Original Article
Polymorphisms in the *GCKR* are associated with serum lipid traits, the risk of coronary artery disease and ischemic stroke

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Abstract: The present study was to determine the association of two single nucleotide polymorphisms (SNPs) in the glucokinase regulator gene (*GCKR*) and serum lipid levels, and the risk of coronary artery disease (CAD) and ischemic stroke (IS). Genotypes of the *GCKR* rs1260326 and rs8179206 in 1736 unrelated subjects (CAD, 584; IS, 555; and healthy controls; 597) were determined by the Snapshot technology platform. The genotypic and allelic frequencies of rs1260326 and rs8179206 were not different among the three groups ($P > 0.05$). The subjects with rs1260326TT genotype had higher serum low-density lipoprotein cholesterol (LDL-C) levels in controls, and higher triglyceride (TG) levels in CAD patients than the subjects with CC and CT genotypes after adjustment for age, sex, body mass index, blood pressure, alcohol consumption, and cigarette smoking ($P < 0.05$). The rs1260326TT genotype was also associated with decreased risk of IS in females (OR = 0.37, 95% CI: 0.18-0.76, $P = 0.007$). The present study shows that the *GCKR* rs1260326TT genotype is associated with high LDL-C in controls, high TG levels in CAD patients, and a decreased risk of IS in females.

Keywords: Glucokinase regulator, single nucleotide polymorphism, lipids, coronary artery disease, ischemic stroke

Introduction

Both coronary artery disease (CAD) and ischemic stroke (IS) are the leading causes of morbidity and death in the developed countries, and are also the major cause of long-term disability in survivors [1, 2]. Atherogenic dyslipidemia characterized by low levels of high-density lipoprotein cholesterol (HDL-C) and apolipoprotein (Apo) A1, high levels of total cholesterol (TC), triglyceride (TG) and low-density lipoprotein (LDL) particle number is highly associated with increased incidence of the cardiovascular disease [3] and IS [4, 5]. In addition, genetic factors are estimated to account for about 50-80% of the variation in serum lipid levels [6], and 30-60% of the incidence of CAD and IS [7]. Therefore, single nucleotide polymorphisms (SNPs) in the lipid-related genes may have some associations with serum lipid levels, and the risk of CAD and IS [8].

Glucokinase regulator gene (*GCKR*) located on chromosome 2p23, consists of 19 exons and encodes for the glucokinase regulator protein [9]. It regulates the glucokinase's (GCK) activity in the hepatocytes and pancreatic beta cells [10], thus may play a pivotal role in glucose homeostasis [11]. Animal studies indicated that *GCKR* knockout mice have decreased GCK activity, and leading to elevated glucose levels. Adenoviral mediated over-expression of *GCKR* in murine liver increases GCK activity and lowered fasting blood glucose as well as increased TG levels [11-14]. Molecular mechanism showed *GCKR* rs1260326 in central pathways regulating both hepatic TG and glucose metabolism in humans [14].

Recently, a genome-wide association study (GWAS) identified *GCKR* as a potential locus for modulating TG [15]. Subsequent population-based studies and meta-analysis and fine-mapping studies, confirmed that the minor alleles of rs780094 and rs1260326 (Pro446Leu) were associated with higher levels of TG and lower fasting glucose [14, 16-21]. Consistent with this

Table 1. Baseline characteristics of the participants

Characteristic	Control (n = 597)	Case		$P_{vs.controls}$	
		CAD (n = 584)	IS (n = 555)	CAD	IS
Male/female	434/163	428/156	405/150	0.861	0.991
Age, years	61.32±9.76	62.23±10.63	62.86±12.11	0.121	0.051
Body mass index, kg/m²	22.41±2.86	23.84±3.38	24.71±22.04	0.000	0.015
Systolic blood pressure, mmHg	130.60±20.01	133.03±23.33	147.72±22.15	0.059	0.000
Diastolic blood pressure, mmHg	82.94±13.37	79.18±14.21	83.78±12.97	0.000	0.284
Pulse pressure, mmHg	49.81±14.70	53.49±18.08	64.02±17.92	0.000	0.000
Cigarette smoking, n (%)	236 (39.5)	250(42.8)	233(42.0)	0.253	0.398
Alcohol consumption, n (%)	235 (39.4)	131(22.4)	157(28.3)	0.000	0.000
Total cholesterol, mmol/L	4.93±1.11	4.53±1.19	4.52±1.15	0.000	0.010
Triglyceride, mmol/L	1.42±1.87	1.66±1.11	1.67±1.36	0.007	0.000
HDL-C, mmol/L	1.90±0.50	1.14±0.34	1.23±0.40	0.000	0.000
LDL-C, mmol/L	2.74±0.80	2.71±1.00	2.68±0.90	0.550	0.245
Apolipoprotein (Apo) A1, g/L	1.41±0.28	1.04±0.52	1.02±0.22	0.000	0.000
ApoB, g/L	0.91±0.22	0.91±0.27	0.89±0.25	0.995	0.320
ApoA1/ApoB	1.63±0.49	1.36±2.45	1.26±0.60	0.013	0.000

CAD, coronary artery disease; IS, ischemic stroke; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

notion and the observed associations, the rs1260326 446Leu was recently reported *in vitro* to increase hepatic GCK activity. However, the associations between the SNPs in *GCKR* and serum lipid levels, especially the risk of CAD and IS are still needed to determine in different populations. Therefore, the aim of the present study was to detect the association of *GCKR* rs1260326 and rs8179206 SNPs and serum lipid levels and the risk of CAD and IS in the Guangxi Han population.

Materials and methods

Study population

A total of 584 patients with CAD and 555 patients with IS were recruited from hospitalized patients in the First Affiliated Hospital, Guangxi Medical University. All of the enrolled CAD patients were evaluated by coronary angiography due to suspected CAD or unrelated conditions requiring angiographic evaluation; the coronary angiograms were analyzed by two experienced interventional cardiologists. CAD was defined as significant coronary stenosis (≥ 50%) in at least one of the three main coronary arteries or their major branches (branch diameter ≥ 2 mm). Subjects with congenital heart disease and type I diabetes mellitus were excluded. All of the enrolled IS patients received

a strict neurological examination and brain magnetic resonance imaging. The diagnosis of IS was according to the International Classification of Diseases (9th Revision). Patients with a transient ischemic attack, embolic brain infarction, stroke caused by inflammatory disease, cardioembolic stroke, autoimmune disease, or serious chronic diseases were excluded from this study. Subjects with a past history of CAD were also excluded from the study [22]. A total of 597 healthy controls matched by age, gender, and geographical area were included. The controls were judged to be free of CAD and IS by questionnaires, medical history, and clinical examination. All individuals enrolled were from the Han population in Guangxi, China. A standard questionnaire was used to ascertain general information and medical history from all participants. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University. Informed consent was obtained from all subjects after receiving a full explanation of the study.

Genotyping and biochemical analysis

All of the biochemical assays and genotyping in CAD and IS patients were performed after hospitalization, and all of the venous blood samples were obtained from the patients and con-

Table 2. Genotype distribution and allele frequencies of rs1260326 and rs8179206 in cases and controls

Genotype or allele	Control [n (%)]	CAD [n (%)]	IS [n (%)]	CAD OR (95% CI)	CAD P	IS OR (95% CI)	IS P
rs1260326							
CC	209 (35.0)	198 (33.9)	197 (35.5)	1		1	
CT	279 (46.7)	265 (45.4)	251 (45.2)	1.12 (0.81-1.55)	0.485	1.04 (0.74-1.48)	0.815
TT	109 (18.3)	121 (20.7)	107 (19.3)	1.25 (0.82-1.91)	0.299	1.38 (0.89-2.15)	0.149
P		0.565	0.851				
C	697 (58.4)	661 (56.6)	645 (58.1)	1		1	
T	497 (41.6)	507 (43.4)	465 (41.8)	1.08 (0.91-1.27)	0.381	1.01 (0.86-1.19)	0.897
P		0.381	0.897				
HWE (P)	0.349	0.065	0.088				
rs8179206							
AA	581 (97.3)	564 (96.6)	533 (96.0)	1		1	
GA	16 (2.7)	20 (3.4)	21 (3.8)	1.46 (0.53-3.99)	0.462	1.73 (0.64-4.70)	0.281
GG	0	0	1 (0.2)			0.091 (0.00-)	1.000
P		0.457	0.330				
A	1178 (98.7)	1148 (98.2)	1087 (97.9)	1		1	
G	16 (1.3)	20 (1.8)	23 (2.1)	1.28 (0.66-2.49)	0.461	1.56 (0.82-2.96)	0.177
P		0.372	0.173				
HWE (P)	0.740	0.673	0.111				

trols after at least 12 h of fasting. Genomic DNA was isolated from peripheral blood leukocytes using the phenol-chloroform method. We selected the SNPs across the region of the *GCKR* from NCBI dbSNP Build 132 (http://www.Ncbi.nlm.nih.gov/SNP/). Genotyping of the rs1260326 and rs8179206 SNPs was performed by the Snapshot technology platform. The levels of serum TC, TG, HDL-C, and LDL-C in the samples were determined by enzymatic methods with commercially available kits. Serum ApoA1 and ApoB levels were detected by an immunoturbidimetric immunoassay using a commercial kit.

Diagnostic criteria

The normal values of serum TC, TG, HDL-C, LDL-C, ApoA1, ApoB and ApoA1/ApoB ratio in our Clinical Science Experiment Center were 3.10-5.17, 0.56-1.70, 0.91-1.81, 2.70-3.20 mmol/L, 1.00-1.78, 0.63-1.14 g/L, and 1.00-2.50; respectively. The individuals with TC > 5.17 mmol/L and/or TG > 1.70 mmol/L were defined as hyperlipidemic. Hypertension was diagnosed according to the criteria from the 1999 World Health Organization-International Society of Hypertension Guidelines for the management of hypertension [23]. The diagnostic criteria of

overweight and obesity were according to the Cooperative Meta-analysis Group of China Obesity Task Force. Normal weight, overweight and obesity were defined as a BMI < 24, 24-28 and > 28 kg/m^2, respectively [24]. Dyslipidemia was defined according to World Health Organization criteria: TG $\geq$ 1.7 mmol/L and HDL-C < 0.9 mmol/L for men or < 1.0 mmol/L for women.

Statistical analyses

All statistical analyses were performed using the statistical software package SPSS 16.0 (SPSS Inc. Chicago, IL, USA). A standard goodness-of-fit test was used to test the Hardy-Weinberg equilibrium. A chi-square analysis was used to evaluate the difference in genotype distribution and sex ratio between the groups. The general characteristics between the cases and controls were tested using Student's unpaired *t*-test. The association between genotypes and serum lipid parameters was tested by analysis of covariance (ANCOVA). Sex, age, body mass index (BMI), blood pressure, alcohol consumption, and cigarette smoking were adjusted for the statistical analysis. ORs and 95% CIs were calculated using unconditional logistic regression. A two-

Table 3. Association of *GCKR* rs1260326 SNP and the risk of CAD and IS stratified by gender

Genotype or allele	Control [n (%)]	CAD [n (%)]	IS [n (%)]	CAD		IS	
				OR (95% CI)	P	OR (95% CI)	P
Male							
CC	156 (35.9)	144 (33.3)	135 (33.9)	1		1	
CT	210 (48.3)	202 (46.8)	176 (44.2)	1.11 (0.81-1.52)	0.527	0.99 (0.72-1.36)	0.947
TT	69 (15.9)	86 (19.9)	87 (21.0)	1.28 (0.84-1.93)	0.248	1.36 (0.91-2.05)	0.139
χ^2		2.490	4.954				
P		0.288	0.084				
Female							
CC	53 (32.9)	54 (35.5)	61 (40.1)	1		1	
CT	68 (44.2)	63 (41.4)	73 (48.0)	0.93 (0.54-1.60)	0.794	0.87 (0.51-1.46)	0.591
TT	40 (24.8)	35 (23.0)	18 (11.8)	0.95 (0.51-1.78)	0.880	0.37 (0.18-0.76)	0.007
χ^2		4.275	8.832				
P		0.872	0.012				

Table 4. Association of the genotypes and serum lipid levels in the combined population

Genotype	n	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	ApoA1 (g/L)	ApoB (g/L)	ApoA1/ApoB
rs1260326								
CC	604	4.63±1.17	1.52±1.53	1.42±0.52	2.68±0.90	1.17±0.35	0.90±0.24	1.46±1.72
CT	795	4.67±1.06	1.56±1.52	1.46±0.58	2.73±0.86	1.16±0.32	0.90±0.24	1.44±1.60
TT	337	4.72±1.38	1.70±1.39	1.37±0.48	2.75±0.99	1.16±0.62	0.92±0.26	1.32±0.51
P		0.589	0.197	0.100	0.416	0.932	0.489	0.346
CC + CT	1399	4.66±1.11	1.55±1.52	1.45±0.56	2.70±0.88	1.17±0.34	0.90±0.24	1.42±1.65
TT	337	4.72±1.38	1.70±1.39	1.37±0.48	2.75±0.99	1.16±0.62	0.92±0.26	1.32±0.51
P		0.629	0.143	0.018	0.468	0.533	0.491	0.186
rs8179206								
AA	1678	4.66±1.17	1.58±1.51	1.43±0.54	2.71±0.90	1.16±0.41	0.90±0.24	1.43±1.53
GA	57	4.74±1.21	1.56±1.24	1.43±0.53	2.79±1.02	1.15±0.31	0.89±0.32	1.44±0.72
GG	1	6.43±	1.42±	1.31±	4.71±	1.03±	1.29±	0.80±
P		0.291	0.994	0.973	0.090	0.909	0.244	0.893
AA	1678	4.66±1.17	1.58±1.51	1.43±0.54	2.71±0.90	1.16±0.41	0.90±0.24	1.43±1.53
GA + GG	58	4.74±1.22	1.54±1.22	1.42±0.52	2.80±1.04	1.14±0.30	0.90±0.32	1.45±0.71
P		0.639	0.857	0.892	0.473	0.738	0.902	0.935

tailed *P* value less than 0.05 was considered to be statistically significant. The pattern of pairwise linkage disequilibrium (LD) between the selected SNPs were measured by D' and r^2 using the SHEsis software.

Results

Baseline characteristics

The baseline characteristics of participants in this study are presented in **Table 1**. The mean age, ratio of males to females, serum LDL-C and ApoB levels, and the percentages of sub-

jects who smoked cigarette were not different between controls and CAD or IS patients (*P* > 0.05 for all). The CAD patients had higher BMI, pulse pressure and serum TG levels, but lower levels of diastolic blood pressure, serum TC, HDL-C, ApoA1, the ratio of ApoA1 to ApoB, and the percentages of subjects who consumed alcohol (*P* < 0.05-0.001) than the controls. The IS patients had higher BMI, systolic blood pressure, pulse pressure and serum TG levels, and lower levels of serum TC, HDL-C, ApoA1, the ratio of ApoA1 to ApoB, and the percentages of subjects who consumed alcohol (*P* < 0.05-0.001) than the controls.

Table 5. Association of the genotypes and serum lipid levels in the controls and cases

Genotype	Control				CAD				IS			
	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
rs1260326												
CC	4.85±1.20	1.49±2.18	1.90±0.49	2.64±0.74	4.49±1.18	1.53±1.01	1.14±0.32	2.68±1.00	4.53±1.10	1.57±1.06	1.19±0.34	2.71±0.96
CT	4.96±1.01	1.34±1.84	1.93±0.52	2.77±0.75	4.52±1.11	1.67±1.12	1.16±0.37	2.72±0.99	4.53±1.00	1.70±1.44	1.26±0.48	2.69±0.83
TT	5.02±1.18	1.42±1.19	1.83±0.47	2.89±0.95	4.60±1.38	1.81±1.26	1.12±0.39	2.74±1.05	4.50±1.51	1.81±1.69	1.22±0.32	2.59±0.94
P	0.353	0.665	0.213	0.022	0.715	0.038	0.504	0.854	0.975	0.354	0.181	0.516
rs8179206												
AA	4.93±1.12	1.43±1.90	1.89±0.50	2.74±0.80	4.51±1.18	1.65±1.09	1.14±0.34	2.70±0.99	4.53±1.15	1.69±1.40	1.23±0.40	2.69±0.90
GA	4.93±1.00	0.91±0.26	2.01±0.47	2.79±0.74	5.12±1.43	2.24±1.71	1.19±0.30	3.12±1.33	4.30±0.93	1.36±0.63	1.26±0.41	2.41±0.68
GG									6.43	1.42	1.31	4.71
P	0.998	0.362	0.292	0.839	0.110	0.173	0.590	0.086	0.174	0.614	0.924	0.051

TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Genotypic and allelic frequencies

The genotypes of rs1260326 and rs8179206 SNPs were in Hardy-Weinberg equilibrium in the controls and patients (**Table 2**). There were no significant differences in the genotypic and allelic frequencies between controls and CAD or IS patients (**Table 2**; *P* > 0.05). When the analysis was stratified by gender, the genotypic frequency of the rs1260326 SNP was significant different between control and IS patient in females but not in males (**Table 3**; *P* < 0.05).

GCKR polymorphisms and the risk of CAD and IS

There was not significant association of rs1260326 and rs8179206 SNPs and the risk of CAD or IS in different genetic models (**Table 2**). But the TT genotype of the rs1260326 SNP was associated with decreased risk of IS in females (OR = 0.37, 95% CI: 0.18-0.76, *P* = 0.007; **Table 3**).

Genotypes and serum lipid levels

As shown in **Table 4**, the subjects with TT genotype of rs1260326 in the total population had lower serum HDL-C levels than those with CT/CC genotypes (*P* = 0.018). For controls, the subjects with TT genotype of rs1260326 were associated with higher serum LDL-C than the subjects with CC genotype (*P* = 0.022). For the CAD patients, the subjects with TT genotype of rs1260326 were associated with higher serum TG levels than the subjects with CC genotype (*P* = 0.038; **Table 5**). There was not significant association of rs8179206 SNP and serum lipid levels (**Tables 4** and **5**).

Discussion

The present study showed that the TT genotype of rs1260326 SNP was associated with increased risk of dyslipidemia and decreased serum HDL-C levels in the total population [17, 19, 25]. We also found that the subjects with TT genotype of rs1260326 SNP had higher serum LDL-C levels in controls and higher serum TG levels in CAD patients than the subjects with CC/CT genotypes. The results of the present study in the CAD patients were consistent with those of several previous studies in the Finnish, Swedish, Danish, Amish, and French populations [15, 16, 24-26]. The association between variants in *GCKR* and TG was initially identified

through an agnostic GWAS approach [15]. Further, some studies showed that the rs1260326 SNP was inversely associated with TG levels and glucose levels, and affected GCK activity in liver through diminished regulation by Fructose 6-phosphate [10]. This is predicted to increase glycolytic flux and hence glucose uptake by the liver. This enhanced rate of glycolysis may raise levels of malonyl-CoA, leading to increase TG levels of de novo lipogenesis and cholesterol synthesis and export. This perturbation of hepatic metabolism could account for the reduced glucose and raised TG seen in TT genotype of rs1260326 SNP [13, 27]. Elevated fasting TG levels are also associated with reduced levels of HDL-C, which is suggested to mark reduced removal of cholesterol from the arterial wall [28]. In this study, no such association was found in the controls and IS patients. It is possible that the impact of uncertain variant and a genetic background and lifestyle and diet caused the associations in the CAD group [21, 28, 29]. Different populations have different relationship between polymorphisms and serum lipid levels, which suggested our sample size may be not enough, further investigations are expended to confirm these interactions.

The SNPs of *GCKR* rs780093 and rs780094 were strong LD with rs1260326 SNP [16, 26, 30]. The SNP of rs8179206 was LD with rs1260326 (D' = 0.252; r^2 = 0.001). The results of several previous association studies between *GCKR* polymorphisms and atherosclerosis related diseases are not entirely consistent. Shen et al. [26] showed that the *GCKR* rs1260326 T allele was associated with higher atherogenic risk. Lian et al. [30] demonstrated *GCKR* rs780093 as a risk factor for CAD in individuals aged 65 and older. On the contrary, Varbo et al. [19] found that the *GCKR* rs1260326 SNP did not influence the risk of ischemic heart disease or myocardial infarction. In the Ludwigshafen Risk and Cardiovascular Health Study, no association was found with respect to coronary stenosis [31]. Járomi et al. [32] could not detect any association of rs1260326 with the susceptibility of stroke. Bi et al. [33] also found that the rs780094 SNP had no association with the incidence of CAD or stroke.

In the present study, we showed that the rs1260326T allele frequency (42.3%) was simi-

lar with that in HapMap CEU (European) population (42.0%). However, the T allele frequency of rs1260326 SNP is different among Chinese population (63.4%), Sub-Saharan African (10.3%), and Japanese population (59.3%) in the HapMap project database. Studies have found that *GCKR* is associated with increased levels of TG, unfavorable factor for CAD concurrent with lower levels of fasting plasma glucose, favorable factor for CAD [14, 17]. In the present study, although the rs1260326 SNP associated with unfavorable serum lipid profiles, we found that the TT genotype associated with decreased IS risk, thus, the lower glucose levels may also help to explain the inconsistent association of *GCKR* rs1260326 SNP and the risk of IS. The ambiguous results propose that the risk of CAD and IS associated with *GCKR* may vary based on the proportion of variance of the subjects' glycemic and lipid traits. In addition, the number of subjects for minor allele of SNPs was too small to interpret the associations of SNPs and the risk of diseases. Therefore, larger sample size and multi-ethnic population studies are needed to confirm our results.

Conclusion

Our data indicate that the TT genotype of rs1260326 SNP is associated with increased serum LDL-C levels in controls, increased serum TG levels in CAD patients, and decreased serum HDL-C levels in the total population, but with decreased risk of IS in females.

Acknowledgements

Disclosure of conflict of interest

None.

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References

[1] WHO publishes definitive atlas on global heart disease and stroke epidemic. Indian J Med Sci 2004; 58: 405-406.

[2] Bake S, Selvamani A, Cherry J and Sohrabji F. Blood brain barrier and neuroinflammation are critical targets of IGF-1-mediated neuroprotection in stroke for middle-aged female rats. PLoS One 2014; 9: e91427.

[3] Bosomworth NJ. Approach to identifying and managing atherogenic dyslipidemia: a metabolic consequence of obesity and diabetes. Can Fam Physician 2013; 59: 1169-1180.

[4] Tanne D, Yaari S and Goldbourt U. High-density lipoprotein cholesterol and risk of ischemic stroke mortality. A 21-year follow-up of 8586 men from the Israeli Ischemic Heart Disease Study. Stroke 1997; 28: 83-87.

[5] Soyama Y, Miura K, Morikawa Y, Nishijo M, Nakanishi Y, Naruse Y, Kagamimori S and Nakagawa H. High-density lipoprotein cholesterol and risk of stroke in Japanese men and women: the Oyabe Study. Stroke 2003; 34: 863-868.

[6] Heller DA, de Faire U, Pedersen NL, Dahlen G and McClearn GE. Genetic and environmental influences on serum lipid levels in twins. N Engl J Med 1993; 328: 1150-1156.

[7] Marenberg ME, Risch N, Berkman LF, Floderus B and de Faire U. Genetic susceptibility to death from coronary heart disease in a study of twins. N Engl J Med 1994; 330: 1041-1046.

[8] Keavney B, Palmer A, Parish S, Clark S, Youngman L, Danesh J, McKenzie C, Delepine M, Lathrop M, Peto R and Collins R. Lipid-related genes and myocardial infarction in 4685 cases and 3460 controls: discrepancies between genotype, blood lipid concentrations, and coronary disease risk. Int J Epidemiol 2004; 33: 1002-1013.

[9] Van Schaftingen E. A protein from rat liver confers to glucokinase the property of being antagonistically regulated by fructose 6-phosphate and fructose 1-phosphate. Eur J Biochem 1989; 179: 179-184.

[10] Beer NL, Tribble ND, McCulloch LJ, Roos C, Johnson PR, Orho-Melander M and Gloyn 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] Farrelly D, Brown KS, Tieman A, Ren J, Lira SA, Hagan D, Gregg R, Mookhtiar KA and Hariharan N. Mice mutant for glucokinase regulatory protein exhibit decreased liver glucokinase: a sequestration mechanism in metabolic regulation. Proc Natl Acad Sci U S A 1999; 96: 14511-14516.

[12] Slosberg ED, Desai UJ, Fanelli B, St Denny I, Connelly S, Kaleko M, Boettcher BR and Caplan SL. Treatment of type 2 diabetes by adenoviral-mediated overexpression of the glucokinase regulatory protein. Diabetes 2001; 50: 1813-1820.

[13] O'Doherty RM, Lehman DL, Telemaque-Potts S and Newgard CB. Metabolic impact of glucokinase overexpression in liver: lowering of blood glucose in fed rats is accompanied by hyperlipidemia. Diabetes 1999; 48: 2022-2027.

[14] Orho-Melander M, Melander O, Guiducci C, Perez-Martinez P, Corella D, Roos C, Tewhey R, Rieder MJ, Hall J, Abecasis G, Tai ES, Welch C, Arnett DK, Lyssenko V, Lindholm E, Saxena R, de Bakker PI, Burtt N, Voight BF, Hirschhorn JN, Tucker KL, Hedner T, Tuomi T, Isomaa B, Eriksson KF, Taskinen MR, Wahlstrand B, Hughes TE, Parnell LD, Lai CQ, Berglund G, Peltonen L, Vartiainen E, Jousilahti P, Havulinna AS, Salomaa V, Nilsson P, Groop L, Altshuler D, Ordovas JM and Kathiresan S. 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.

[15] Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PI, Chen H, Roix JJ, Kathiresan S, Hirschhorn JN, Daly MJ, Hughes TE, Groop L, Altshuler D, Almgren P, Florez JC, Meyer J, Ardlie K, Bengtsson Bostrom K, Isomaa B, Lettre G, Lindblad U, Lyon HN, Melander O, Newton-Cheh C, Nilsson P, Orho-Melander M, Rastam L, Speliotes EK, Taskinen MR, Tuomi T, Guiducci C, Berglund A, Carlson J, Gianniny L, Hackett R, Hall L, Holmkvist J, Laurila E, Sjogren M, Sterner M, Surti A, Svensson M, Tewhey R, Blumenstiel B, Parkin M, Defelice M, Barry R, Brodeur W, Camarata J, Chia N, Fava M, Gibbons J, Handsaker B, Healy C, Nguyen K, Gates C, Sougnez C, Gage D, Nizzari M, Gabriel SB, Chirn GW, Ma Q, Parikh H, Richardson D, Ricke D and Purcell S. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 2007; 316: 1331-1336.

[16] Sparso T, Andersen G, Nielsen T, Burgdorf KS, Gjesing AP, Nielsen AL, Albrechtsen A, Rasmussen SS, Jorgensen T, Borch-Johnsen K, Sandbaek A, Lauritzen T, Madsbad S, Hansen T and Pedersen O. The GCKR rs780094 polymorphism is associated with elevated fasting serum triacylglycerol, reduced fasting and OGTT-related insulinaemia, and reduced risk of type 2 diabetes. Diabetologia 2008; 51: 70-75.

[17] Vaxillaire M, Cavalcanti-Proenca C, Dechaume A, Tichet J, Marre M, Balkau B and Froguel P. 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.

[18] Tam CH, Ma RC, So WY, Wang Y, Lam VK, Germer S, Martin M, Chan JC and Ng MC. Interaction effect of genetic polymorphisms in glucokinase (GCK) and glucokinase regulatory protein (GCKR) on metabolic traits in healthy Chinese adults and adolescents. Diabetes 2009; 58: 765-769.

[19] Varbo A, Benn M, Tybjaerg-Hansen A, Grande P and Nordestgaard BG. TRIB1 and GCKR polymorphisms, lipid levels, and risk of ischemic heart disease in the general population. Arterioscler Thromb Vasc Biol 2011; 31: 451-457.

[20] Alfred T, Ben-Shlomo Y, Cooper R, Hardy R, Deary IJ, Elliott J, Harris SE, Kivimaki M, Kumari M, Power C, Starr JM, Kuh D and Day IN. Associations between a polymorphism in the pleiotropic GCKR and Age-related phenotypes: the HALCyon programme. PLoS One 2013; 8: e70045.

[21] Sotos-Prieto M, Guillen M, Vicente Sorli J, Portoles O, Guillem-Saiz P, Ignacio Gonzalez J, Qi L and Corella D. Relevant associations of the glucokinase regulatory protein/glucokinase gene variation with TAG concentrations in a high-cardiovascular risk population: modulation by the Mediterranean diet. Br J Nutr 2013; 109: 193-201.

[22] Wu DF, Yin RX, Cao XL, Chen WX, Aung LH, Wang W, Huang KK, Huang P, Zeng XN and Wu J. Scavenger receptor class B type 1 gene rs5888 single nucleotide polymorphism and the risk of coronary artery disease and ischemic stroke: a case-control study. Int J Med Sci 2013; 10: 1771-1777.

[23] Chalmers J, MacMahon S, Mancia G, Whitworth J, Beilin L, Hansson L, Neal B, Rodgers A, Ni Mhurchu C and Clark T. 1999 World Health Organization-International Society of Hypertension Guidelines for the management of hypertension. Guidelines sub-committee of the World Health Organization. Clin Exp Hypertens 1999; 21: 1009-1060.

[24] Zhou BF. Predictive values of body mass index and waist circumference for risk factors of certain related diseases in Chinese adults--study on optimal cut-off points of body mass index and waist circumference in Chinese adults. Biomed Environ Sci 2002; 15: 83-96.

[25] Hishida A, Morita E, Naito M, Okada R, Wakai K, Matsuo K, Nakamura K, Takashima N, Suzuki S, Takezaki T, Mikami H, Ohnaka K, Watanabe Y, Uemura H, Kubo M, Tanaka H and Hamajima N. Associations of apolipoprotein

A5 (APOA5), glucokinase (GCK) and glucokinase regulatory protein (GCKR) polymorphisms and lifestyle factors with the risk of dyslipidemia and dysglycemia in Japanese - a cross-sectional data from the J-MICC Study. Endocr J 2012; 59: 589-599.

[26] Shen H, Pollin TI, Damcott CM, McLenithan JC, Mitchell BD and Shuldiner AR. Glucokinase regulatory protein gene polymorphism affects postprandial lipemic response in a dietary intervention study. Hum Genet 2009; 126: 567-574.

[27] Rees MG, Wincovitch S, Schultz J, Waterstradt R, Beer NL, Baltrusch S, Collins FS and Gloyn AL. Cellular characterisation of the GCKR P446L variant associated with type 2 diabetes risk. Diabetologia 2012; 55: 114-122.

[28] Cuchel M and Rader DJ. Macrophage reverse cholesterol transport: key to the regression of atherosclerosis? Circulation 2006; 113: 2548-2555.

[29] Pollin TI, Jablonski KA, McAteer JB, Saxena R, Kathiresan S, Kahn SE, Goldberg RB, Altshuler D and Florez JC. Triglyceride response to an intensive lifestyle intervention is enhanced in carriers of the GCKR Pro446Leu polymorphism. J Clin Endocrinol Metab 2011; 96: E1142-1147.

[30] Lian J, Guo J, Chen Z, Jiang Q, Ye H, Huang X, Yang X, Ba Y, Zhou J and Duan S. Positive association between GCKR rs780093 polymorphism and coronary heart disease in the aged Han Chinese. Dis Markers 2013; 35: 863-868.

[31] Kozian DH, Barthel A, Cousin E, Brunnhofer R, Anderka O, Marz W, Bohm B, Winkelmann B, Bornstein SR and Schmoll D. Glucokinase-activating GCKR polymorphisms increase plasma levels of triglycerides and free fatty acids, but do not elevate cardiovascular risk in the Ludwigshafen Risk and Cardiovascular Health Study. Horm Metab Res 2010; 42: 502-506.

[32] Jaromi L, Csongei V, Polgar N, Szolnoki Z, Maasz A, Horvatovich K, Farago B, Sipeky C, Safrany E, Magyari L, Kisfali P, Mohas M, Janicsek I, Lakner L and Melegh B. Functional variants of glucokinase regulatory protein and apolipoprotein A5 genes in ischemic stroke. J Mol Neurosci 2010; 41: 121-128.

[33] Bi M, Kao WH, Boerwinkle E, Hoogeveen RC, Rasmussen-Torvik LJ, Astor BC, North KE, Coresh J and Kottgen A. Association of rs780094 in GCKR with metabolic traits and incident diabetes and cardiovascular disease: the ARIC Study. PLoS One 2010; 5: e11690.