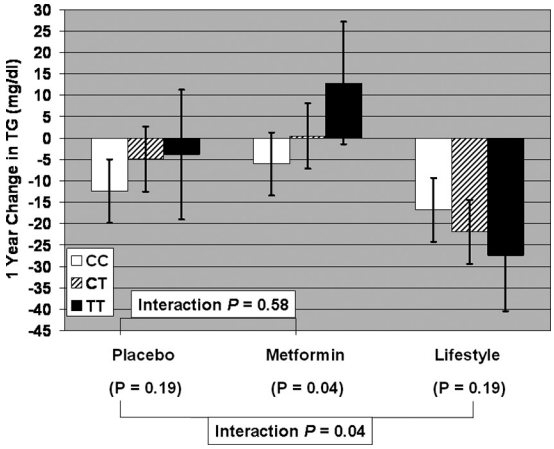

FIG. 1. Ethnicity-adjusted, 1-yr changes in TG (milligrams per deciliter) by treatment group and rs1260326/P446L genotype (mean and 95% CI). *P* values in *parentheses* refer to additive genetic effects.

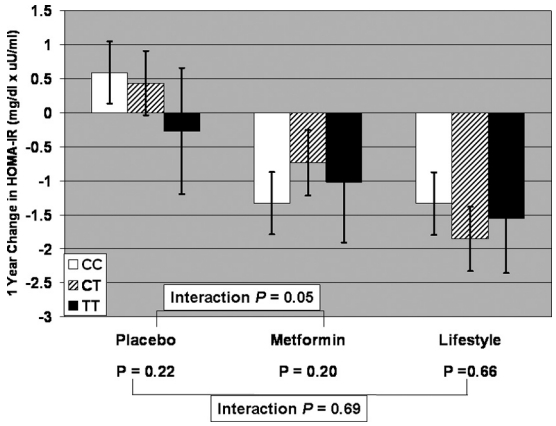

FIG. 2. Ethnicity-adjusted, 1-yr changes in HOMA-IR (milligrams per deciliter × microunits per milliliter) by treatment group and rs1260326/P446L genotype (mean and 95% CI). *P* values in *parentheses* refer to additive genetic effects.

of subjects in the troglitazone arm, there were no interactions with any traits.

Discussion

In the DPP, we observed no significant association between either *GCKR* SNP or progression to diabetes (although there was a slight trend consistent with previous reports), nor was there any interaction between the variants and treatment group with respect to this outcome. This result was not surprising, given the relatively modest effect seen in previous studies and the limited power of the DPP to detect such effects (20, 24).

We have confirmed that the intronic (rs780094) and coding (rs1260326/P446L) SNP are strongly correlated in all ethnicities except African-Americans. As predicted, the coding SNP shows a stronger association with TG in African-Americans than does the intronic SNP (17). These findings enhance support for P446L as a causal variant. However, the association of TG levels with the coding variant in African-Americans is not statistically significant, and the effect size is still greatly attenuated. It is possible that a third, unknown variant may be responsible for the associations in the other ethnic groups. Alternatively [and perhaps more likely, given the recent functional evidence for P446L (12)], the impact of this variant may be diminished against a genetic background conferring greatly reduced TG, although a statistically significant SNP × ethnicity interaction was not observed.

We have replicated the previous finding of association of the rs1260326 446L variant of *GCKR* with increased TG and CRP levels and decreased glucose. We extend these findings by showing that increased physical activity, dietary changes, and weight loss lead to a greater decrease in TG in carriers of the *GCKR* 446L allele, resulting in a reduction in the differences in TG between carriers and noncarriers. In the absence of intervention, the 446L variant has previously been shown to be associated with greater increases in TGs over a 23-yr period (4); our findings suggest that increased physical activity and weight loss partially mitigate this effect. The reduced impact of the 446L allele on TG after a lifestyle intervention likely results from the reduced availability of free fatty acids to participate in the lipogenesis that would otherwise be enhanced by increased GCK-mediated glycolysis. No interaction was observed between metformin and *GCKR* genotype on lipids, which is not surprising, given that metformin did not have a marked TG-lowering effect in general.

In the metformin group, carriers of the glucose-raising allele (P446) showed greater responsiveness to metformin as evidenced by a larger decrease in HOMA-IR, with an accompanying trend toward a greater decrease in insulin levels. This finding may be a result of the GCK-stimulating effect of metformin, which has been observed in hepatocytes (25), overpowering the impact of the *GCKR* P446 variant on decreased GCK activity and, if replicated, represents a potentially relevant pharmacogenetic interaction. That such an interaction was not observed in the lifestyle group may be due to the lack of a direct effect of lifestyle change on GCK activity.

In summary, in the DPP, intensive lifestyle change appears to reduce the deleterious impact of the *GCKR* 446L variant on TG levels without significantly affecting its relationship with insulin sensitivity. Metformin seems to have a greater insulin-sensitizing effect on carriers of the *GCKR* P446 allele.

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