Association of Adiponectin Gene Polymorphisms With the Risk of Coronary Artery Disease in Patients With Nonalcoholic Fatty Liver Disease in a Chinese Han Population

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Received 2016 February 24; Revised 2016 May 11; Accepted 2016 May 31.

Abstract

Background: Cardiovascular events are an independent risk factor for nonalcoholic fatty liver disease (NAFLD), which is the leading cause of mortality in NAFLD patients. Several recent studies demonstrated that adiponectin (Ad) polymorphisms were involved in the progression of NAFLD and coronary artery disease (CAD). However, reports on the association between Ad polymorphisms and the risk of developing CAD in NAFLD patients are lacking in a Northern Han Chinese population.

Objectives: The present study was designed to evaluate the association between Ad gene polymorphisms (rs266729 and rs2241766) and the risk of developing CAD in Northern Han Chinese patients with NAFLD.

Materials and Methods: In this case-control study, using the polymerase chain reaction (PCR), Adrs266729 and rs2241766 gene polymorphisms were genotyped in B-type ultrasonography-proven NAFLD patients, with (n = 246) or without (n = 247) CAD and in healthy controls (n = 304). Serum lipid profiles were determined using biochemical methods. Statistical analyses were performed using SPSS 17.0 statistical software.

Results: There were significant differences in the Adrs266729 G allele between the NAFLD patients with and without CAD (P < 0.05). In addition, there was a significant difference in the Adrs2241766 G allele of the NAFLD patients compared with that of the controls (P < 0.05). In the NAFLD CAD population, carriers of the G allele of Adrs266729 had higher serum triglycerides (TG), total cholesterol (TC), fasting plasma glucose (FPG), and low-density lipoprotein (LDL) levels and a lower Ad level than their noncarrier counterparts (P = 0.031, P = 0.034, P = 0.007, P < 0.001, and P < 0.001, respectively). NAFLD patients without CAD had higher TG and serum FPG values and a lower Ad level than their noncarrier counterparts (P = 0.014, P = 0.038, and P < 0.001, respectively). In the NAFLD patients with/without CAD, the carriers of the G allele of Adrs2241766 had higher TG levels (P = 0.039 and P = 0.042, respectively) than those of their noncarrier counterparts.

Conclusions: In this Northern Chinese Han population, the Adrs266729 and rs2241766 G alleles were closely associated with the occurrence of NAFLD. However, only NAFLD patients who carried the Adrs266729 G allele had an increased risk of developing CAD.

Keywords: Adiponectin, Polymorphisms, Genetic, Nonalcoholic Fatty Liver Disease, Coronary Artery Disease

1. Background

Nonalcoholic fatty liver disease (NAFLD) is the most common and prevalent liver disease worldwide (1), the prevalence of the global has reached 25.24% (22.10 - 28.65) (2). Accumulated evidence suggests that coronary artery disease (CAD) is closely related to the severity and progression of NAFLD, and it is the leading cause of mortality in NAFLD (3). Adams et al. (4) showed that cardiovascular disease accounted for about 25% of deaths in patients with NAFLD versus 13% of deaths in those with other liver diseases. Adiponectin (Ad), a 30-kDa peptide hormone, is almost exclusively secreted by adipose tissue (5). The human Ad gene is located on chromosome 3q27, containing three exons and two introns and encoding 244 amino acids (6). Recently, several single-nucleotide polymorphisms of the Ad gene, such as rs266729 (C > G) and rs2241766 (T > G), were shown to be significantly associated with (6). As one of the most abundant fat-derived biologically active proteins (7, 8), Ad plays an important role in the regulation of lipid metabolism and glucose metabolism, especially in improving insulin sensitivity, which is a key factor in the pathogenesis of NAFLD. Previous studies revealed a strong association between the presence of Ad polymorphisms and the risk of developing NAFLD, especially in patients of Chinese origin (9-16). The role of Ad polymorphisms...
in the susceptibility to CAD has been widely studied, but the results are inconsistent (17-19). Considering the high prevalence of CAD-related mortality in NAFLD patients (3), identifying the potential association between Ad polymorphisms and the relative risk of developing CAD in NAFLD patients is extremely important.

2. Objectives

In the present study, we selected and genotyped two Ad gene polymorphisms, rs266729 (C > G) and rs2241766 (T > G), in NAFLD patients, with or without CAD and in healthy controls. We then investigated the association of these two Ad polymorphisms with the risk of developing CAD among NAFLD patients in a Chinese Han population.

3. Materials and Methods

3.1. Study Subjects

This case-control study was approved by the ethics committee on human research of Qingdao municipal hospital (Qingdao, China). Written informed consent was obtained from each subject prior to starting the study, and all the subjects in the study were of Northern Han Chinese origin. This study was performed in accordance with the principles of the declaration of Helsinki and its appendices (20).

From April 2010 to May 2015, we selected 493 unrelated adult Chinese patients of both genders who had been diagnosed with NAFLD using B-type ultrasonography. Of those, 246 had CAD (127 males, 118 females, mean age of 61.54 ± 9.74 years), and 247 did not have CAD (127 males, 118 females, mean age of 62.13 ± 9.74 years). We also selected 304 healthy sex- and age-matched controls (152 males, 152 females, mean age of 61.31 ± 9.40 years). Data on the patients and healthy controls were collected from the department of gastroenterology and cardiology of Qingdao municipal hospital. NAFLD was diagnosed by a standard clinical evaluation, according to the criteria of the AASID (21). The diagnosis of CAD was based on a percutaneous coronary angiogram, which was evaluated by two experienced interventional cardiologists. CAD was defined as the presence of at least 50% stenosis in at least one of the coronary arteries. The control volunteers were confirmed as being healthy by echocardiography, medical history, and general and laboratory examinations at the same hospital. Subjects who had cardiac disorders, other liver diseases, infectious disease, diabetes mellitus, concurrent major renal disease, or a history of medication were excluded.

3.2. Baseline Demographic and Biochemical Analyses

We used a standard study questionnaire to obtain basic clinicopathological information (name, age, gender, height, weight, smoker, hypertension). The body mass index (BMI) was calculated using the following equation: BMI = weight/height$^2$ (kg/m$^2$) (22).

After a 12-hour overnight fast, blood samples were collected from each subject in ethylene diamine tetraacetic acid-containing tubes. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride (TG), total cholesterol (TC), fasting plasma glucose (FPG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were measured using routine enzymatic methods (23). The concentration of total Ad was obtained by a radioimmunoassay, using a human Ad RIA kit.

3.3. Genomic DNA Extraction and Genotyping

Genomic DNA was extracted using a genomic DNA purification kit (Beijing Biatek biotechnology, Beijing, China) for peripheral blood, according to the manufacturer’s instructions and stored at -20°C until use. The primers for PCR amplification of the fragments containing rs266729 and rs2241766 were synthesized by Shanghai Sangon Biotech company: Ad 5'-CAGACACTTGCCCTGCCCTCTGT3' and 5'-TGCCACATCTCACACCTTGGAGA-3' for rs266729; 5'-ACATGTTGAGTCTCCAGGGCCTCAG-3' and 5'-CTTCTCACCAGGGGTGCCATCT-3' for rs2241766. The PCR amplification profile was as follows: pre-denaturation at 94°C for 5 minutes, 35 cycles, denaturation at 94°C for 20 seconds, annealing at 62°C for 30 seconds, extending at 72°C for 30 seconds, and finally extending at 72°C for 5 minutes to terminate the reaction. Target amplified fragments were then detected by gel electrophoresis in 2% gel with a 197-base and 218-base pair product, respectively. The Ad genotypes were detected by direct DNA sequencing using the ABI Prism sequence detection system ABI3730 (Foster City, CA, USA).

3.4. Statistical Analysis

Differences in characteristics of the different groups were analyzed using the Student’s t test or the $\chi^2$ test. Baseline characteristics are expressed as the mean ± standard deviation (S.D.), and categorical data are presented as percentages and numbers. The quantitative variables were distributed normally. Genotype and allele frequencies were assessed by counting the DNA sequencing data of each subject. Differences in the distributions of the patients versus those of the controls were analyzed by Pearson’s $\chi^2$ test or Fisher’s exact test, where appropriate. The Hardy-Weinberg equilibrium between expected
and observed genotype distributions was estimated using the $\chi^2$ test. Logistic regression analysis was used to evaluate the association between the polymorphisms and presence/absence of CAD. The association was also determined by estimating the odds ratios (ORs), with their 95% confidence intervals (CIs). These statistical analyses were performed using SPSS 17.0 statistical software (SPSS Inc., Chicago, IL, USA). All P values of less than 0.05 were considered statistically significant.

4. Results

4.1. Characteristics of the Study Participants

The baseline characteristics of the three groups (NAFLD with CAD, NAFLD without CAD, and healthy controls) according to the experimental requirements are shown in Table 1. There were no significant age, gender, or height differences between the three groups ($P > 0.05$). The prevalence of the weight, increased BMI, hypertension, serum ALT and AST levels, serum TG, TC, and LDL levels, FPG, decreased HDL and Ad levels in NAFLD subjects (with and without CAD) were significantly higher than in the controls ($P < 0.05$).

Importantly, NAFLD patients with CAD had a higher prevalence of smoking and hypertension, increased weight, BMI, ALT, AST, TG, TC, FPG, and LDL, decreased HDL levels and Ad levels compared to the healthy controls (all $P < 0.05$). In the NAFLD patients without CAD, we found an increased prevalence of smoking and hypertension, higher TG and LDL levels, and lower HDL and Ad levels compared to those with CAD (all $P < 0.05$).

4.2. Ad rs266729 and rs2241766 Genotype and Allele Distribution

The genotype distributions of Ad rs266729 and rs2241766 were correlated with the Hardy-Weinberg equilibrium in both the patients and controls (all $P > 0.05$) (Table 2). As shown in Table 3, there was a significant difference in the genotypic and allelic distributions of the NAFLD patients and their control counterparts (OR: 0.414, 95% CI: 0.291 - 0.590, $P < 0.001$; OR: 0.610, 95% CI: 0.427 - 0.873, $P = 0.007$; OR: 0.473, 95% CI: 0.353 - 0.633, $P < 0.001$, OR: 0.633, 95% CI: 0.468 - 0.855, $P = 0.003$, respectively) at position Ad rs266729. Moreover, there was a statistically significant difference at position Ad rs266729 between the NAFLD patients with CAD versus those without CAD (OR: 0.679, 95% CI: 0.474 - 0.970, $P = 0.033$; OR: 0.747, 95% CI: 0.561 - 0.994, $P = 0.045$, respectively). At position Ad rs2241766, there was a strongly statistical difference observed between the NAFLD patients and their control counterparts (OR: 0.675, 95% CI: 0.481 - 0.949, $P = 0.023$; OR: 0.681, 95% CI: 0.485 - 0.956, $P = 0.026$; OR: 0.730, 95% CI: 0.557 - 0.956, $P = 0.022$, OR: 0.720, 95% CI: 0.549 - 0.943, $P = 0.017$, respectively). Nevertheless, there was no statistically significant difference in the Ad rs2241766 polymorphism between the NAFLD patients with CAD and without CAD ($P = 0.965$ and 0.921, respectively).

4.3. Association of the Ad Polymorphisms with Clinical Parameters in NAFLD Patients

We compared the Ad rs266729 and rs2241766 genotypes with the clinical characteristics of the NAFLD patients, with or without CAD and the control group to estimate whether the rs266729 and rs2241766 gene polymorphisms were correlated with clinical parameters (Table 4). With regard to position rs266729, the serum TG, TC, FPG, and LDL levels of the NAFLD CAD patients with a CG+GG genotype were significantly higher than those with a CC genotype ($P = 0.031$, OR: 0.034, $P = 0.007$, and $P < 0.001$, respectively). With regard to Ad rs2241769 (CG+GG) the genotype of NAFLD patients with CAD showed lower Ad levels in serum than those with the CC genotype ($P < 0.001$). Furthermore, CAD-free NAFLD patients with the rs266729 (CG+GG) genotype exhibited higher TG and serum FPG levels and lower serum Ad levels than those with the CC genotype ($P = 0.014$, $P = 0.038$, and $P < 0.001$, respectively). In both the NAFLD groups, the serum TG levels of those with the rs24241766 GT+GG genotype were higher than those with the rs2241766 TT genotype ($P = 0.039$ and $P = 0.042$, respectively). No other statistically significant differences were observed between the controls, with regards to the presence of the rs2421766 gene polymorphism ($P > 0.05$).

5. Discussion

In this study, we correlated the expression of the Ad rs266729 and rs2241766 gene polymorphisms with the risk of developing CAD in NAFLD patients for the first time. The main finding of our study was that the Ad rs266729 G allele and rs2241766 G allele were significantly associated with the occurrence of NAFLD. In addition, the NAFLD patients in this Northern Han Chinese population who carried the Ad rs266729 G allele had an increased risk of developing CAD. Future studies are required to determine whether and how this polymorphism modulates Ad expression, as well as the role of the Ad polymorphism in the pathogenesis of CAD in the setting of NAFLD.

The Ad gene, which is expressed primarily in adipose and vascular tissues, encodes the protein Ad (24). Ad has been shown to have anti-inflammatory effects and antiatherosclerotic properties (25) and to have important roles in insulin sensitivity (7, 8). Overexpression of Ad may contribute to the development of NAFLD, atherosclerosis,
Table 1. Baseline Characteristics of the Study Participants

| Characteristics | Groups |  | P Value |
|-----------------|--------|-----------------|---------|
|                 | CAD+NAFLD (n = 246) | CAD-NAFLD (n = 247) | Control (n = 304) | P1 | P2 | P3 |
| Age, y          | 61.54 ± 10.28 | 62.13 ± 9.74 | 61.31 ± 9.40 | 0.514 | 0.782 | 0.316 |
| Gender, Female/Male | 119/127 | 121/126 | 152/152 | 0.991 | 0.491 | 0.518 |
| Height, m       | 1.68 ± 0.07 | 1.68 ± 0.03 | 1.67 ± 0.08 | 1.032 | 1.342 | 0.782 |
| Weight, kg      | 67.46 ± 7.31 | 67.32 ± 7.42 | 66.54 ± 6.41 | 0.602 | 0.007 | 0.003 |
| Smoker, No. (%) | 115 (46.7) | 90 (36.4) | 100 (32.9) | 0.02 | 0.001 | 0.384 |
| BMI, Kg/m²      | 225.83 ± 3.20 | 26.05 ± 3.36 | 20.75 ± 9.63 | 0.457 | <0.001 | <0.001 |
| Hypertension, No. (%) | 125 (50.8) | 99 (40.1) | 68 (22.4) | 0.624 | <0.001 | <0.001 |
| ALT, U/L        | 41.88 ± 23.80 | 42.09 ± 23.12 | 20.75 ± 9.63 | 0.92 | <0.001 | <0.001 |
| AST, U/L        | 40.57 ± 22.01 | 41.49 ± 22.39 | 20.33 ± 8.13 | 0.647 | <0.001 | <0.001 |
| TG, mmol/L      | 2.34 ± 0.99 | 1.88 ± 0.81 | 1.35 ± 0.58 | <0.001 | <0.001 | <0.001 |
| TC, mmol/L      | 5.22 ± 0.96 | 5.05 ± 0.94 | 4.32 ± 0.93 | 0.057 | <0.001 | <0.001 |
| FPG, mmol/L     | 5.32 ± 0.83 | 5.07 ± 0.53 | 4.93 ± 0.49 | 0.624 | <0.001 | <0.001 |
| HDL, mmol/L     | 2.90 ± 0.86 | 2.70 ± 0.86 | 2.61 ± 0.75 | 0.011 | <0.001 | 0.021 |
| Adiponectin, ug/mL | 12.63 ± 2.83 | 15.31 ± 3.72 | 19.32 ± 3.21 | <0.001 | <0.001 | <0.001 |

Abbreviations: CAD+NAFLD, nonalcoholic fatty liver disease patients with coronary artery disease; CAD-NAFLD, NAFLD patients without CAD; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TG, triglycerides; TC, total cholesterol; FPG, fasting plasma glucose; HDL, high-density lipoprotein; LDL, low-density lipoprotein; P1, CAD+NAFLD vs. CAD-NAFLD; P2, CAD+NAFLD vs. control; P3, CAD-NAFLD vs. control.

Table 2. Results of the Hardy-Weinberg Equilibrium

| Gene Locus Groups | χ² | P |
|-------------------|----|---|
| rs266729          |    |   |
| CAD+NAFLD         | 0.314 | 0.575 |
| CAD-NAFLD         | 3.017 | 0.082 |
| Controls          | 3.324 | 0.068 |
| rs2241766         |    |   |
| CAD+NAFLD         | 0.813 | 0.367 |
| CAD-NAFLD         | 1.829 | 0.176 |
| Controls          | 3.310 | 0.069 |

and CAD by affecting the metabolism of major lipids, reducing insulin receptor signaling, and blocking insulin actions (16, 26, 27). Hashemi et al. (28) reported that the prevalence of ADRS266729 in Iranian patients with NAFLD was higher than that of controls. Similar findings were reported in some Chinese populations (16). Our results also showed that the frequency of the Ad rs266729 G allele was higher in the NAFLD groups than in the control group. Moreover, the Ad polymorphism at position rs266729 G allele increased TG, TC, and LDL levels in patients with NAFLD. These results are consistent with other Chinese data (16). In addition, the serum Ad level was decreased in those with the G allele and the Ad polymorphism at position rs266729. In common with the findings of the present study, Wang et al. (29) reported that variant alleles at rs266729 were associated with lower Ad levels.

Although the results indicate that the G allele of Ad rs266729 may contribute to the development of NAFLD, there is no consensus on whether rs2241766 is a risk factor for the prevalence of NAFLD. Tokushige et al. (30) discovered that the Ad rs2241766 genotype GG was related to the progression of NAFLD in Japanese patients. However, Wang et al. (31) revealed that this genotype was not significantly associated with NAFLD in a Southern Han Chinese population. Interestingly, in the current study, we found that Ad rs2241766 was associated with NAFLD in a Northern Han Chinese population. However, further studies with larger samples and different ethnicities or races are needed to confirm the findings (27).

CAD is a multifactorial disorder. The results of previous studies on the association between Ad gene polymorphisms and the risk of CAD are conflicting. One study of a Northeast Han Chinese population showed that the pres-
ence of the Ad rs2082940 variant was significantly associated with CAD (7). Specifically, these investigators found that the Ad rs2082940 G allele seemed to protect against the progression of CAD. On the other hand, Tong et al. (17) found that the presence of the G allele of the rs266729 was strongly correlated with an increased prevalence of CAD in Europeans. Hoenle et al. (32) reached the same conclusion in a study of an Australian population. A recent meta-analysis concluded that there was a strong association between Ad rs266729 and cardiovascular disease (33). In agreement with previous studies, Foucan et al. (34) showed that French patients carrying the Ad rs2241766 G allele had an increased risk of CAD. Other studies have reported discordant findings because of differences in ethnicity, phenotypes, and environments (16, 33). In the present study, the frequency of the Ad rs266729 G allele in NAFLD patients with CAD was substantially higher than in those without CAD. In addition, NAFLD patients with CAD who carried the Ad rs266729 G allele had increased serum TG, TC, and LDL levels compared to those who did not carry the G allele. The Ad rs2241766 polymorphism was not associated with the risk of developing CAD, but it had detrimental effects on lipid levels in NAFLD patients with CAD, indicating that the Ad G allele could lead to insulin resistance in CAD patients and that it may increase the risk of cardiovascular complications. Several previous studies showed that high concentrations of serum Ad decreased the risk of CAD in male diabetic patients (35), the risk of myocardial infarction in healthy men (36), and cardiovascular disease outcomes in patients with end-stage renal failure (37). In addition, Ad increased the production of endothelial NO, which has well-documented antithrombotic, antiatherogenic, and vasodilatory actions (38, 39). Thus, the literature demonstrates that Ad may have potential benefits in the treatment and prevention of cardiovascular disease (40). Further studies are needed to associate the Ad single-nucleotide polymorphism with expression of the Ad protein.

Some limitations of our study should be acknowledged. First, the entire study population was racially homogeneous, and the participants were all Han. Moreover, the population was highly selected. Therefore, our results may not be applicable to other populations. Second, we used ultrasonography to diagnose NAFLD because of the difficulty in obtaining a liver biopsy. Third, our study did not correlate Ad polymorphisms with the level of expression and insulin resistance or disease severity in NAFLD patients. Finally, larger sample sizes and diverse ethnic groups are needed in future studies to confirm the present data.

In conclusion, we found that the presence of the Ad rs266729 G allele increased the risk of CAD in NAFLD patients. The Ad rs2241766 G allele may be a risk factor for the development of NAFLD but not CAD in these patients. Additional studies are needed to delineate the mechanisms underlying the potential roles of these Ad gene polymorphisms in the risk of CAD in NAFLD.

### Table 3. Distribution of the Adiponectin rs266729 and rs2241766 Polymorphisms in the Study Groups

| Genotype       | CAD-NAFLD | CAD-NAFLD | Controls | OR (95%CI) | χ² | P1 | OR (95%CI) | χ² | P2 | OR (95%CI) | χ² | P3 |
|----------------|-----------|-----------|----------|------------|----|----|------------|----|----|------------|----|----|
| rs266729       |           |           |          |            |    |    |            |    |    |            |    |    |
| CC             | 127 (51.6)| 96 (45.6) | 109 (60.9)| 1.04 (0.97 - 1.12) | 0.09 | 0.95 | 0.75 (0.68 - 0.84) | 5.04 | 0.025 | 0.81 (0.69 - 0.96) | 4.81 | 0.032 |
| GG+GT          | 119 (48.4)| 348 (51.4)| 348 (39.1)| 1.04 (0.98 - 1.10) | 0.09 | 0.95 | 0.75 (0.68 - 0.84) | 5.04 | 0.025 | 0.81 (0.69 - 0.96) | 4.81 | 0.032 |
| Allele C       | 119 (48.4)| 348 (51.4)| 348 (39.1)| 1.04 (0.98 - 1.10) | 0.09 | 0.95 | 0.75 (0.68 - 0.84) | 5.04 | 0.025 | 0.81 (0.69 - 0.96) | 4.81 | 0.032 |
| Allele G       | 119 (48.4)| 348 (51.4)| 348 (39.1)| 1.04 (0.98 - 1.10) | 0.09 | 0.95 | 0.75 (0.68 - 0.84) | 5.04 | 0.025 | 0.81 (0.69 - 0.96) | 4.81 | 0.032 |

Footnotes

Authors’ Contribution: Study concept and design, Shui-Xian Du, Lin-Lin Lu; acquisition of the data, Shui-Xian Du and Yang Liu; analysis and interpretation of the data, Shui-Xian Du; drafting of the manuscript, Shui-Xian Du and Lin-Lin Lu; critical revision of the manuscript for important intellectual content, Shi-Ying Xuan and Yong-Ning Xin; statistical analysis, Shui-Xian Du, Yang Liu, and Lin-Lin Lu; administrative, technical, and material support; Yong-Ning Xin and Quan-Jiang Dong; study supervision, Shi-Ying Xuan and Yong-Ning Xin.

Funding/Support: This study was supported by the Qing-dao Livelihood, science and technology project, China.
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Table 4. Comparison of Adiponectin rs266729 and rs2241766 Genotypes With Clinicopathological Parameters

| Parameters               | rs266729 | rs2241766 |
|--------------------------|----------|-----------|
| Age, y                   | 46.54 ± 11.04 | 46.39 ± 12.08 | 46.39 ± 11.21 | 45.94 ± 11.09 |
| Gender, Female/Male      | 57/62    | 62/65     | 59/64        | 60/66        |
| Height, m                | 1.67 ± 0.06 | 1.67 ± 0.08 | 1.67 ± 0.07  | 1.68 ± 0.02  |
| Weight, kg               | 67.53 ± 6.31 | 65.98 ± 7.02 | 68.21 ± 5.98 | 67.34 ± 6.56 |
| BMI, kg/m²               | 24.29 ± 3.76 | 25.31 ± 3.12 | 23.67 ± 4.62 | 24.54 ± 3.21 |
| Hypertension, No. (%)    | 60 (50.4) | 61 (48.0) | 58 (48.3)    | 60 (47.6)    |
| ALT, U/L                 | 44.52 ± 21.43 | 43.62 ± 22.36 | 44.31 ± 21.54 | 43.96 ± 20.09 |
| AST, U/L                 | 41.34 ± 20.31 | 40.56 ± 21.09 | 40.99 ± 21.21 | 39.62 ± 20.97 |
| TG, mmol/L               | 2.85 ± 0.18 | 2.21 ± 0.96 | 2.83 ± 1.00  | 2.68 ± 0.96  |
| TC, mmol/L               | 5.62 ± 1.34 | 5.21 ± 0.99 | 5.67 ± 1.02  | 5.38 ± 1.01  |
| FPG, mmol/L              | 5.33 ± 0.71 | 5.01 ± 0.61 | 5.27 ± 0.61  | 5.17 ± 0.59  |
| HDL, mmol/L              | 1.37 ± 0.41 | 1.34 ± 0.42 | 1.43 ± 0.39  | 1.41 ± 0.41  |
| LDL, mmol/L              | 3.43 ± 0.94 | 2.96 ± 0.89 | 3.29 ± 0.97  | 3.21 ± 0.71  |
| Adiponectin, ug/mL       | 13.62 ± 4.32 | 16.82 ± 4.47 | 13.38 ± 3.21 | 14.98 ± 3.92 |

CAD-NAFLD

| Parameters               | rs266729 | rs2241766 |
|--------------------------|----------|-----------|
| Age, y                   | 62.87 ± 9.65 | 62.09 ± 9.87 | 62.41 ± 9.51 | 62.08 ± 9.61 |
| Gender, Female/Male      | 49/47    | 71/78     | 58/62        | 61/64        |
| Height, m                | 1.67 ± 0.06 | 1.67 ± 0.05 | 1.66 ± 0.08  | 1.67 ± 0.06  |
| Weight, kg               | 66.58 ± 6.71 | 67.32 ± 7.01 | 66.42 ± 6.52 | 66.67 ± 6.81 |
| Smoker, No. (%)          | 39 (40.6) | 65 (43.0) | 41 (34.2)    | 46 (36.2)    |
| BMI, kg/m²               | 24.89 ± 3.62 | 25.01 ± 3.01 | 24.42 ± 3.31 | 24.65 ± 3.45 |
| Hypertension, No. (%)    | 38 (39.6) | 60 (39.7)  | 43 (35.8)    | 47 (37.0)    |
| ALT, U/L                 | 41.02 ± 21.32 | 40.61 ± 20.56 | 41.45 ± 20.67 | 41.21 ± 20.81 |
| AST, U/L                 | 41.34 ± 22.09 | 40.21 ± 21.89 | 40.01 ± 21.09 | 40.56 ± 21.82 |
| TG, mmol/L               | 2.23 ± 0.81 | 2.01 ± 0.75 | 2.21 ± 0.76  | 2.17 ± 0.69  |
| TC, mmol/L               | 5.04 ± 0.91 | 5.05 ± 0.89 | 5.04 ± 0.84  | 5.04 ± 0.69  |
| FPG, mmol/L              | 5.21 ± 0.61 | 4.94 ± 0.57 | 5.19 ± 0.21  | 5.16 ± 0.22  |
| HDL, mmol/L              | 1.34 ± 0.32 | 1.36 ± 0.41 | 1.32 ± 0.25  | 1.35 ± 0.32  |
| LDL, mmol/L              | 2.63 ± 0.67 | 2.61 ± 0.68 | 2.59 ± 0.65  | 2.61 ± 0.67  |
| Adiponectin, ug/mL       | 13.26 ± 4.21 | 15.56 ± 4.31 | 14.26 ± 3.89 | 15.63 ± 4.01 |

Controls

| Parameters               | rs266729 | rs2241766 |
|--------------------------|----------|-----------|
| Age, y                   | 61.09 ± 9.21 | 61.23 ± 9.31 | 62.21 ± 9.02 | 61.59 ± 9.11 |
| Gender, Female/Male      | 41/44    | 111/108   | 57/62        | 95/90        |
| Height, m                | 1.68 ± 0.07 | 1.67 ± 0.09 | 1.68 ± 0.07  | 1.68 ± 0.08  |
| Weight, kg               | 66.65 ± 6.32 | 67.32 ± 5.94 | 67.01 ± 6.01 | 67.21 ± 5.45 |
| Smoker, No. (%)          | 29 (34.1) | 79 (36.1)  | 45 (37.8)    | 64 (34.6)    |

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|                  | Group 1          | Group 2          | Group 3          | Group 4          |
|------------------|------------------|------------------|------------------|------------------|
| **BMI, kg/m²**   | 23.11 ± 3.21     | 22.97 ± 3.01     | 24.01 ± 3.41     | 23.07 ± 3.01     |
| **Hypertension, No. (%)** | 31 (36.5) | 80 (36.5) | 37 (31.1) | 62 (33.5) |
| **ALT, U/L**     | 20.31 ± 7.21     | 20.56 ± 6.91     | 21.21 ± 7.09     | 20.56 ± 6.18     |
| **AST, U/L**     | 19.71 ± 7.33     | 20.81 ± 8.07     | 19.76 ± 7.85     | 19.81 ± 8.02     |
| **TG, mmol/L**   | 1.21 ± 0.41      | 1.41 ± 0.52      | 1.29 ± 0.33      | 1.36 ± 0.41      |
| **TC, mmol/L**   | 4.32 ± 0.81      | 4.02 ± 0.71      | 4.01 ± 0.36      | 4.28 ± 0.52      |
| **FPG, mmol/L**  | 4.86 ± 0.42      | 4.93 ± 0.31      | 4.92 ± 0.41      | 4.94 ± 0.44      |
| **HDL, mmol/L**  | 1.54 ± 0.46      | 1.55 ± 0.41      | 1.54 ± 0.42      | 1.54 ± 0.42      |
| **LDL, mmol/L**  | 2.59 ± 0.76      | 2.61 ± 0.71      | 2.61 ± 0.76      | 2.60 ± 0.68      |
| **Adiponectin, ug/ml** | 18.78 ± 3.21 | 19.02 ± 3.18 | 19.01 ± 2.97 | 19.36 ± 3.02 |

*Values are expressed as mean ± SD unless otherwise indicated.

aP = 0.039.
bP = 0.034.
cP = 0.007.
dP < 0.001.
eP = 0.014.
fP = 0.042.
gP = 0.035.
hP < 0.001.
iP = 0.038.
jP < 0.001.