ORIGINAL ARTICLE

The Common P446L Polymorphism in *GCKR* Inversely Modulates Fasting Glucose and Triglyceride Levels and Reduces Type 2 Diabetes Risk in the DESIR Prospective General French Population

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OBJECTIVE—Hepatic glucokinase (GCK) is a key regulator of glucose storage and disposal in the liver, where its activity is competitively modulated, with respect to glucose, by binding to glucokinase regulatory protein (GCKR) in the presence of fructose 6-phosphate. Genome-wide association studies for type 2 diabetes identified *GCKR* as a potential locus for modulating triglyceride levels. We evaluated, in a general French population, the contribution of the *GCKR* rs1260326-P446L polymorphism to quantitative metabolic parameters and to dyslipidemia and hyperglycemia risk.

RESEARCH DESIGN AND METHODS—Genotype effects of rs1260326 were studied in 4,833 participants from the prospective DESIR (Data from an Epidemiological Study on the Insulin Resistance syndrome) cohort both at inclusion and using the measurements at follow-up.

RESULTS—The minor T-allele of rs1260326 was strongly associated with lower fasting glucose (-1.43% per T-allele; $P = 8 \times 10^{-13}$) and fasting insulin levels (-4.23%; $P = 3 \times 10^{-7}$), lower homeostasis model assessment of insulin resistance index (-5.69%; $P = 1 \times 10^{-8}$), and, conversely, higher triglyceride levels (3.41%; $P = 1 \times 10^{-4}$) during the 9-year study. These effects relate to a lower risk of hyperglycemia (odds ratio [OR] 0.79 [95% CI 0.70–0.88]; $P = 4 \times 10^{-5}$) and of incident cases during the study (hazard ratio [HR] 0.83 [0.74–0.95]; $P = 0.005$). Moreover, an additive effect of *GCKR* rs1260326(T) and *GCK* (-30G) alleles conferred lower fasting glycemia ($P = 1 \times 10^{-13}$), insulinemia ($P = 5 \times 10^{-6}$), and hyperglycemia risk ($P = 1 \times 10^{-6}$).

CONCLUSIONS—*GCKR*-L446 carriers are protected against type 2 diabetes despite higher triglyceride levels and risk of dyslipidemia, which suggests a potential molecular mechanism by which these two components of the metabolic syndrome can be dissociated. *Diabetes* 57:2253–2257, 2008

The enzyme glucokinase (GCK) is the major glucose sensor of the pancreatic β-cells, where it adapts insulin secretion to blood glucose levels. GCK also participates in regulating glycogen synthesis and gluconeogenesis in the liver (1). GCK activity is allosterically controlled in hepatocytes by the glucokinase regulatory protein (GCKR), which reversibly binds to GCK and inhibits its activity in the presence of fructose 6-phosphate. GCKR acts as a competitive inhibitor of GCK with respect to glucose and is suppressed by the specific metabolite fructose 1-phosphate (2).

In GCKR-deficient mice, the disruption of this regulation and the subsequent decrease in GCK activity leads to altered glucose metabolism and impaired postprandial glycemic control (3,4), although no change in fasting blood glucose concentration is observed. Adenoviral-mediated hepatic overexpression of GCKR in mice with high-fat diet–induced diabetes improves fasting and glucose-induced glycemia and leads to a concomitant increase in insulin sensitivity and triglyceride levels and a decrease in leptin levels (5). Heterozygous mutations in *GCK* resulting in a reduction of enzymatic activity are responsible for a subtype of monogenic diabetes (maturity-onset diabetes of the young-2) (1,6). Previous genetic studies at the *GCKR* locus have not reported intragenic mutations associated with type 2 diabetes in humans (7,8).

The Diabetes Genetics Initiative (DGI) genome-wide association study for type 2 diabetes and quantitative metabolic traits reported an intronic polymorphism of *GCKR* (rs780094) explaining interindividual variability in plasma triglyceride (TG) levels and a trend toward association with lower fasting glycemia, less insulin resistance, and lower risk for type 2 diabetes (9). The HapMap II CEU data (www.hapmap.org) showed that rs780094 is in strong linkage disequilibrium ($r^2 = 0.932$) with a non-synonymous *GCKR* variant (Pro446Leu, rs1260326) that we previously identified by the DNA sequencing of French individuals (7). Marju Orho-Melander and colleagues recently communicated their fine-mapping data showing the strongest signal for TG levels at the coding single nucleotide polymorphism (SNP) (rs1260326; $P = 1.5 \times 10^{-9}$), with lesser associations to fasting glycemia and insulin sensitivity (M. Orho-Melander, O. Melander, V. Lyssenko, for the DGI, unpublished data). Similar findings for the intronic variant rs780094 were reported in a Danish population (10).

TABLE 1
Anthropometric and metabolic characteristics of participants not treated with either hypoglycemic or lipid-lowering drugs at inclusion in the study, with data stratified according to *GCKR* rs1260326 genotype

		CC	CT	TT	$P_{additive}$	Δ% (95% CI)
n	4,363	1,383	2,125	855		
Sex ratio (male/female)	2,135/2,228	666/717	1,044/1,081	425/430		
Age (years)	46.5 ± 9.79	46.3 ± 9.83	46.7 ± 9.8	46.5 ± 9.69		
BMI (kg/m²)	24.5 ± 3.77	24.4 ± 3.64	24.6 ± 3.94	24.5 ± 3.53	0.8	0.09 (−0.50 to 0.68)
Waist-to-hip ratio	0.85 ± 0.09	0.85 ± 0.09	0.85 ± 0.09	0.85 ± 0.09	0.2	−0.16 (−0.42 to 0.09)
Fasting glucose (mmol/l)	5.31 ± 0.68	5.37 ± 0.74	5.30 ± 0.64	5.24 ± 0.68	9×10^{-9}	−1.27 (−1.70 to −0.84)
Fasting insulin (pmol/l)	46.0 ± 29.07	46.8 ± 27.49	46.8 ± 31.47	42.9 ± 24.86	5×10^{-5}	−3.90 (−5.72 to −2.04)
HOMA-B	89.7 ± 61.27	87.6 ± 50.89	90.7 ± 57.29	90.9 ± 82.58	0.9	0.11 (−2.19 to 2.46)
HOMA-IR	1.82 ± 1.27	1.88 ± 1.33	1.84 ± 1.31	1.68 ± 1.07	4×10^{-6}	−5.45 (−7.66 to −3.18)
Total cholesterol (mmol/l)	5.74 ± 1.03	5.75 ± 1.04	5.73 ± 1.02	5.74 ± 1.03	0.5	−0.24 (−0.94 to 0.46)
LDL cholesterol (mmol/l)	3.58 ± 0.92	3.61 ± 0.95	3.56 ± 0.91	3.57 ± 0.94	0.2	−0.75 (−1.82 to 0.33)
HDL cholesterol (mmol/l)	1.64 ± 0.43	1.64 ± 0.43	1.64 ± 0.43	1.63 ± 0.41	0.8	0.14 (−0.86 to 1.14)
TG (mmol/l)	1.16 ± 1.08	1.12 ± 1.2	1.17 ± 1.08	1.21 ± 0.83	5×10^{-4}	3.69 (1.61 to 5.82)

Data are means ± SD or n unless otherwise indicated. The P values indicated are nominal P-values. Δ% is the percentage of variation of the trait per supplementary minor T-allele. P values and Δ% are from linear regression models adjusted for age, sex, and BMI. HOMA-B, homeostasis model assessment of β-cell function; HOMA-IR, homeostasis model assessment of insulin resistance; TG, triglycerides.

We evaluated the association between *GCKR* rs1260326-P446L and TG levels in a middle-aged general French population with a follow-up of 9 years (11). Given the key role of GCKR in hepatic glucose metabolism, we also assessed the effect of rs1260326 on glucose homeostasis parameters and on the risk of impaired fasting glycemia and type 2 diabetes. Furthermore, as we previously showed a strong association of the *GCK* (−30A) promoter variant (rs1799884) to increased fasting glycemia and type 2 diabetes risk in the same study cohort (11), we also assessed possible additive effects of *GCKR* rs1260326-P446L and *GCK* −30G/A SNPs on fasting glucose, insulin, TG levels, and hyperglycemia risk.

RESEARCH DESIGN AND METHODS

Participants in the Data from an Epidemiological Study on the Insulin Resistance syndrome (DESIR) study were clinically and biologically evaluated at inclusion and at 3-, 6-, and 9-year follow-up visits (11,12). All subjects included in the study signed an informed consent form, and the protocol was approved by the ethics committee of Bicêtre Hospital. Because ethnic origin could not be legally documented at the beginning of the DESIR study, the proportion of subjects having non-European ancestry was estimated as 0.30% by a STRUCTURE analysis of 328 SNPs in 654 selected subjects, as previously published (11). Moreover, all individuals born outside France were excluded from this study. Overall, 4,833 individuals of the DESIR cohort were analyzed, of whom 3,877 were examined during the entire 9-year study.

Glycemic status was defined according to 1997 American Diabetes Association criteria: normoglycemia, defined as fasting plasma glucose (FPG) <6.1 mmol/l without hypoglycemic treatment; impaired fasting glucose (IFG), defined as FPG between 6.1 and 6.99 mmol/l without hypoglycemic treatment; and type 2 diabetes, defined as FPG ≥7.0 mmol/l and/or treatment with antidiabetic agents.

Dyslipidemia was defined according to World Health Organization criteria: TG ≥1.7 mmol/l, HDL cholesterol <0.9 mmol/l for men or <1.0 mmol/l for women, or current treatment with lipid-lowering drugs. Biological parameters were assessed as previously described (11,12).

SNP genotyping. Genotyping of rs1260326 was performed using TaqMan Technology (Applied Biosystems, Foster City, CA). A successful genotyping rate of 99% was achieved in the whole cohort sample. Duplicate samples were assayed with a concordance rate ≥99%. The genotype distribution of rs1260326 was in Hardy-Weinberg equilibrium ($P > 0.05$). The rs1799884 SNP was genotyped as previously reported (11).

Statistical analyses. The effect of the rs1260326 genotype on quantitative parameters was assessed in untreated individuals both at baseline and using repeated measures at 3-, 6-, and 9-year follow-up visits (11). All quantitative traits were log transformed before analyses and adjusted for age, sex, and BMI. We used linear regression models for analyses at baseline and mixed models for analyses of repeated measures.

The risk of hyperglycemia, type 2 diabetes, and dyslipidemia at the end of the study was assessed by logistic regression models, including age, sex, and BMI as covariates. Cox proportional hazards regression models were used to estimate the genotype effects on the incidence of hyperglycemia, type 2 diabetes, and dyslipidemia. The models included sex and BMI as covariates. Mean BMI was estimated for all available measures for censored individuals and for all available measures before diagnosis for case subjects. Survival time was considered equivalent to an individual's age.

The additive effect of *GCKR* rs1260326 and *GCK* (−30G/A) rs1799884 on FPG, insulin, and TG levels was assessed in individuals untreated at baseline by a linear regression model including the number of risk alleles, age, sex, and BMI as covariates in order to quantify the combined genotype effect per supplementary at-risk allele (as previously described [11]). In the same way, the additive effect of both variants on the risk of hyperglycemia at the end of the 9-years' follow-up was explored using a logistic regression model. In these analyses, we considered the T-allele of *GCKR* rs1260326 and the G-allele of *GCK* rs1799884 as protective against hyperglycemia and type 2 diabetes. We then compared the models, including the number of risk alleles as a covariate to the null model including no SNP covariate, by using likelihood ratio. All statistical analyses were performed using R (version 2.6.0) combined with mgcv, survival, and nlme packages.

RESULTS

The *GCKR* rs1260326-P446L variant was genotyped in a total of 4,833 participants of the DESIR study and showed an overall minor allele frequency of 43.9%. We first analyzed the relationship of rs1260326 with lipid and glucose homeostasis parameters in 4,363 individuals who were not treated with hypoglycemic or lipid-lowering drugs at inclusion. The minor T-allele of rs1260326 was associated with increased TG levels both at baseline ($P = 5 \times 10^{-4}$; Table 1) and from the repeated measures during the 9-year follow-up ($P = 1 \times 10^{-4}$; Table 2). No associations were observed with total, HDL, or LDL cholesterol levels (Table 1).

Conversely, the same T-allele of rs1260326 was strongly associated with lower FPG ($P = 9 \times 10^{-9}$) and fasting serum insulin ($P = 5 \times 10^{-5}$) and a lower homeostasis model assessment of insulin resistance (HOMA-IR) index ($P = 4 \times 10^{-6}$) at baseline (Table 1). The genotype effect was even more significant when repeated measures over the 9-year study were analyzed for these parameters ($P = 8 \times 10^{-13}$ for FPG, $P = 3 \times 10^{-7}$ for insulin, and $P = 1 \times 10^{-8}$ for HOMA-IR) (Table 2). No effect on the β-cell insulin secretion index was seen in this population, in agreement with the hypothesis that the *GCKR* coding variant would

TABLE 2
Effects of *GCKR* rs1260326 on TG levels and glucose homeostasis parameters using up to four repeated measurements over the 9-year follow-up study

	Number of observations	P	Δ% (95% CI)	Overall mean ± SD		
				CC	CT	TT
Fasting plasma glucose (mmol/l)						
additive	13,941	8×10^{-13}	−1.43 (−1.81 to −1.04)	5.41 ± 0.70	5.34 ± 0.69	5.27 ± 0.64
dominant		2×10^{-10}	−1.92 (−2.50 to −1.33)			
recessive		2×10^{-7}	−1.85 (−2.54 to −1.17)			
Fasting serum insulin (pmol/l)						
additive	13,915	3×10^{-7}	−4.23 (−5.80 to −2.63)	52.9 ± 37.48	51.5 ± 36.66	47.9 ± 31.57
dominant		3×10^{-5}	−5.19 (−7.54 to −2.77)			
recessive		3×10^{-5}	−6.13 (−8.86 to −3.32)			
HOMA-IR						
additive	12,042	1×10^{-8}	−5.69 (−7.57 to −3.79)	2.15 ± 1.75	2.06 ± 1.70	1.90 ± 1.53
dominant		1×10^{-6}	−7.20 (−9.98 to −4.34)			
recessive		7×10^{-6}	−7.87 (−11.10 to −4.52)			
HOMA-B						
additive	12,031	1	−0.04 (−1.99 to 1.95)	101.3 ± 69.15	103.1 ± 80.51	101.4 ± 77.25
dominant		0.7	0.57 (−2.39 to 3.61)			
recessive		0.6	−0.90 (−4.30 to 2.63)			
Fasting plasma TG (mmol/l)						
additive	13,955	1×10^{-4}	3.41 (1.68 to 5.16)	1.12 ± 0.87	1.19 ± 0.97	1.21 ± 0.73
dominant		1×10^{-3}	4.24 (1.61 to 6.94)			
recessive		1×10^{-3}	5.00 (1.90 to 8.19)			

The P values indicated are nominal P values. Δ%, percentage of variation of the trait per supplementary minor T-allele. P values and Δ% are from mixed models adjusted for age, sex, and BMI. HOMA-B, homeostasis model assessment of β-cell function; HOMA-IR, homeostasis model assessment of insulin resistance; TG, triglycerides.

enhance insulin sensitivity through improved regulation of hepatic glucose metabolism. The characteristics of the 470 individuals treated with hypoglycemic and/or lipid-lowering medications or for whom data were unknown and were thus excluded from the quantitative trait analyses are shown in online appendix Table 1 (available online at http://dx.doi.org/db07-1807).

Furthermore, the rs1260326 T-allele was strongly associated with a decreased risk of hyperglycemia in the whole cohort at the end of the study (OR 0.79 [95% CI 0.70–0.88]; $P = 4 \times 10^{-5}$) and with decreased incidence during the 9-year follow-up (based on 516 incident cases of hyperglycemia, HR 0.83 [95% CI 0.74–0.95]; $P = 0.005$) (Table 3). Similarly, the rs1260326 T-allele conferred a significantly lower risk of type 2 diabetes in this population (OR 0.79 [0.65–0.95] at the end of the study, $P = 0.01$).

With regard to dyslipidemia, according to World Health Organization criteria, 2,102 subjects showed abnormal TG or HDL cholesterol profiles or were treated with a lipid-lowering drug at least once during the study, and 2,731 remained normolipidemic. The rs1260326 T-allele was modestly associated with dyslipidemia risk (OR 1.12 [95% CI 1.02–1.22]; $P = 0.01$) (Table 3). When considering hypertriglyceridemia (TG ≥1.7 mmol/l) alone, a similar risk effect was observed (1.12 [1.01–1.23]; $P = 0.03$ [data not shown]).

We also analyzed the combined effects of *GCKR* rs1260326-P446L with the *GCK* −30G/A SNP: when comparing carriers of four risk alleles with the reference group with zero or one allele, fasting glycemia was significantly lower by 0.20 mmol/l ($P = 1 \times 10^{-13}$) (Fig. 1A), fasting insulinemia was significantly lower by 6.19 pmol/l ($P = 5 \times 10^{-6}$) (data not shown), and hyperglycemia (including type 2 diabetes) risk decreased to OR 0.49 ($P = 1 \times 10^{-6}$) (Fig. 1B). Models including the number of risk alleles as a covariate were significantly better than those with no SNP covariate ($P = 1.5 \times 10^{-13}$ for fasting glucose, $P = 5.4 \times 10^{-6}$ for fasting insulin, and $P = 1.2 \times 10^{-6}$ for hyperglycemia or type 2 diabetes risk). As expected, no additive effect on TG levels was observed when combining the risk alleles of *GCK* −30G/A and *GCKR* rs1260326-P446L.

DISCUSSION

Our data show that the *GCKR* P446L polymorphism is associated with increased TG levels in a general French population, which extends previous findings from intronic tagged SNPs in Finnish and Swedish populations (9) and in the Danish population-based Inter99 study (10). The minor T-allele of rs1260326-P446L explained 0.2% of the TG variance in our study population, showing a more modest effect than that reported in the Scandinavian samples, where the T-allele of rs780094 explained between 0.4–1.2% of the trait variance (ref. 9 and M. Orho-Melander, O. Melander, V. Lyssenko, for the DGI, unpublished data). In the Northern European populations investigated for the *GCKR* intronic variant, the mean BMI values were higher than in the DESIR participants (26–30 vs. 24.5 kg/m², respectively), and the TG levels showed more variability according to genotypes (9,10). This may explain the differences in the degree of association for TG levels between the French DESIR study and other Northern European populations.

Conversely, the nonsynonymous rs1260326-P446L variant has a much stronger effect on the glucose homeostasis parameters in the DESIR cohort than in the Finnish, Swedish, and Danish populations, although the effects were seen in the same direction (9,10). The lower fasting glycemia in the T-allele carriers (even greater than the levels seen for the *GCK* −30G/A promoter variant in the

TABLE 3
Association with hyperglycemia, type 2 diabetes, and dyslipidemia in the DESIR population at the end of the study and over the 9-year follow-up

	n	n (frequency in each genotypic group)			Minor allele frequency (%)	Overall risk at the end of the study*	Incidence risk over the 9-year follow-up†
		CC	CT	TT			
Hyperglycemia status							
Normoglycemia	2,883	856 (0.30)	1,444 (0.50)	583 (0.20)	45.27		
End-of-study cases	948	338 (0.36)	449 (0.47)	161 (0.17)	40.66	4×10^{-5}, 0.79 (0.70–0.88)	
Incident cases	516	187 (0.36)	238 (0.46)	91 (0.18)	40.70		5×10^{-3}, 0.83 (0.74–0.95)
Type 2 diabetes status							
Normoglycemia	3,397	1,048 (0.31)	1,680 (0.49)	669 (0.20)	44.42		
End-of-study cases	306	103 (0.34)	153 (0.50)	50 (0.16)	41.34	0.01, 0.79 (0.65–0.95)	
Incident cases	187	67 (0.36)	88 (0.47)	32 (0.17)	40.64		0.2, 0.87 (0.71–1.08)
Dyslipidemia status							
Normolipidemia	2,731	898 (0.33)	1,319 (0.48)	514 (0.19)	42.97		
End-of-study cases	2,102	611 (0.29)	1,057 (0.50)	434 (0.21)	45.79	0.01, 1.12 (1.02–1.22)	
Incident cases	959	293 (0.31)	472 (0.49)	194 (0.20)	44.84		0.9, 0.99 (0.91–1.09)

Overall risk data are P, OR (95% CI) and incident risk data are P, HR (95% CI). Hyperglycemia and type 2 diabetes were defined according to 1997 American Diabetes Association criteria and dyslipidemia according to World Health Organization criteria. Hyperglycemia was defined as impaired fasting glucose or type 2 diabetes. *OR from logistic regression models adjusted for age, sex, and BMI. †HR from Cox proportional hazards models adjusted for age, sex, and BMI.
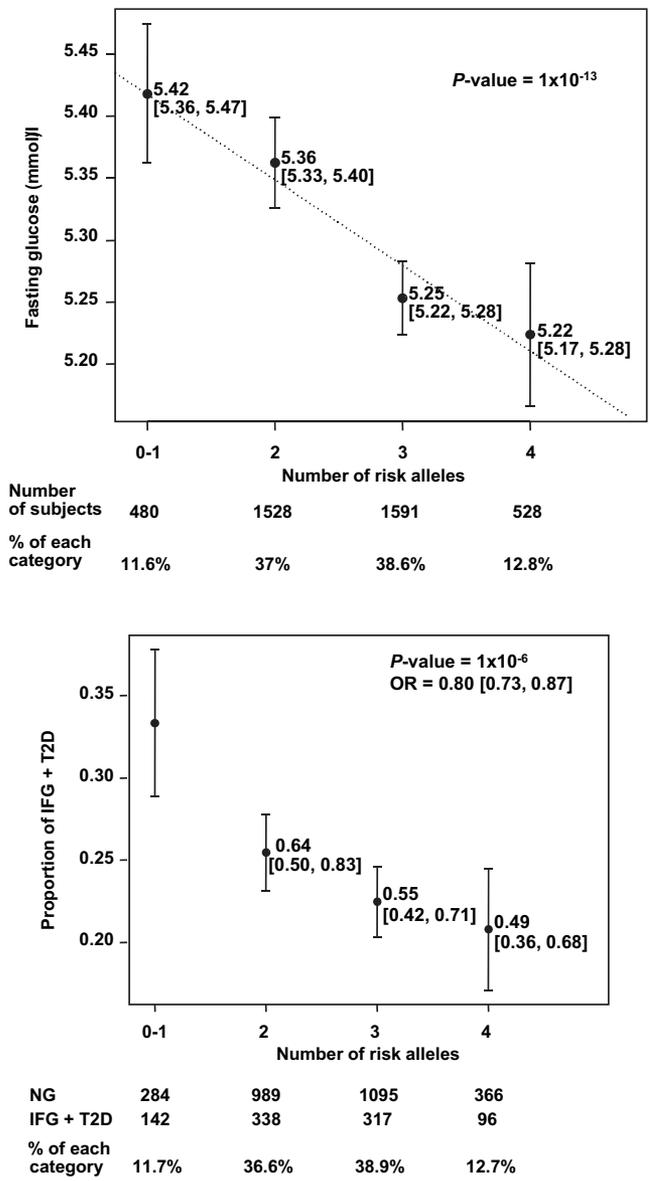
FIG. 1. Additive effects of *GCKR* rs1260326 (*T*) and *GCK* rs1799884 −30G alleles on fasting glucose (*A*) and risk of hyperglycemia (*B*). Data in *A* are presented as means [95% CI] of fasting glucose (millimoles per liter) according to the number of risk alleles. The *P* value corresponds to the coefficient for the number of risk alleles in the linear regression model including age, sex, and BMI as covariates. Data in *B* are presented as ORs [95% CI] of hyperglycemia, defined as impaired fasting glucose or type 2 diabetes at the end of the follow-up study, according to the number of risk alleles. The *P* value corresponds to the coefficient for the number of risk alleles in the logistic regression model including age, sex, and BMI as covariates.

same cohort [11]) is likely due to improved hepatic glucose disposal and insulin sensitivity; this may result from a direct effect on the hepatic glucose metabolism, as GCKR closely interacts with GCK in the hepatocytes, depending on glucose concentrations, to regulate the amount and activity of GCK and, consequently, the hepatic glucose disposal (13).

Whatever the mechanism of action of *GCKR* genetic variation, it leads to a lower risk of fasting hyperglycemia and type 2 diabetes in this study. Both *GCKR*-P446L and *GCK* −30G/A SNPs strongly modulate FPG, with significant additive effects (they lower FPG by 0.20 mmol/l in the general population and decrease hyperglycemia risk), supporting the hypothesis that these frequent variants may

have a non-negligible impact on human health. Indeed, there is strong evidence that even small changes in FPG, well below the IFG threshold of 6.1 mmol/l, may be associated with risk of cardiovascular morbidity and mortality (14–16). In this context, the *GCK* (−30A) allele was previously shown to be associated with type 2 diabetes and increased risk for coronary heart diseases in both diabetic and nondiabetic samples (17).

It is tempting to propose that the P446L variant replacing a residue conserved between mammals might be the causative variant. However, one previous study having investigated the sensitivity of the GCKR-L446 mutant to fructose 6- and 1-phosphate, using rat GCKR, did not report any effect compared with the wild-type protein (7). Differential regulatory features between human and rat GCKR (18) could have masked a functional consequence of the L446 variant, or a more subtle, distinct defect acting on either GCK or another target protein remains to be discovered. Alternatively, other SNPs may be etiological, as the common P446L variant lies in a large haplotype block covering at least 400 Kb of genomic sequence (www.hapmap.org).

In conclusion, our study in the DESIR prospective cohort shows that carriers of the *GCKR*-L446 variant have lower fasting glycemia and insulin resistance and are protected against the development of diabetes despite higher TG levels and a risk of dyslipidemia. This suggests, for the first time, a molecular mechanism by which these two components of the so-called metabolic syndrome can be dissociated. Based on rodent models, such as the adenoviral-mediated hepatic overexpression of GCK or GCKR in mice with diet-induced diabetes (5,19), more active GCKR may result in improved interaction with GCK, leading to more efficiently releasable pools of GCK enzyme, with subsequent beneficial effects on glucose metabolism but otherwise with a concomitant alteration of lipid profile.

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