Association of GCKR Gene Polymorphisms with the Risk of Nonalcoholic Fatty Liver Disease and Coronary Artery Disease in a Chinese Northern Han Population

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Abstract

Background and Aims: Accumulated studies have evaluated the effects of glucokinase regulatory protein (GCKR) gene polymorphisms on the risk of nonalcoholic fatty liver disease (NAFLD) and coronary artery disease (CAD), but the association of GCKR polymorphisms with the risk of NAFLD and CAD in the Chinese Han population have remained unclear. The aim of this study was to investigate the association between GCKR gene polymorphisms (rs780094 and rs1260326) and the risk of NAFLD and CAD in NAFLD patients in a Chinese Northern Han population. Methods: GCKR rs780094 and rs1260326 gene polymorphisms were genotyped by polymerase chain reaction sequencing for B-type ultrasonography-proven NAFLD patients with (n = 82) or without (n = 142) CAD, and in healthy controls (n = 152). Serum lipid profiles’ levels were determined using biochemical methods. Statistical analyses were conducted using SPSS 22.0 statistical software. Results: As the results showed, significant differences in the serum lipid profiles existed between each group. No significant differences were observed in the distributions of genotypes and alleles of GCKR rs780094 and rs1260326 in each group. The GCKR rs780094 T and rs1260326 T allele carriers possessed decreased body mass index value, and serum fasting plasma glucose and TG levels in the overall subjects, respectively. In addition, the GCKR rs780094 T allele carriers possessed decreased serum fasting plasma glucose level in the controls and NAFLD + CAD patients. Conclusions: GCKR rs780094 and rs1260326 polymorphisms were found to be not associated with the risk of NAFLD nor of CAD in NAFLD patients in this Chinese Northern Han population. GCKR rs780094 T and rs1260326 T alleles could affect the body mass index value and serum fasting plasma glucose and triglyceride levels.

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most prevalent chronic liver disease in the world and is regarded as a severe public health concern. The overall prevalence of NAFLD is approximately 25% worldwide and is 23%, 31% and 27% in Europe, South America and Asia, respectively. Many factors contribute to the development of NAFLD, such as aging, hyperlipidemia, insulin resistance, type 2 diabetes, diet, genetics and so on. Accumulated lines of evidence have suggested that coronary artery disease (CAD) plays an important role in the progression of NAFLD and is associated with the severity of NAFLD.

CAD has become the most important cause of mortality among NAFLD patients. Although some defects of liver biopsy exist, such as invasive, sample errors and operator dependence, it remains the gold standard for diagnosis of NAFLD. In recent years, some noninvasive diagnostic methods have been developed and their effects have been tested in different countries and populations. Genome-wide association studies (GWAS) have identified several important single nucleotide polymorphism (SNP) sites which are tightly associated with the risk of development of NAFLD; these include PNPLA3 rs738409, TM6SF2 rs58542926, and LYPLAL1 rs12137855. In consideration of the differences of genetic background of NAFLD patients, it is necessary to identify the relationship of gene polymorphisms with the risk of NAFLD in different countries and races.

Glucokinase (GCK) is a phosphorylating enzyme which can regulate hepatic glucose metabolism and activate hepatic lipogenesis. The GCKR gene encodes the glucokinase...
regulatory protein (GCKRP), which can bind to GCK allosterically and regulate the activity of GCK. In 2011, Speliotes et al. identified the GCKR rs780094 by GWAS as a significant SNP site associated with the risk of NAFLD. Subsequent studies in different districts reported controversial conclusions of GCKR rs780094 in NAFLD. The GCKR rs780094 T allele was found to be tightly associated with susceptibility to NAFLD in Americans and Malaysians, acting as a risk factor for NAFLD. However, no significant association between GCKR rs780094 and the risk of NAFLD was observed in the community-based study in Suzhou and Shanghai, and in obese adolescents in Beijing, China. On the contrary, Lin et al. reported that GCKR rs780094 was associated with an increased risk of NAFLD in obese children in Taiwan. Therefore, large-scale population-based studies in different districts are needed to investigate the effect of GCKR rs780094 on the development of NAFLD in China.

In addition, GWAS analyses conducted in Finnish, Swedish and Danish populations found a strong linkage disequilibrium of GCKR rs780094 with GCK rs1260326. In Japan, Kawaguchi et al. found that GCKR rs1260326 was a significant risk factor for the development of NAFLD. In France, Petit et al. reported that GCKR rs1260326 influences the liver fat content in patients with type 2 diabetes. Di et al. reported that GCKR rs1260326 could increase the liver fat content, although the detailed role of GCKR rs1260326 in NAFLD remains controversial. Besides, Simons et al. conducted a meta-analysis to investigate the relationship of GCKR polymorphisms with the risk of CAD, and a tight association of GCKR variants with the risk of CAD was observed.

In consideration of the unclear effects of GCKR rs780094 and rs1260326 on NAFLD in Chinese, and the tight association of CAD and NAFLD, it is of interest to explore the effects of GCKR rs780094 and rs1260326 on the development of NAFLD and the development of CAD in NAFLD patients from this population.

The aim of this study was to explore the relationship between GCKR gene polymorphisms (rs780094 and rs1260326) with the risk of NAFLD, the risk of CAD in NAFLD patients in a Chinese Northern Han population, and the effects of GCKR rs780094 and rs1260326 on the levels of serum lipid profiles.

Methods

Subjects

This case-control study was conducted according to the principles of the Declaration of Helsinki and its appendices. This study was approved by the ethics committee of Qingdao Municipal Hospital (Qingdao, China). All the subjects included in this study were of the Chinese Northern Han population, and each signed a written informed consent form.

From June 2018 to December 2018, 225 unrelated adult Chinese Northern Han patients with NAFLD, of both genders, that were diagnosed by B-type ultrasonography were recruited. Among these NAFLD patients, 82 suffered from CAD (41 males, 41 females; mean age of 55.62 ± 7.187 years) and 142 did not have CAD (75 males, 67 females; mean age of 50.14 ± 11.842 years). A total of 152 healthy controls, matched for sex and age (75 males, 77 females; mean age of 51.38 ± 13.276 years) were included. All the patients were recruited from the Departments of Gastroenterology and Cardiology and the Health Examination Center of Qingdao Municipal Hospital.

Standard clinical evaluations of the NAFLD patients were performed according to the criteria of American Association for the Study of Liver Diseases. Subjects with the following symptoms were excluded from the NAFLD cohort: 1) excessive alcohol intake (males >210 g/w, females >140 g/w); 2) viral hepatitis, such as hepatitis B or hepatitis C; 3) drug-induced hepatitis; 4) other endocrine and metabolic disorders, such as renal disease. CAD was diagnosed using the findings from percutaneous coronary angiogram conducted by two experienced interventional cardiologists, and defined as the presence of at least 50% stenosis in at least one of the coronary arteries. The healthy controls were also subjected to the same diagnosis routines for NAFLD and CAD, as well as the laboratory and general examinations.

Biochemical analyses

The basic clinical pathological information (gender, age, height, and weight) was obtained by a standard study questionnaire. The body mass index (BMI) was calculated as mass weight (kg)/height2 (m2). All the subjects underwent a 12-h overnight fast, and then the blood samples were collected into ethylene diaminetetraacetic acid-containing tubes. Serum fasting plasma glucose (FPG), total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutamyltransferase (r-GT), alkaline phosphatase (ALP), and bilirubin (BIL) were measured using standard clinical laboratory techniques.

Genomic DNA extraction and genotyping

Genomic DNA was extracted and stored as previously described. The primers for PCR amplification containing rs780094 were 5′-ACGTGGATGAGTGTTGGAGATTACAGGACG-3′ and 5′-ACGTTGGATGAGGGCCCATTTTACGAC-3′, rs1260326 5′-ACGGTTGGATGACCTTGGGTCCCTTTGTACG-3′ and 5′-ACGTTGGATGACCCATCCACAC-3′ and were synthesized by Beijing BoMiao Biotech Company (China). The PCR amplification profile was as follows: predenaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 20 s, annealing at 56°C for 30 s, extending at 72°C for 60 s, followed by the final extension at 72°C for 3 min. The target amplified fragments were detected by 2% gel electrophoresis, with the predicted fragment length. The genotypes of rs780094 and rs1260326 were detected by direct DNA sequencing using the Applied Biosystems Inc. (USA) Veriti-384 Prism Sequence Detection System, and the raw data were analyzed using MassARRAY TYPER4.0 software (Agena Bioscience, USA). Genotyping was performed in a blinded fashion and the success rates were >95%.

Statistical analysis

Statistical analysis was conducted using the SPSS 22.0 statistical software (SPSS Inc., USA). Student’s t-test or χ2 test were used to analyzed the differences in characteristics of different groups. First, continuous variables were tested by normal distribution and F test; if yes, the baseline characteristics were expressed as the mean ± standard deviation (SD), and if no, the baseline characteristics were expressed as the median ± quartile. Hardy–Weinberg equilibrium was between expected and observed genotype distributions and was estimated using the χ2 test. Genotype and allele frequencies were
assessed by counting the DNA sequencing data, and the distributions in NAFLD versus controls were analyzed by Pearson’s χ² test or Fisher’s exact test, where appropriate. Association between polymorphisms and NAFLD/CAD were analyzed by logistic regression and odds ratios (ORs) with p-value <0.05 was considered statistically significant.

Results

Characteristics of the study participants

The baseline characteristics of the three groups (NAFLD, NAFLD with CAD, and healthy controls) according to the experimental requirements are shown in Table 1. There were no significant differences in age or gender among the three groups (all p > 0.05). The increased serum levels of BMI, TC, TG, LDL, ALT, AST, r-GT, and ALP were observed in the NAFLD patients compared to the healthy controls (all p < 0.05). In the NAFLD + CAD group, the BMI value and serum FPG were higher than in the non-carriers in the overall series (all p < 0.05), respectively. In addition, the serum level of FPG in rs780094 T carriers was higher than in rs1260326 T allele carriers than in the non-carriers in the NAFLD + CAD group (p < 0.05). No marked difference of other clinical parameters was observed between the carriers and non-carriers of the rs780094 T or rs1260326 T allele on the clinical characteristics of NAFLD patients, NAFLD + CAD patients, and overall series were analyzed. As the results show in Table 4 and Table 5, the BMI value and serum FPG and TG levels were significantly lower in the rs780094 T and rs1260326 T allele carriers than in the non-carriers in the overall series (all p < 0.05), respectively. In addition, the serum level of FPG in rs780094 T Carriers was higher than in non-carriers in the NAFLD + CAD group (p < 0.05). No marked difference of other clinical parameters was observed between the carriers and non-carriers of the rs780094 T or rs1260326 T allele in each group (p > 0.05).

Association of GCKR polymorphisms with clinical parameters in each group

The effects of GCKR rs780094 T or rs1260326 T allele on the clinical characteristics of NAFLD patients, NAFLD + CAD patients, and overall series were analyzed. As the results showed in Table 4 and Table 5, the BMI value and serum FPG and TG levels were significantly lower in the rs780094 T and rs1260326 T allele carriers than in the non-carriers in the overall series (all p < 0.05), respectively. In addition, the serum level of FPG in rs780094 T Carriers was higher than in non-carriers in the NAFLD + CAD group (p < 0.05). No marked difference of other clinical parameters was observed between the carriers and non-carriers of the rs780094 T or rs1260326 T allele in each group (p > 0.05).

Table 2. Results of the Hardy-Weinberg equilibrium test

| Gene locus groups | CC | CT | TT | χ² | p value |
|-------------------|----|----|----|----|---------|
| rs780094          |    |    |    |    |         |
| Controls          | 26 | 81 | 45 | 1.040 | 0.308   |
| NAFLD             | 26 | 71 | 45 | 0.047 | 0.828   |
| NAFLD + CAD       | 18 | 39 | 25 | 0.014 | 0.708   |

| rs1260326         |    |    |    |    |         |
| Controls          | 23 | 82 | 46 | 1.890 | 0.169   |
| NAFLD             | 24 | 73 | 45 | 0.372 | 0.542   |
| NAFLD + CAD       | 15 | 41 | 26 | 0.028 | 0.868   |

Abbreviations: NAFLD, patients with nonalcoholic fatty liver disease; NAFLD + CAD, patients with nonalcoholic fatty liver disease and coronary artery disease.

Data were compared by chi-square test.
### Table 3. Distribution of the GCKR rs780094 and rs1260326 polymorphisms in the study groups^a,b^.

| Genotype          | Controls | NAFLD | NAFLD + CAD | χ² | OR (95% CI) | P₁ | χ² | OR (95% CI) | P₂ |
|-------------------|----------|-------|-------------|----|------------|----|----|------------|----|
| rs780094          |          |       |             |    |            |    |    |            |    |
| CC                | 26 (17.1%) | 26 (17.1%) | 18 (22.0%) | 0.837 (0.455–1.538) | 0.819 | 0.073 | 0.914 | 0.012 | 0.877 (0.470–1.636) | 0.711 |
| CT + TT           | 126 (82.9%) | 116 (81.7%) | 64 (80.0%) | 0.754 (0.375–1.573) | 0.509 | 0.247 | 0.619 | 0.351 | 0.877 (0.470–1.636) | 0.711 |
| Alleles           |          |       |             |    |            |    |    |            |    |
| C                 | 133 (43.8%) | 123 (43.3%) | 75 (45.7%) | 0.982 (0.709–1.361) | 0.351 | 0.532 | 0.787 | 0.073 | 0.914 | 0.012 |
| T                 | 171 (56.2%) | 161 (56.7%) | 89 (54.3%) | 1.003 (0.736–1.361) | 0.908 | 1.083 | 1.255 | 0.819 | 0.365 | 0.437 |
| rs1260326         |          |       |             |    |            |    |    |            |    |
| CC                | 23 (15.1%) | 24 (16.9%) | 15 (18.3%) | 0.877 (0.470–1.636) | 0.711 | 0.679 | 1.256 | 0.351 | 0.877 (0.470–1.636) | 0.711 |
| CT + TT           | 129 (84.9%) | 118 (83.1%) | 67 (81.7%) | 0.821 (0.579–1.172) | 0.437 | 0.073 | 0.787 | 0.073 | 0.914 | 0.012 |
| Alleles           |          |       |             |    |            |    |    |            |    |
| C                 | 128 (42.1%) | 121 (42.6%) | 71 (43.3%) | 1.031 (0.736–1.416) | 0.15 | 0.902 | 1.256 | 0.819 | 0.365 | 0.437 |
| T                 | 176 (57.9%) | 163 (57.4%) | 93 (56.7%) | 1.031 (0.736–1.416) | 0.15 | 0.902 | 1.256 | 0.819 | 0.365 | 0.437 |

Abbreviations: NAFLD, patients with nonalcoholic fatty liver disease; NAFLD + CAD, patients with nonalcoholic fatty liver disease and coronary artery disease.

Data were compared by chi-square test. Values are expressed as n(%).

### Discussion

NAFLD can be caused by many risk factors, such as diet, genetics and so on. Genetic susceptibility genes are a type of risk factor that can contribute to the development of NAFLD. In this study, we investigated the relationship between GCKR rs780094 and rs1260326 gene polymorphisms and the risk of NAFLD in a general Chinese Northern Han population, and the risk of CAD in NAFLD patients in this population for the first time. Our results showed that the genotype distributions of GCKR rs780094 and rs1260326 were not associated with the risk of NAFLD, and not associated with the risk of CAD in NAFLD patients in this Chinese Northern Han population. In addition to the BMI value and serum FPG and TG levels, the T allele of GCKR rs780094 and rs1260326 also did not significantly affect the clinical parameters in NAFLD patients and NAFLD + CAD patients. These data suggest that GCKR rs780094 and rs1260326 polymorphisms are not associated with the risk of NAFLD in the general Chinese Northern Han population, and GCKR rs780094 and rs1260326 polymorphisms do not increase the risk of CAD in NAFLD patients in the Chinese Northern Han population.

Accumulated studies have explored the effects of GCKR polymorphisms on the risk of NAFLD but the results have been inconsistent. Some previous studies and our study suggested that GCKR rs780094 and rs1260326 polymorphisms are not associated with the risk of NAFLD, the probable reason for this finding may be due to the ethnic differences among the NAFLD patients studied. The relationship between GCKR polymorphism and NAFLD or nonalcoholic steatohepatitis may be modified by the change and confluence of race, therefore the effects of a GCKR polymorphism may become more dominant and significant in European populations than they are in Asian populations. Therefore, the GCKR rs780094 T allele might increase the risk of NAFLD in European populations but not in Asian populations. A higher prevalence of NAFLD has been observed in some Indians that possess European ancestry, as opposed to those that possess Asian ancestry, which might explain the above hypothesis.

The GCKR gene encodes GCKRP, an enzyme that plays a regulatory role in hepatic GCK activity. The GCKR rs1260326 functional variant P446L increases GCK activity by down-regulating the level of fructose 6-phosphate. Increased GCK activity is tightly accompanied by elevated hepatic glycolytic flux, de novo lipogenesis, and hepatic TG level. In this study, our results showed that the GCKR rs780094 and rs1260326 T allele were significantly associated with decreasing serum FPG level among the overall patients and the NAFLD + CAD patients. These results suggest that GCKR rs780094 and rs1260326 may repress the activity of GCKRP to decrease the serum FPG level.

Previous studies also investigated the association of GCKR polymorphisms with the risk of CAD. In a dietary intervention study, Shen et al. found that the GCKR rs1260326 T allele may increase the risk of atherosclerosis. Lian et al. demonstrated the positive association of GCKR rs780093 with the risk of CAD in an older Chinese Han population, through a case-control study. On the contrary, Járomi et al. did not detect any association of GCKR rs1260326 with the susceptibility of stroke. Bi et al. also found that the GCKR rs780094
Table 4. Clinical characteristics of GCKR rs260326 T carriers and non-carriers in the study population

| Characteristic | Controls, Carriers, n = 126 | Non-carriers, n = 26 | p value |
|----------------|-----------------------------|---------------------|---------|
| BMI (kg/m²)    | 22.91 ± 1.04                | 24.44 ± 1.30        | 0.003   |
| FPG (mmol/L)   | 4.45 ± 1.00                 | 4.74 ± 1.00         | 0.056   |
| TC (mmol/L)    | 5.06 ± 2.00                 | 5.99 ± 1.01         | 0.046   |
| HDL (mmol/L)   | 1.08 ± 0.00                 | 1.04 ± 0.00         | 0.796   |
| TG (mmol/L)    | 1.04 ± 0.00                 | 1.14 ± 0.00         | 0.025   |
| LDL (mmol/L)   | 1.29 ± 0.00                 | 1.61 ± 0.00         | 0.676   |
| AST (U/L)      | 16.89 ± 9.60                | 20.22 ± 12.00       | 0.014   |
| ALT (U/L)      | 69.92 ± 26.00               | 77.06 ± 36.00       | 0.121   |
| r-GT (mmol/L)  | 12.70 ± 6.00                | 11.50 ± 4.00        | 0.587   |
| ALP (mmol/L)   | 69.71 ± 26.00               | 80.25 ± 32.00       | 0.281   |
| BIL (mmol/L)   | 12.50 ± 6.00                | 11.70 ± 5.00        | 0.593   |

Abbreviations: NAFLD, Non-alcoholic fatty liver disease; NAFLD + CAD, Non-alcoholic fatty liver disease and coronary artery disease.

Table 5. Clinical characteristics of GCKR rs260326 T carriers and non-carriers in the study population

| Characteristic | Controls, Carriers, n = 129 | Non-carriers, n = 23 | p value |
|----------------|-----------------------------|---------------------|---------|
| BMI (kg/m²)    | 22.96 ± 1.04                | 24.46 ± 1.30        | 0.003   |
| FPG (mmol/L)   | 4.49 ± 1.00                 | 4.74 ± 1.00         | 0.056   |
| TC (mmol/L)    | 5.03 ± 2.00                 | 5.94 ± 1.01         | 0.046   |
| HDL (mmol/L)   | 1.08 ± 0.00                 | 1.04 ± 0.00         | 0.796   |
| TG (mmol/L)    | 1.04 ± 0.00                 | 1.14 ± 0.00         | 0.025   |
| LDL (mmol/L)   | 1.29 ± 0.00                 | 1.61 ± 0.00         | 0.676   |
| AST (U/L)      | 16.81 ± 9.60                | 20.22 ± 12.00       | 0.014   |
| ALT (U/L)      | 69.71 ± 26.00               | 77.06 ± 36.00       | 0.121   |
| r-GT (mmol/L)  | 12.70 ± 6.00                | 11.50 ± 4.00        | 0.587   |
| ALP (mmol/L)   | 69.71 ± 26.00               | 80.25 ± 32.00       | 0.281   |
| BIL (mmol/L)   | 12.50 ± 6.00                | 11.70 ± 5.00        | 0.593   |

Abbreviations: NAFLD, Non-alcoholic fatty liver disease; NAFLD + CAD, Non-alcoholic fatty liver disease and coronary artery disease.
SNP had no significant association with the incidence of CAD or stroke. SNPs of GCKR rs780094 showed strong linkage disequilibrium with rs1260326.²⁴,³⁹,⁴⁰ Our results showed that GCKR rs780094 and rs1260326 genotype distributions were not associated with the occurrence of CAD in NAFLD patients but that the rs780094 T allele could decrease the serum FPG levels in the NAFLD + CAD group. These results indicated that GCKR rs780094 and rs1260326 were not the risk factor of CAD in NAFLD patients but that the rs780094 T allele could affect serum FPG level in the NAFLD + CAD patients of our Chinese Northern Han population. Some limitations of our study should be acknowledged. First, all the subjects in this study were of the Chinese Northern Han population, so that the applicability of our conclusion to other ethnic populations requires further study for confirmation. Second, ultrasonography was used to diagnose NAFLD because of the difficulty in conducting liver biopsy. Third, our study did not grade the severity of NAFLD patients. Finally, a greater number of subjects should be included in the further studies to verify this conclusion in the Chinese Han population and other ethnic populations.

Conclusions

In summary, we investigated the relationship of GCKR rs780094 and rs1260326 gene polymorphisms with the risk of NAFLD as well as the risk of CAD in NAFLD patients in the Chinese Northern Han population. We concluded that GCKR rs780094 and rs1260326 gene polymorphisms did not associate with the risk of NAFLD nor with the risk of CAD in NAFLD patients in the Chinese Northern Han population. In addition, the GCKR rs780094 T and rs1260326 T alleles did affect the BMI value and serum FPG and TG levels in the overall subjects and NAFLD + CAD patients.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Study concept and design (YX and SX), acquisition of the data (HG, SL, ZZ, XY, and QL), analysis and interpretation of the data (HG, SL, and ZZ), drafting of the manuscript (HG and SL), critical revision of the manuscript for important intellectual content (YX and SX) supervision (YX and SX). All the authors read and approved the final manuscript.

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