A Common Gene Variant in Glucokinase Regulatory Protein Interacts With Glucose Metabolism on Diabetic Dyslipidemia: the Combined CODAM and Hoorn Studies

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OBJECTIVE

Small molecules that disrupt the binding between glucokinase and glucokinase regulatory protein (GKRP) are potential new glucose-lowering targets. They stimulate hepatic glucose disposal by increasing glucokinase activity in the liver. It can, however, be anticipated that increased hepatic glucokinase activity might be accompanied by the development of hypertriglyceridemia, particularly in type 2 diabetes. We examined whether the strength of association between rs1260326, a common, functional gene variant in GKRP, and plasma lipids is affected by glucose metabolism.

RESEARCH DESIGN AND METHODS

rs1260326 was genotyped in subjects with normal glucose metabolism (*n* = 497), subjects with impaired glucose metabolism (*n* = 256), and patients with type 2 diabetes (*n* = 351) in the combined Hoorn and Cohort on Diabetes and Atherosclerosis Maastricht (CODAM) studies.

RESULTS

The strength of association between the rs1260326 minor T allele and plasma triglycerides increased from normal glucose metabolism to impaired glucose metabolism to type 2 diabetes (*P* for interaction = 0.002). The inverse relation between rs1260326 and plasma HDL cholesterol was again most prominent in type 2 diabetes (*P* for interaction = 0.004). Similar trends were observed when the Hoorn and CODAM cohorts were analyzed separately. Comparable results were obtained when glucose metabolism strata were replaced by continuous indices of glucose metabolism, i.e., HbA_{1c} and fasting plasma glucose.

CONCLUSIONS

These findings illustrate that common gene variants, such as rs1260326, can have substantial effect sizes when they are studied in specific populations, such as type 2 diabetes. Moreover, our results shed light on potential side effects of small molecule disruptors of the GKRP-glucokinase complex, especially when glucose control is suboptimal.

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Glucokinase is the principal, rate-limiting enzyme in glycolysis that phosphorylates glucose in the pancreas and liver. It is therefore an important regulator of insulin secretion and hepatic glucose disposal, respectively (1). Loss-of-function mutations in the glucokinase gene result in maturity-onset diabetes of the young type 2 (2,3). Given its major role in glucose metabolism, efforts have been undertaken to develop glucokinase activators as a potential new class of glucose-lowering drugs (4). Clinical studies have demonstrated that these systemic activators are capable of lowering plasma glucose levels, albeit at the expense of hypoglycemia (5,6). This undesired side effect could be circumvented by organ-specific glucokinase activators, which only act in the liver, not in the pancreas (7).

Lloyd et al. (8) recently reported a different approach to stimulate hepatic glucokinase activity only. They developed a small molecule that disrupts the binding between glucokinase and glucokinase regulatory protein (GKRP), a liver-specific protein that binds and inactivates glucokinase. Disruption of the GKRP-glucokinase complex facilitates migration of glucokinase to the cytosolic space where glycolysis and consequently hepatic glucose disposal are stimulated (1). Mice treated with these small molecules indeed displayed lower plasma glucose levels, without hypoglycemia (8).

It can, however, be anticipated that an increased hepatic glucose disposal will affect intrahepatic glucose metabolism, such as stimulation of glycogenesis as well as de novo lipogenesis. This may accelerate the development of nonalcoholic fatty liver disease and hypertriglyceridemia (9). Clinical studies with small-molecule GKRP-glucokinase disruptors are therefore eagerly awaited.

Meanwhile, lessons can be learned from humans carrying gene variants that are known to encode GKRP (*GCKR*) that binds glucokinase less effectively (10). We recently reviewed the metabolic effects of such common, functional variants in *GCKR* and showed that the rs780094 and rs1260326 minor alleles are associated with not only decreased plasma glucose levels but also increased de novo lipogenesis, nonalcoholic fatty liver disease, and plasma triglycerides (9). Notably, many of these studies have been carried out in individuals with normal glucose metabolism. Based on enzyme kinetics and expression, it can be anticipated that the metabolic consequences of common variants in *GCKR* are different in patients with type 2 diabetes, although the exact effects may be difficult to predict. Glucokinase has a relatively low affinity for glucose ($S_{0.5} \approx 7.5$ mmol/L) (11), implicating that it is more active when plasma glucose levels are within the diabetic range (12). This would suggest that the effect size of the *GCKR* minor allele variant on plasma triglycerides is more pronounced in patients with type 2 diabetes compared with individuals without type 2 diabetes. On the other hand, glucokinase expression has been shown to be reduced in liver biopsies derived from humans with type 2 diabetes (13).

The aim of the current study was therefore to examine whether the association between *GCKR* and plasma lipids differs between individuals with normal glucose metabolism, impaired glucose metabolism, and type 2 diabetes.

RESEARCH DESIGN AND METHODS

Study Population

Study participants were derived from the Hoorn follow-up cohort and the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM). In brief, Hoorn and CODAM are prospective cohorts designed to study determinants and (cardiovascular) complications of type 2 diabetes. The original Hoorn study was conducted in a random sample (n = 2,484) of the general population of Hoorn, the Netherlands, between 1989 and 1992 (14). In 2000, a follow-up study was performed in a subset of participants with normal and impaired glucose metabolism and type 2 diabetes (n = 836 in total) (15,16). The CODAM study was carried out in individuals with elevated risk for type 2 diabetes (n = 574) from the Dutch Monitoring Project for Cardiovascular Diseases (MORGEN) between 1999 and 2000 (17–19).

Since comparable research protocols have been used in the Hoorn and CODAM studies, both cohorts were merged into one larger cohort in this and previous studies (20–23). The current study consisted of 1,286 individuals who were available for genotyping. The Hoorn and CODAM studies did differ in terms of lipid-modifying medication use, i.e., participants of the CODAM study were asked to stop lipid-modifying medication 2 weeks prior to their visit (of whom 40 did not stop), whereas subjects in the Hoorn study were allowed to continue their lipid-modifying medication. Since plasma lipids were the main outcome variable of this study, all individuals from the Hoorn and CODAM studies taking lipid-modifying medication at time of visit were excluded from analyses (n = 153 in total).

Written informed consent was given by all participants. Approval for the Hoorn and CODAM studies was provided by the Human Investigations Review Committee of VU University Medical Center and Maastricht University Medical Center, respectively.

Measurements

Height was determined standing upright against a stadiometer and weight was measured using an electronic weight scale without shoes. BMI was calculated as weight divided by height squared. Waist circumference was ascertained with a measuring tape at the level of umbilicus. Blood samples were drawn after an overnight fast for measurement of glycated hemoglobin (HbA_{1c}), plasma glucose, insulin, total cholesterol, HDL cholesterol, and triglycerides, as described elsewhere (24,25). LDL cholesterol was calculated by the Friedewald formula. The degree of insulin resistance (HOMA2-IR) was estimated with the HOMA2 calculator (http://www.dtu.ox.ac.uk). Participants underwent a standard 75-g oral glucose tolerance test and were subsequently divided into three groups; normal glucose metabolism, impaired glucose metabolism (which comprised impaired fasting glycemia and impaired glucose tolerance), and type 2 diabetes, according to the World Health Organization criteria (26). Of the 1,286 individuals who were genotyped, an oral glucose tolerance test was not performed in 11 participants; they were therefore excluded from analyses.

Genotyping

Genotyping of rs1260326, which has been shown to be a functional variant that is in strong linkage disequilibrium with rs780094 (10,27), was done in the CODAM study as part of a genome-wide association study array, with the use of a HumanOmniExpress BeadChip assay (Illumina, San Diego, CA). In the Hoorn

study, a validated Invitrogen TaqMan assay was used (Thermo Fisher Scientific, Waltham, MA). Genotyping was successful in 98% (695 of 712) and 99% (573 of 574) of the Hoorn and CODAM samples, respectively. The minor allele frequency (T allele) was 0.38 in the overall population (0.38, 0.39, and 0.39 in the normal glucose metabolism, impaired glucose metabolism, and type 2 diabetes subgroups, respectively). Genotype distribution was in Hardy-Weinberg equilibrium ($P = 0.76$; χ^2 test).

Statistics

Data are presented as mean ± SD or as median (interquartile range) in case of nonnormal distribution. Nonnormally distributed variables were log transformed before further analyses. Differences in characteristics between subgroups (i.e., normal glucose metabolism, impaired glucose metabolism, and type 2 diabetes) were analyzed using linear or logistic regression, with adjustments for age, sex, and cohort (i.e., the Hoorn and CODAM studies). Associations between the rs1260326 minor T allele and plasma lipid levels were analyzed using linear regression, with adjustments for age, sex, and cohort, and stratified by glucose metabolism subgroups. An additive mode of inheritance was assumed, based on previous reports (28). To investigate whether the strength of the association between the rs1260326 minor T allele and plasma lipids differed between the three subgroups of glucose metabolism, interaction terms (i.e., rs1260326 * glucose metabolism subgroups) were used. Interactions were considered statistically significant at $P < 0.10$. The remaining results were considered statistically significant at $P < 0.05$. All statistical analyses were carried out by the IBM Statistical Package of Social Sciences (SPSS) version 22 for Windows (SPSS Inc., Chicago, IL).

RESULTS

Characteristics of Overall Study Population and Glucose Metabolism Subgroups

General characteristics of the overall study population, as well as stratified by levels of glucose metabolism, are displayed in Table 1. After adjustment for age, sex, and cohort, individuals with impaired glucose metabolism and type 2 diabetes displayed higher levels of HbA_{1c}, fasting plasma glucose, insulin, and triglycerides, HOMA2-IR, BMI, waist circumference, systolic and diastolic blood pressure, and use of blood pressure–lowering medication when compared with individuals with normal glucose metabolism. In contrast, plasma HDL cholesterol concentrations decreased as glucose tolerance deteriorated.

Association Between the rs1260326 Minor T Allele and Plasma Lipid Levels in Glucose Metabolism Subgroups

In the overall population, the rs1260326 minor T allele was significantly associated with plasma triglycerides but not with plasma total cholesterol, LDL cholesterol, and HDL cholesterol levels (Table 2). There was no gene-age or gene-sex interaction on these plasma lipid levels (data not shown). In contrast, stratification by glucose metabolism stage clearly demonstrated that the strength of association between the rs1260326 minor T allele and plasma triglycerides increased from normal glucose metabolism to impaired glucose metabolism to type 2 diabetes (unstandardized β: 0.006, 0.018, and 0.067, respectively; P for interaction = 0.002) (Table 2). Furthermore, it also showed a significant, inverse association of the minor T allele with plasma HDL cholesterol in the type 2 diabetes subgroup as well as a significant interaction (P for interaction = 0.004) (Table 2). Additional adjustment for waist circumference (or BMI) and plasma insulin (or HOMA2-IR) did not essentially change these outcomes (data not shown). An interaction was not observed when fasting plasma glucose levels were used as an outcome parameter ($P = 0.45$).

Table 1—Characteristics of the overall population and stratified by glucose metabolism stage

	Overall (n = 1,104)	Normal glucose metabolism (n = 497)	Impaired glucose metabolism (n = 256)	Type 2 diabetes (n = 351)
Male/female, n	611/493	271/226	144/112	196/155
Age (years)	64 ± 9	63 ± 9	66 ± 8*	65 ± 8
BMI (kg/m^2)	28.1 ± 4.3	26.9 ± 3.7	28.2 ± 4.3*	29.6 ± 4.6*†
Waist circumference (cm)	98 ± 12	94 ± 11	99 ± 11*	103 ± 12*†
Fasting glucose (mmol/L)	5.8 (5.3–6.7)	5.3 (5.1–5.6)	6.1 (5.6–6.4)*	7.3 (6.7–8.3)*†
HbA_{1c} (%)	6.0 ± 0.8	5.7 ± 0.4	5.8 ± 0.4*	6.7 ± 1.0*†
HbA_{1c} (mmol/mol)	42 ± 8.5	38 ± 4.6	40 ± 4.4*	49 ± 10.6*†
Insulin (pmol/L)	65 (45–99)	53 (39–74)	69 (49–99)*	90 (62–131)*†
HOMA2-IR	1.3 (0.9–1.9)	1.0 (0.7–1.4)	1.3 (1.0–1.9)*	1.8 (1.3–2.7)*†
Glucose-lowering medication (% yes)	10	0	0.8	31*†
Total cholesterol (mmol/L)	5.5 ± 1.0	5.5 ± 1.0	5.6 ± 1.0	5.5 ± 1.1†
LDL cholesterol (mmol/L)	3.5 ± 0.9	3.5 ± 0.9	3.6 ± 0.9	3.4 ± 0.9*†
HDL cholesterol (mmol/L)	1.3 ± 0.4	1.4 ± 0.4	1.3 ± 0.4*	1.2 ± 0.3*†
Triglycerides (mmol/L)	1.4 (1.0–1.9)	1.2 (0.9–1.6)	1.4 (1.0–2.0)*	1.6 (1.2–2.2)*†
Systolic blood pressure (mmHg)	142 ± 20	137 ± 19	144 ± 19*	147 ± 20*†
Diastolic blood pressure (mmHg)	83 ± 10	81 ± 10	83 ± 11*	85 ± 10*
Antihypertensive medication (% yes)	35	24	36*	50*†
Smoking (% yes)	18	19	18	16

Data are expressed as mean ± SD or as median (interquartile range), unless otherwise stated. *$P < 0.05$ vs. normal glucose metabolism. †$P < 0.05$ vs. impaired glucose metabolism. Analyzed with linear and logistic regression with adjustment for age, sex, and cohort (Hoorn/CODAM studies).

Table 2—Associations between rs1260326 and plasma lipids in the overall population and stratified by glucose metabolism stage

Dependent variables	Overall (*n* = 1,104)		Normal glucose metabolism (*n* = 497)		Impaired glucose metabolism (*n* = 256)		Type 2 diabetes (*n* = 351)		*P* for interaction#
	β	95% CI	β	95% CI	β	95% CI	β	95% CI	
Total cholesterol	0.032	−0.052; 0.117	0.045	−0.073; 0.164	−0.136	−0.300; 0.029	0.154	−0.016; 0.323	0.3
LDL cholesterol	0.000	−0.075; 0.076	0.024	−0.088; 0.136	−0.189	−0.342; −0.036	0.122	−0.015; 0.258	0.4
HDL cholesterol	−0.024	−0.054; 0.006	0.012	−0.033; 0.057	0.020	−0.043; 0.082	−0.096	−0.143; −0.050	0.004
Triglycerides (log)	0.030	0.012; 0.048	0.006	−0.017; 0.029	0.018	−0.016; 0.053	0.067	0.032; 0.102	0.002

Analyzed with linear regression with inclusion of age, sex, cohort (Hoorn/CODAM), and rs1260326 as independent variables and plasma lipids as dependent variables. #Interaction between rs1260326 and glucose metabolism stage.

Of note, almost similar results, albeit less significant, were observed when these analyses were repeated in the Hoorn and CODAM studies separately (*P* for interaction on plasma triglycerides = 0.017 and 0.167, respectively; *P* for interaction on plasma HDL cholesterol = 0.042 and 0.026, respectively). Furthermore, sensitivity analyses to evaluate the impact of exclusion of individuals taking lipid-modifying medication (*n* = 153) yielded comparable outcomes (*P* for interaction = 0.013 and = 0.005 for plasma triglycerides and HDL cholesterol, respectively; *n* = 1257).

Association Between Plasma Triglycerides and HDL Cholesterol and Continuous Metabolic Traits Stratified by rs1260326

Replacement of glucose metabolism strata by continuous indices of glucose metabolism, i.e., fasting plasma glucose and HbA_{1c} levels, also yielded significant interactions with the rs1260326 minor T allele on plasma triglycerides (*P* for interaction = 0.002 and 0.01, respectively) (Table 3 and Fig. 1*A*). In contrast, we did not observe a statistically significant interaction between other metabolic parameters (BMI, waist circumference, plasma insulin, and HOMA2-IR) and rs1260326 on plasma triglycerides, although the strength of association between the anthropometric variables and plasma triglycerides increased from CC to TT carriers (*P* for interaction of 0.13 for both waist circumference and BMI) (Table 3). Comparable results were observed for plasma HDL cholesterol levels (Table 3 and Fig. 1*B*).

CONCLUSIONS

The current study extends previous findings on the relation between *GCKR* and plasma lipids by demonstrating that rs1260326 interacts with indices of glucose metabolism, i.e., glucose tolerance state, fasting plasma glucose, and HbA_{1c} levels, on plasma triglycerides and HDL cholesterol, two prominent features of diabetic dyslipidemia (29). Patients with moderately controlled type 2 diabetes (HbA_{1c} 8.0% [64 mmol/mol]) carrying two T alleles displayed higher plasma triglycerides levels than homozygous carriers of the C allele (2.2 vs. 1.6 mmol/L), whereas no differences were observed in healthy individuals (as illustrated in Fig. 1*A*).

Although such an interaction has not been reported before, there are studies that corroborate our findings. In a meta-analysis, Orho-Melander et al. (27) convincingly demonstrated that the minor T allele of rs780094, which is in strong linkage disequilibrium with rs1260326, is associated with plasma triglycerides. Of interest, the authors noted that the difference in plasma triglycerides between homozygous carriers of the C and T allele was substantially greater in a cohort that consisted of patients with type 2 diabetes only, i.e., the Skania Diabetes 2000 Registry (delta triglycerides: 0.54 mmol/L), in comparison with the general population, e.g., the Malmö Preventive Project (delta triglycerides: 0.11 mmol/L) (30). In addition, Cole et al. (31) recently showed that rs1260326 interacts with BMI on plasma lipids. The current study failed to replicate this observation, but it should be noted that the *P* value for interaction was 0.13. The fact that indices of glucose metabolism displayed a more significant interaction with rs1260326 strongly suggests that glucose metabolism is more closely linked to the pathophysiological pathway underlying these interactions than obesity per se.

Although this pathway has not been elucidated, previous stable isotope studies suggest a prominent role for hepatic de novo lipogenesis from glucose. First, Adiels et al. (32) showed that the production of triglyceride-rich VLDL particles is associated with plasma glucose levels, which is in line with the currently observed associations between indices of glucose metabolism and plasma triglycerides. Furthermore, as glucose tolerance deteriorates, de novo lipogenesis becomes a more important source for VLDL production (33). In theory, increased metabolic flux, as a consequence of decreased binding of GKRP to glucokinase, would further drive de novo lipogenesis by providing glucose-6-phosphate to be converted to fatty acids. In statistical terms, this biological mechanism would be reflected by an interaction between indices of glucose metabolism and rs1260326. Indeed, individuals carrying the rs1260326 minor T allele are characterized by increased de novo lipogenesis when compared with homozygous carriers of the C allele (34). Finally, the negative interaction between the rs1260326 minor T allele and indices of glucose metabolism with plasma HDL cholesterol is most likely accounted for by the well-documented negative association between plasma triglycerides and HDL cholesterol levels as a consequence of cholesteryl ester transfer protein (CETP)–mediated exchange of cholesteryl esters and triglycerides between VLDL and HDL particles (29).

The outcomes of this study have several clinically relevant consequences. First, although it is a well-accepted rule of thumb that gene variants with a high minor allele frequency, such as rs1260326, generally have small effect sizes (35), the current study clearly demonstrates that common gene variants can have substantial effect sizes

when they are studied in specific populations, such as in type 2 diabetes. In fact, the difference in plasma triglycerides between those with type 2 diabetes that are homozygous carriers of the C and T allele was identical to the therapeutic effects of fenofibrate in patients with diabetic dyslipidemia in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) study (36). These observations also raise the possibility of undesired side effects of small molecule disruptors of the GKRP-glucokinase complex. Previous genetic studies have shown to be instrumental in forecasting the clinical effects of newly developed drugs. One illustrative example are gene variants in proprotein subtilisin kexin type 9 (PCSK9), which have correctly predicted the beneficial LDL cholesterol–lowering and cardiovascular disease–reducing effects of PCSK9 antagonists (37,38). Our study suggests that the glucose-lowering effects of small molecule disruptors of the GKRP-glucokinase complex will be accompanied by aggravation of dyslipidemia, in particular when glucose regulation is suboptimal. This will seriously limit their applicability in type 2 diabetes treatment and therefore warrants special attention when this new group of drugs is introduced to human subjects in phase I and II studies.

Although the results from this study deserve replication, we believe that the observed interactions are unlikely the consequence of a type I statistical error. First, similar interactions between rs1260326 and glucose metabolism stage on plasma triglycerides and HDL cholesterol were found when the Hoorn and CODAM cohorts were analyzed separately. Furthermore, the hitherto-mentioned, previous genetic reports on *GCKR* and the biological plausibility further support the validity of the current observations.

In conclusion, we demonstrated that the strength of association between a common gene variant in *GCKR* with plasma triglycerides and HDL cholesterol is stronger in patients with type 2 diabetes than healthy control subjects. These findings illustrate the added value of studying the effect sizes of common gene variants in selected study populations, such as type 2 diabetes, and question the applicability of small molecule

Table 3—Associations between metabolic variables and (log) plasma triglycerides and HDL cholesterol in the overall population and stratified by rs1260326

	Overall (n = 1,104)		CC (n = 420)		CT (n = 518)		TT (n = 166)		
Independent variables	β	95% CI	β	95% CI	β	95% CI	β	95% CI	P for interaction§
Plasma triglycerides									
BMI	0.013	0.010; 0.016	0.009	0.005; 0.013	0.016	0.012; 0.020	0.013	0.006; 0.021	0.13
Waist circumference	0.006	0.005; 0.007	0.004	0.003; 0.006	0.006	0.005; 0.008	0.007	0.004; 0.010	0.13
Fasting plasma glucose (log)	0.693	0.560; 0.826	0.452	0.243; 0.660	0.784	0.579; 0.989	0.984	0.672; 1.296	0.002
HbA_{1c}	0.060	0.045; 0.076	0.037	0.013; 0.061	0.066	0.041; 0.090	0.085	0.052; 0.118	0.01
Plasma insulin (log)	0.338	0.295; 0.381	0.297	0.227; 0.368	0.364	0.301; 0.426	0.345	0.231; 0.458	0.40
HOMA2-IR	0.061	0.052; 0.071	0.063	0.045; 0.081	0.062	0.049; 0.075	0.053	0.031; 0.076	0.45
Plasma HDL cholesterol									
BMI	−0.022	−0.026; −0.017	−0.021	−0.029; −0.013	−0.024	−0.031; −0.018	−0.016	−0.029; −0.004	0.70
Waist circumference	−0.009	−0.011; −0.008	−0.010	−0.013; −0.007	−0.009	−0.012; −0.007	−0.008	−0.013; −0.004	0.30
Fasting plasma glucose (log)	−0.922	−1.157; −0.688	−0.460	−0.846; −0.074	−1.209	−1.556; −0.863	−1.248	−1.808; −0.688	0.007
HbA_{1c}	−0.093	−0.119; −0.066	−0.053	−0.097; −0.008	−0.114	−0.156; −0.073	−0.124	−0.182; −0.067	0.04
Plasma insulin (log)	−0.484	−0.561; −0.407	−0.478	−0.608; −0.348	−0.495	−0.605; −0.385	−0.459	−0.661; −0.258	0.84
HOMA2-IR	−0.089	−0.106; −0.072	−0.106	−0.139; −0.072	−0.083	−0.106; −0.061	−0.081	−0.120; −0.042	0.28

Analyzed with linear regression with inclusion of age, sex, cohort (Hoorn/CODAM), and metabolic parameter as independent variables and (log) plasma triglycerides/HDL cholesterol as dependent variable. §Interaction between rs1260326 and metabolic parameter.

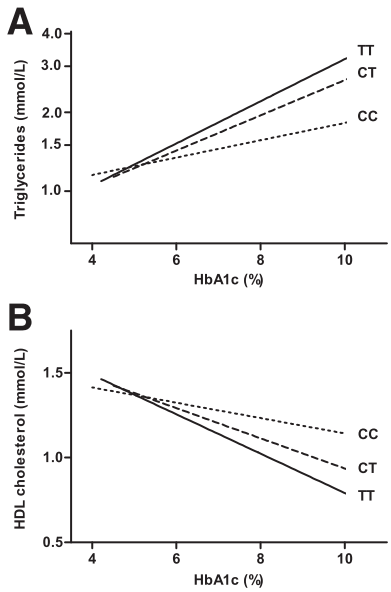

Figure 1—Association between plasma HbA_{1c} and plasma triglycerides (*A*) and HDL cholesterol levels (*B*), stratified by rs1260326. Plasma triglycerides are presented on a log scale.

disruptors of the GKRP-glucokinase complex in type 2 diabetes.

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References

1. Hale C, Lloyd DJ, Pellacani A, Véniant MM. Molecular targeting of the GK-GKRP pathway in diabetes. Expert Opin Ther Targets 2015;19:129–139
2. Froguel P, Vaxillaire M, Sun F, et al. Close linkage of glucokinase locus on chromosome 7p to early-onset non-insulin-dependent diabetes mellitus. Nature 1992;356:162–164
3. Hattersley AT, Turner RC, Permutt MA, et al. Linkage of type 2 diabetes to the glucokinase gene. Lancet 1992;339:1307–1310
4. Grimsby J, Sarabu R, Corbett WL, et al. Allosteric activators of glucokinase: potential role in diabetes therapy. Science 2003;301:370–373
5. Meininger GE, Scott R, Alba M, et al. Effects of MK-0941, a novel glucokinase activator, on glycemic control in insulin-treated patients with type 2 diabetes. Diabetes Care 2011;34:2560–2566
6. Wilding JP, Leonsson-Zachrisson M, Wessman C, Johnsson E. Dose-ranging study with the glucokinase activator AZD1656 in patients with type 2 diabetes mellitus on metformin. Diabetes Obes Metab 2013;15:750–759
7. Pfefferkorn JA, Guzman-Perez A, Litchfield J, et al. Discovery of (S)-6-(3-cyclopentyl-2-(4-(trifluoromethyl)-^{1}H-imidazol-1-yl)propanamido) nicotinic acid as a hepatoselective glucokinase activator clinical candidate for treating type 2 diabetes mellitus. J Med Chem 2012;55:1318–1333
8. Lloyd DJ, St Jean DJ Jr, Kurzeja RJ, et al. Antidiabetic effects of glucokinase regulatory protein small-molecule disruptors. Nature 2013;504:437–440
9. Brouwers MC, Jacobs C, Bast A, Stehouwer CD, Schaper NC. Modulation of glucokinase regulatory protein: a double-edged sword? Trends Mol Med 2015;21:583–594
10. Beer NL, Tribble ND, McCulloch LJ, et al. The P446L variant in GCKR associated with fasting plasma glucose and triglyceride levels exerts its effect through increased glucokinase activity in liver. Hum Mol Genet 2009;18:4081–4088
11. Agius L. Glucokinase and molecular aspects of liver glycogen metabolism. Biochem J 2008;414:1–18
12. Liu S, Ammirati MJ, Song X, et al. Insights into mechanism of glucokinase activation: observation of multiple distinct protein conformations. J Biol Chem 2012;287:13598–13610
13. Haeusler RA, Camastra S, Astiarraga B, Nannipieri M, Anselmino M, Ferrannini E. Decreased expression of hepatic glucokinase in type 2 diabetes. Mol Metab 2014;4:222–226
14. Mooy JM, Grootenhuis PA, de Vries H, et al. Prevalence and determinants of glucose intolerance in a Dutch caucasian population. The Hoorn study. Diabetes Care 1995;18:1270–1273
15. Henry RM, Kostense PJ, Spijkerman AM, et al.; Hoorn Study. Arterial stiffness increases with deteriorating glucose tolerance status: the Hoorn study. Circulation 2003;107:2089–2095
16. Spijkerman AM, Adriaanse MC, Dekker JM, et al. Diabetic patients detected by population-based stepwise screening already have a diabetic cardiovascular risk profile. Diabetes Care 2002;25:1784–1789
17. Kershaw KN, Droomers M, Robinson WR, Carnethon MR, Daviglus ML, Monique Verschuren WM. Quantifying the contributions of behavioral and biological risk factors to socioeconomic disparities in coronary heart disease incidence: the MORGEN study. Eur J Epidemiol 2013;28:807–814
18. van den Berg SW, Jansen EH, Kruijshoop M, et al. Paraoxonase 1 phenotype distribution and activity differs in subjects with newly diagnosed type 2 diabetes (the CODAM study). Diabet Med 2008;25:186–193
19. Kruijshoop M, Feskens EJ, Blaak EE, de Bruin TW. Validation of capillary glucose measurements to detect glucose intolerance or type 2 diabetes mellitus in the general population. Clin Chim Acta 2004;341:33–40
20. van Moorsel D, van Greevenbroek MM, Schaper NC, et al. BclI glucocorticoid receptor polymorphism in relation to cardiovascular variables: the Hoorn and CODAM studies. Eur J Endocrinol 2015;173:455–464
21. Engelen L, Ferreira I, Gaens KH, et al. The association between the -374T/A polymorphism of the receptor for advanced glycation endproducts gene and blood pressure and arterial stiffness is modified by glucose metabolism status: the Hoorn and CoDAM studies. J Hypertens 2010;28:285–293
22. Geelen CC, van Greevenbroek MM, van Rossum EF, et al. BclI glucocorticoid receptor polymorphism is associated with greater body fatness: the Hoorn and CODAM studies. J Clin Endocrinol Metab 2013;98:E595–E599
23. Engelen L, Ferreira I, Brouwers O, et al. Polymorphisms in glyoxalase 1 gene are not associated with vascular complications: the Hoorn and CoDAM studies. J Hypertens 2009;27:1399–1403
24. van Woudenbergh GJ, Theofylaktopoulou D, Kuijsten A, et al. Adapted dietary inflammatory index and its association with a summary score for low-grade inflammation and markers of glucose metabolism: the Cohort study on Diabetes and Atherosclerosis Maastricht (CODAM) and the Hoorn study. Am J Clin Nutr 2013;98:1533–1542
25. Jacobs M, van Greevenbroek MM, van der Kallen CJ, et al. Low-grade inflammation can partly explain the association between the metabolic syndrome and either coronary artery disease or severity of peripheral arterial disease: the CODAM study. Eur J Clin Invest 2009;39:437–444
26. World Health Organization. Part 1: diagnosis and classification of diabetes mellitus. In *Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. Report of a WHO Consultation*. Geneva, World Health Org., 1999
27. Orho-Melander M, Melander O, Guiducci C, et al. Common missense variant in the glucokinase regulatory protein gene is associated with increased plasma triglyceride and C-reactive protein but lower fasting glucose concentrations. Diabetes 2008;57:3112–3121
28. Vaxillaire M, Cavalcanti-Proença C, Dechaume A, et al.; DESIR Study Group. 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. Diabetes 2008;57:2253–2257
29. Taskinen MR. Diabetic dyslipidaemia: from basic research to clinical practice. Diabetologia 2003;46:733–749
30. Lindholm E, Agardh E, Tuomi T, Groop L, Agardh CD. Classifying diabetes according to the new WHO clinical stages. Eur J Epidemiol 2001;17:983–989
31. Cole CB, Nikpay M, Lau P, et al. Adiposity significantly modifies genetic risk for dyslipidemia. J Lipid Res 2014;55:2416–2422

32. Adiels M, Borén J, Caslake MJ, et al. Overproduction of VLDL1 driven by hyperglycemia is a dominant feature of diabetic dyslipidemia. Arterioscler Thromb Vasc Biol 2005;25:1697–1703
33. Vedala A, Wang W, Neese RA, Christiansen MP, Hellerstein MK. Delayed secretory pathway contributions to VLDL-triglycerides from plasma NEFA, diet, and de novo lipogenesis in humans. J Lipid Res 2006;47:2562–2574
34. Santoro N, Caprio S, Pierpont B, Van Name M, Savoye M, Parks EJ. Hepatic de novo lipogenesis in obese youth is modulated by a common variant in the GCKR gene. J Clin Endocrinol Metab 2015;100:E1125–E1132
35. Manolio TA, Collins FS, Cox NJ, et al. Finding the missing heritability of complex diseases. Nature 2009;461:747–753
36. Ginsberg HN, Elam MB, Lovato LC, et al.; ACCORD Study Group. Effects of combination lipid therapy in type 2 diabetes mellitus. N Engl J Med 2010;362:1563–1574
37. Cohen JC, Boerwinkle E, Mosley TH Jr, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. N Engl J Med 2006;354:1264–1272
38. Robinson JG, Farnier M, Krempf M, et al.; ODYSSEY LONG TERM Investigators. Efficacy and safety of alirocumab in reducing lipids and cardiovascular events. N Engl J Med 2015;372:1489–1499