Relationship between AGT rs2493132 polymorphism and the risk of coronary artery disease in patients with NAFLD in the Chinese Han population

-1-2

Relationship between *AGT* rs2493132 polymorphism and the risk of coronary artery disease in patients with NAFLD in the Chinese Han population

Mengzhen Dong[1],[*], Shousheng Liu[2],[3],[*],
Mengke Wang[1], Yifen Wang[1],
Yongning Xin[1],[2],[3],[4] and Shiying Xuan[1],[3]

Abstract

Objective: To investigate the relationship between angiotensin (*AGT*) rs2493132 gene polymorphism and the risk of developing non-alcoholic fatty liver disease (NAFLD) and coronary artery disease (CAD) in the Chinese Han population.

Methods: Polymerase chain reaction was performed to determine *AGT* genotypes. Anthropometric and clinical data were investigated and statistically analyzed in the clinical laboratory department of Qingdao Municipal Hospital.

Results: The *AGT* rs2493132 CT + TT genotype was an important risk factor for CAD in patients with NAFLD and NAFLD + CAD in healthy controls. The *AGT* rs2493132 T allele increased the risk of NAFLD + CAD in healthy controls. The *AGT* rs2493132 CT + TT genotype and T allele also significantly increased the risk of CAD in patients with NAFLD after adjustments for age, sex, and body mass index. In addition, *AGT* rs2493132 T allele carriers showed higher total cholesterol (TC) and low-density lipoprotein (LDL) levels compared with non-carriers.

Conclusions: The *AGT* rs2493132 CT + TT genotype and T allele significantly increased the risk of developing CAD in patients with NAFLD in the Chinese Han population. The *AGT* rs2493132 T allele was associated with increased serum TC and LDL levels.

[4]Department of Gastroenterology, Qingdao Municipal Hospital, Qingdao University, Qingdao, China

*These authors contributed equally to this work.

Corresponding author:
Yongning Xin, Department of Infectious Disease, Qingdao Municipal Hospital, Qingdao University, 1 Jiaozhou Road, Qingdao, Shandong Province 266011, China.
Email: xinyongning@163.com

[1]Department of Infectious Disease, Qingdao Municipal Hospital, Qingdao University, Qingdao, China
[2]Clinical Research Center, Qingdao Municipal Hospital, Qingdao University, Qingdao, China
[3]Digestive Disease Key Laboratory of Qingdao, Qingdao, China

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent form of chronic liver disease, with an estimated prevalence as high as 29.62% in the Asian population.[1] As a hepatic manifestation of metabolic syndrome, NAFLD has been shown to be an independent risk factor for cardiovascular disease (CVD).[2–5] The occurrence rate of coronary artery disease (CAD) has increased by 4.7-fold in adults with NAFLD, and the severity of CAD is significantly associated with the presence of NAFLD.[6] Multiple factors contribute to the pathogenesis of NAFLD and CAD.[7] Accumulated evidence suggests that gene polymorphism is an important risk factor not only for the occurrence of NAFLD or CAD in the general population but also for the incidence of CAD in patients with NAFLD.[3,8] Simons et al. investigated the effect of three variants in NAFLD susceptibility genes on the risk of developing CAD in patients with NAFLD.[9] The results showed that some variants provided a protective effect for the development of CAD, whereas others exerted a neutral effect on CAD risk. Glucokinase regulatory protein (GCKR) participates in de novo lipogenesis, and previous case-control studies suggested that *GCKR* rs780094 was significantly related to NAFLD.[10,11] A recent meta-analysis (including 274,625 individuals) demonstrated a significant association between *GCKR* rs780094 and the risk of CAD.[12] Therefore, the different effects of gene polymorphisms on plasma lipids may be a vital mediator between NAFLD and the risk of CAD.[3]

Angiotensinogen (AGT) plays an essential role in blood pressure regulation.[13–15] In addition, AGT is potentially involved in metabolic disorders.[14,16] For example, Tao et al. found that hepatocyte-specific AGT deficiency protected mice from western diet-induced liver steatosis and insulin resistance.[16] Recently, several clinical studies have reported a relationship between *AGT* gene polymorphism and the regulation of serum lipid metabolism. The *AGT* rs699 CC genotype was found to be significantly associated with high-density lipoprotein (HDL) levels in patients with peripheral arterial diseases.[17] Khamlaoui et al. reported that the *AGT* M235T genotype was associated with the serum levels of low-density lipoprotein (LDL) and total cholesterol (TC) and increased the risk of overweight-obesity.[18] Ji et al. suggested that *AGT* rs2493132 was associated with hypertension in the Chinese Han population, indicating that this polymorphism might be a risk factor for CAD.[19]

Although the association of *AGT* gene polymorphisms with serum lipid metabolism and CAD has been investigated in several studies, the exact relationship between *AGT* gene polymorphism and the risk of NAFLD and CAD remains unclear. This study was designed to investigate the effect of *AGT* rs2493132 on the risk of developing NAFLD and CAD in the Chinese Han population and determine the influence of *AGT* rs2493132 on serum lipid profiles.

Materials and methods

Study subjects

This study was approved by the Ethics Committee of Qingdao Municipal Hospital (approval number: 2017LSZD20) and conducted in accordance with the principles of the Helsinki Declaration and its appendices.[20] All participants filled out an informed consent form before this study. From July 2017 to December 2018, patients with NAFLD and CAD (NAFLD + CAD), patients with NAFLD only, and healthy controls were recruited at Qingdao Municipal Hospital. The diagnosis of NAFLD was based on the guidelines of the American Association for the Study of Liver Disease.[21] CAD was diagnosed by a percutaneous coronary angiography and defined as ≥50% stenosis in at least one coronary artery. Subjects with other liver diseases, such as autoimmune hepatitis, drug-induced hepatitis, viral hepatitis, hepatocellular carcinoma, were excluded from this study.

Biochemical analyses

Demographic characteristics (age and sex) were assessed by a questionnaire, and anthropometric parameters (height and weight) and blood pressure were measured using standard methods. Body mass index (BMI) was calculated as weight (kg)/height (m)^2. Blood samples were collected from each subject's median vein for biochemical analysis following 12 hours of fasting. Serum aspartic aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamine transpeptidase (GGT), triglyceride (TG), TC, HDL, LDL, and fasting blood glucose (FPG) were detected in the clinical laboratory department of our hospital.

Genomic DNA extraction and genotyping

Genomic DNA was extracted from ethylenediaminetetraacetic acid-anticoagulated whole blood using a TIANamp Blood DNA Kit (Tiangen Biochemical Technology, Beijing, China) and stored at −20°C until use. *AGT* rs2493132 was genotyped by polymerase chain reaction with the corresponding primers 5′-ACGTTGGATGGGATTTGACATGAGAGTGGC-3′ and 5′-ACGTTGGATGATTTACCTAGCCTCCGGATG-3′. The ABI Veriti-384 Prism Sequence Detection System (Applied Biosystems, Foster City, CA, USA) was used to detect the rs2493132 genotype by direct DNA sequencing, and MassARRAY TYPER4.0 software (Agena Bioscience, Inc., San Diego, CA, USA) was used to analyze the original data.

Statistical analysis

All analyses were performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA). The Pearson's χ^2 test was used to analyze the Hardy–Weinberg equilibrium in the distributions of genotypes among subjects. Continuous variables were compared by an unpaired Student's t-test and expressed as the mean ± standard deviation when normally distributed. Categorical variables were compared using the Chi-square test. The association between *AGT* rs2493132 and the risk of NAFLD and NAFLD + CAD was determined by calculating the crude and adjusted odds ratios (ORs) and 95% confidence intervals (CI) from the logistic regression analyses. Statistical significance was considered at $P < 0.05$.

Results

Clinical characteristics of the study population

The clinical characteristics of 142 patients with NAFLD (mean age: 40.95 ± 5.96 years), 109 patients with NAFLD + CAD (mean age: 59.05 ± 7.95 years), and 246

healthy controls (mean age: 47.40 ±11.52 years) were summarized in Table 1. Patients with NAFLD had higher BMI, ALT, AST, GGT, TG, systolic blood pressure (SBP), and diastolic blood pressure (DBP) than the healthy controls (all $P < 0.05$). Additionally, patients with NAFLD + CAD exhibited higher BMI, ALT, ALP, TG, and SBP and lower serum levels of TC, HDL, and LDL compared with the healthy controls (all $P < 0.05$). Last, patients with NAFLD + CAD had lower BMI, GGT, TC, HDL, and LDL and higher serum ALP levels and SBP than patients with NAFLD (all $P < 0.05$).

Genotypes and allele distribution of AGT rs2493132

The genotype distributions of *AGT* rs2493132 in the healthy control, NAFLD, and NAFLD + CAD groups were confirmed to be in Hardy–Weinberg equilibrium (Table 2). There was no significant difference in the genotype distributions of *AGT* rs2493132 between the NAFLD and healthy control groups or between the NAFLD + CAD and NAFLD groups. In addition, the distributions of the *AGT* rs2493132 allele between the NAFLD and healthy control groups and between the NAFLD + CAD and NAFLD groups were not significantly different. However, the genotype and allele distributions of *AGT* rs2493132 between the NAFLD + CAD and control groups were significantly different ($P = 0.048$ and $P = 0.015$, respectively) (Table 3). The results of logistic regression analysis suggested that the *AGT* rs2493132 CT+TT genotype was an important risk factor for CAD in patients with NAFLD (OR = 1.785, 95%CI: 1.061–3.003, $P = 0.029$) and NAFLD + CAD in healthy controls (OR = 1.738, 95%CI: 1.082–2.793,

Table 1. Basic characteristics of the study population.

Characteristics	Controls (n = 246)	NAFLD (n = 142)	NAFLD + CAD (n = 109)	P1	P2	P3
Men/Women	149/97	95/47	75/34	0.251	0.139	0.750
Age (year)	47.40 ±11.52	40.95 ± 5.96	59.05 ± 7.95	<0.001	<0.001	<0.001
BMI (kg/m^2)	23.65 ± 3.23	26.82 ± 2.77	25.27 ± 2.61	<0.001	<0.001	<0.001
ALT (U/L)	22.64 ± 18.20	34.60 ± 25.42	30.06 ± 28.16	<0.001	0.003	0.183
AST (U/L)	21.88 ± 6.26	24.16 ± 8.86	23.53 ± 13.96	0.003	0.133	0.670
GGT (U/L)	26.00 ± 14.67	35.80 ± 18.28	29.36 ± 15.70	<0.001	0.061	0.005
ALP (U/L)	70.72 ± 21.08	70.95 ± 19.06	85.60 ± 20.77	0.915	<0.001	<0.001
FPG (mmol/L)	5.36 ± 7.64	5.54 ± 6.28	6.02 ± 2.17	0.817	0.381	0.447
TG (mmol/L)	1.40 ± 0.97	2.22 ± 3.10	2.10 ± 3.85	<0.001	0.008	0.789
TC (mmol/L)	5.26 ± 1.53	5.42 ± 0.95	4.64 ± 1.23	0.259	<0.001	<0.001
HDL (mmol/L)	1.34 ± 0.54	1.24 ± 0.26	1.04 ± 0.27	0.08	<0.001	<0.001
LDL (mmol/L)	3.19 ± 1.42	3.28 ± 0.59	2.81 ± 0.89	0.528	0.011	<0.001
SBP (mmHg)	118.65 ± 13.21	123.53 ± 13.01	133.83 ± 17.40	0.002	<0.001	<0.001
DBP (mmHg)	81.18 ± 11.36	84.32 ± 9.16	80.78 ± 9.70	0.012	0.771	0.030

P1, NAFLD group vs control group; P2, NAFLD + CAD group vs control group; P3, NAFLD + CAD group vs NAFLD group.
BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamine transpeptidase; ALP, alkaline phosphatase; FPG, fasting blood glucose; TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NAFLD, non-alcoholic fatty liver disease; NAFLD + CAD, NAFLD with coronary artery disease; SBP, systolic blood pressure; DBP, diastolic blood pressure.

$P = 0.022$). The *AGT* rs2493132 T allele did not show a significant association with the risk of CAD in patients with NAFLD but did increase the risk of NAFLD + CAD in healthy controls (OR = 1.501, 95%CI: 1.081–2.084, $P = 0.015$). After adjustments for age, sex, and BMI, both the *AGT* rs2493132 CT+TT genotype and T allele were identified as significant risk factors for the development of CAD in patients with NAFLD (OR = 4.718, 95%CI: 1.502–14.824, $P = 0.008$; OR = 2.436, 95%CI: 1.184–5.011, $P = 0.016$, respectively) (Table 4).

Association of the AGT rs2493132 polymorphism with clinical characteristics in the overall patient series

To investigate the effect of *AGT* rs2493132 polymorphism on the clinical parameters of the enrolled subjects, we compared the differences in clinical parameters of the *AGT* rs2493132 T allele carriers and non-carriers among all patients. As shown in Table 5, the serum levels of TC and LDL in the T allele carriers were higher than those in non-carriers ($P = 0.045$ and 0.044, respectively). There was no significant difference in the other parameters, such as ALT, AST, GGT, ALP, TG, or HDL, between the T allele carriers and non-carriers. The above results suggest that the *AGT* rs2493132 T allele variant may increase serum TC and LDL levels in the general population.

Discussion

NAFLD is a multisystem disease and is significantly associated with the risk of CVD.[22] Previous studies demonstrated that NAFLD is an independent risk factor for CAD, and CAD is the leading cause of death among patients with NAFLD.[2,4,22] In a study conducted in Korea, Lee et al. showed that NAFLD was an independent predictor of CAD, and the presence of coronary artery stenosis was significantly correlated with fatty liver disease in a grade-dependent manner ($P = 0.025$).[23] Accumulated studies have demonstrated that gene polymorphisms are significant risk factors for NAFLD and CAD. In the Chinese Han population, Liu et al. reported that genetic variants of tribbles-1 rs17321515 increased the risk of CAD in patients with NAFLD.[24] Cheng et al.

Table 2. Results of the Hardy–Weinberg Equilibrium analysis[a].

Groups	χ^2	P value
Control	0.010	0.921
CAD − NAFLD	1.083	0.298
CAD + NAFLD	0.308	0.579

[a]Data were compared by the Chi-square test.
NAFLD, non-alcoholic fatty liver disease; CAD, coronary artery disease.

Table 3. Distribution of *AGT* rs2493132 genotypes and alleles in the study group.

Genotype	Controls	NAFLD	NAFLD + CAD	χ^2	P1	χ^2	P2	χ^2	P3
Genotype				0.873	0.646	4.807	0.094	6.075	0.048
CC	111	65	35						
CT	109	58	56						
TT	26	19	18						
Allele				0.095	0.758	3.714	0.054	5.917	0.015
C	331	188	126						
T	161	96	92						

P1, NAFLD group vs control group; P2, NAFLD + CAD vs NAFLD; P3, NAFLD + CAD vs. control group.
NAFLD, non-alcoholic fatty liver disease; CAD, coronary artery disease.

Table 4. Odds ratios according to *AGT* rs2493132 genotypes in the study group.

	Unadjusted						Adjusted[a]					
Genotype	OR (95% CI)	P1	OR (95% CI)	P2	OR (95% CI)	P3	OR (95% CI)	P1	OR (95% CI)	P2	OR (95% CI)	P3
Genotype												
CC	—		—		—		—		—		—	
CT+TT	0.974 (0.643–1.475)	0.901	1.785 (1.061–3.003)	0.029	1.738 (1.082–2.793)	0.022	0.748 (0.450–1.245)	0.264	4.718 (1.502–14.824)	0.008	1.583 (0.901–2.780)	0.107
Allele												
C	—		—		—		—		—		—	
T	1.050 (0.770–1.431)	0.758	1.430 (0.993–2.058)	0.054	1.501 (1.081–2.084)	0.015	0.882 (0.604–1.288)	0.516	2.436 (1.184–5.011)	0.016	1.338 (0.907–1.973)	0.142

[a]Binary logistic regression model was used for the adjustment of age, sex, and body mass index.
OR, odds ratio; CI, confidence interval; P1, NAFLD group vs control group; P2, NAFLD + CAD vs control group; P3, NAFLD + CAD vs NAFLD.

showed that tumor necrosis factor-α-238 gene polymorphism also increases the risk of developing CAD in patients with NAFLD in the Chinese Han population.[25] Moreover, variants in patatin-like phospholipase domain-containing protein (*PNPLA3*) and transmembrane 6 superfamily member 2 (*TM6SF2*) in patients predisposed to NAFLD conferred a mild protective effect for CAD, and both *TM6SF2* and *PNPLA3* gene polymorphisms were associated with lower plasma lipid levels.[3,26] These results suggested that gene polymorphisms play an important role in the development of CAD in patients with NAFLD.

A previous study showed that the *AGT* M35T gene polymorphism was strongly associated with an increased risk of heart failure.[27] A study in Saudi Arabia reported that *AGT* variants (rs5051C and rs7079G) were related to CAD.[28] In the current study, we investigated the effect of the *AGT* rs2493132 polymorphism on the risk of NAFLD in the general population and the risk of CAD in patients with NAFLD for the first time. We found that the *AGT* rs2493132 CT+TT genotype but not the *AGT* rs2493132 T allele was significantly associated with the risk of developing CAD in patients with NAFLD. After adjustments for age, sex, and BMI, the *AGT* rs2493132 CT+TT genotype remained associated with the risk of CAD. In addition, the *AGT* rs2493132 T allele significantly increased the risk of developing CAD in patients with NAFLD after adjustments for these parameters.

Dyslipidemia is a common feature of patients with both NAFLD and CAD and is characterized by elevated plasma triacylglycerol and circulating LDL particles and reduced HDL.[29–32] Brouwers et al. suggested that plasma lipids act as major mediators of the relationship between NAFLD and CVD.[3] The analysis of patient characteristics in this study revealed that patients

Table 5. Clinical characteristics of *AGT* rs2493132 T allele carriers and non-carriers in the overall study group.

	Carriers	Non-carriers	*P* value
Male/Female	184/102	135/76	0.935
Age (year)	48.69 ± 11.62	47.33 ± 11.14	0.193
BMI (kg/m^2)	26.16 ± 3.34	24.64 ± 3.12	0.083
ALT (U/L)	27.36 ± 24.15	28.16 ± 22.41	0.710
AST (U/L)	22.95 ± 10.18	22.79 ± 7.40	0.848
GGT (U/L)	29.92 ± 16.78	28.96 ± 16.13	0.536
ALP (U/L)	75.19 ± 20.29	72.37 ± 22.55	0.147
TG (mmol/L)	1.78 ± 1.92	1.81 ± 3.68	0.867
TC (mmol/L)	5.31 ± 1.54	5.06 ± 1.18	0.045
HDL (mmol/L)	1.21 ± 0.36	1.28 ± 0.53	0.108
LDL (mmol/L)	3.25 ± 1.51	3.03 ± 0.75	0.044
FPG (mmol/L)	5.42 ± 5.07	5.75 ± 7.85	0.584
SBP (mmHg)	124.89 ± 15.13	124.63 ± 16.41	0.876
DBP (mmHg)	82.39 ± 9.94	81.99 ± 10.63	0.707

BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamine transpeptidase; ALP, alkaline phosphatase; TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FPG, fasting blood glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure.

with NAFLD exhibit higher BMI, AST, ALT, TG, LDL, and GGT and decreased HDL levels compared with healthy controls, consistent with the results in previous studies.[30,33–35] In the overall patient group in this study, we found that *AGT* rs2493132 T allele carriers were more likely to have higher serum TC and LDL levels compared with non-carriers. A previous study suggested that decreased LDL levels protected patients with NAFLD from CVD, and NAFLD susceptibility genes contributed to the strong relationship between plasma lipids and the risk of CAD.[36,37] These data suggested that *AGT* rs2493132 gene polymorphism may increase the risk of CAD in patients with NAFLD by affecting serum TC and LDL levels.

There were several limitations to this study. First, the diagnosis of NAFLD was determined by ultrasonography rather than liver biopsy due to the limited acceptability of patients to liver biopsies during clinical examinations. Second, CAD usually occurs more often in elderly patients; therefore, the mean age in patients with NAFLD + CAD was higher than in patients with NAFLD and healthy controls. Third, all study subjects in the study were Han Chinese in Qingdao. Therefore, the effects of *AGT* rs2493132 on the risk of NAFLD and CAD should be studied in other countries and ethnicities.

Conclusion

This study investigated the effect of *AGT* rs2493132 polymorphism on the risk of NAFLD and CAD in the Chinese Han population. The results showed that the *AGT* rs2493132 CT+TT genotype was significantly associated with the risk of CAD in patients with NAFLD and NAFLD + CAD in healthy controls. The *AGT* rs2493132 T allele increased the risk of NAFLD + CAD in healthy controls. After adjustments for age, sex, and BMI, the *AGT* rs2493132 CT+TT genotype and T allele remained as significant risk factors for CAD in patients with NAFLD. In

addition, *AGT* rs2493132 T allele carriers showed higher LDL and TC levels compared with non-carriers among all subjects. Our study was the first to investigate the effect of *AGT* rs2493132 polymorphism on the risk of developing NAFLD and CAD. Our results should be verified in other countries and ethnicities in future studies.

Author contributions

Study concept and design: Shiying Xuan and Yongning Xin
Data acquisition: Mengzhen Dong, Shousheng Liu, Mengke Wang, and Yifen Wang
Drafting of the manuscript: Mengzhen Dong, Shousheng Liu, Mengke Wang, and Yifen Wang
Critical revision of the manuscript for important intellectual content: Shiying Xuan and Yongning Xin

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

Funding

ORCID iD

Yongning Xin   https://orcid.org/0000-0002-3692-7655

References

1. Li J, Zou B, Yeo YH, et al. Prevalence, incidence, and outcome of non-alcoholic fatty liver disease in Asia, 1999-2019: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2019; 4: 389–398. doi: 10.1016/S2468-1253(19)30039-1.

2. Targher G, Byrne CD, Lonardo A, et al. Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: A meta-analysis. *J Hepatol* 2016; 65: 589–600. doi: 10.1016/j.jhep.2016.05.013.

3. Brouwers M, Simons N, Stehouwer CDA, et al. Non-alcoholic fatty liver disease and cardiovascular disease: assessing the evidence for causality. *Diabetologia* 2020; 63: 253–260. doi: 10.1007/s00125-019-05024-3.

4. Athyros VG, Tziomalos K, Katsiki N, et al. Cardiovascular risk across the histological spectrum and the clinical manifestations of non-alcoholic fatty liver disease: An update. *World J Gastroenterol* 2015; 21: 6820–6834. doi: 10.3748/wjg.v21.i22.6820.

5. Liu HH, Cao YX, Sun D, et al. Impact of Non-Alcoholic Fatty Liver Disease on Cardiovascular Outcomes in Patients With Stable Coronary Artery Disease: A Matched Case-Control Study. *Clin Transl Gastroenterol* 2019; 10: e00011. doi: 10.14309/ctg.0000000000000011.

6. Öztürk H, Gümrükçüoğlu HA, Yaman M, et al. Hepatosteatosis and carotid intima-media thickness in patients with myocardial infarction. *J Med Ultrason (2001)* 2016; 43: 77–82. doi: 10.1007/s10396-015-0649-x.

7. Wojcik-Cichy K, Koslinska-Berkan E and Piekarska A. The influence of NAFLD on the risk of atherosclerosis and cardiovascular diseases. *Clin Exp Hepatol* 2018; 4: 1–6. doi: 10.5114/ceh.2018.73155.

8. Romeo S, Kozlitina J, Xing C, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008; 40: 1461–1465. doi: 10.1038/ng.257.

9. Simons N, Isaacs A, Koek GH, et al. PNPLA3, TM6SF2, and MBOAT7 Genotypes and Coronary Artery Disease. *Gastroenterology* 2017; 152: 912–913. doi: 10.1053/j.gastro.2016.12.020.

10. Brouwers M, Jacobs C, Bast A, et al. Modulation of Glucokinase Regulatory Protein: A Double-Edged Sword? *Trends Mol Med* 2015; 21: 583–594. doi: 10.1016/j.molmed.2015.08.004.

11. Kitamoto A, Kitamoto T, Nakamura T, et al. Association of polymorphisms in GCKR and TRIB1 with nonalcoholic fatty liver disease and metabolic syndrome traits. *Endocr J* 2014; 61: 683–689. doi: 10.1507/endocrj.ej14-0052.

12. Simons P, Simons N, Stehouwer CDA, et al. Association of common gene variants in glucokinase regulatory protein with cardiorenal disease: A systematic review and meta-

analysis. *PLoS One* 2018; 13: e0206174. doi: 10.1371/journal.pone.0206174.

13. Lu H, Cassis LA, Kooi CW, et al. Structure and functions of angiotensinogen. *Hypertens Res* 2016; 39: 492–500. doi: 10.1038/hr.2016.17.

14. Lu H, Wu C, Howatt DA, et al. Angiotensinogen Exerts Effects Independent of Angiotensin II. *Arterioscler Thromb Vasc Biol* 2016; 36: 256–265. doi: 10.1161/atvbaha.115.306740.

15. Almeida LF, Tofteng SS, Madsen K, et al. Role of the renin-angiotensin system in kidney development and programming of adult blood pressure. *Clin Sci (Lond)* 2020; 134: 641–656. doi: 10.1042/cs20190765.

16. Tao XR, Rong JB, Lu HS, et al. Angiotensinogen in hepatocytes contributes to Western diet-induced liver steatosis. *J Lipid Res* 2019; 60: 1983–1995. doi: 10.1194/jlr.M093252.

17. Junusbekov Y, Bayoglu B, Cengiz M, et al. AGT rs699 and AGTR1 rs5186 gene variants are associated with cardiovascular-related phenotypes in atherosclerotic peripheral arterial obstructive disease. *Ir J Med Sci* 2019. doi: 10.1007/s11845-019-02166-6.

18. Khamlaoui W, Mehri S, Hammami S, et al. Association of angiotensin-converting enzyme insertion/deletion (ACE I/D) and angiotensinogen (AGT M235T) polymorphisms with the risk of obesity in a Tunisian population. *J Renin Angiotensin Aldosterone Syst* 2020; 21: 1470320320907820. doi: 10.1177/1470320320907820.

19. Ji L, Cai X, Zhang L, et al. Association between polymorphisms in the renin-angiotensin-aldosterone system genes and essential hypertension in the Han Chinese population. *PLoS One* 2013; 8: e72701. doi: 10.1371/journal.pone.0072701.

20. Rickham PP. Human Experimentation. Code of Ethics of the World Medical Association. Declaration of Helsinki. *Br Med J* 1964; 2: 177. doi: 10.1136/bmj.2.5402.177.

21. Fan JG, Jia JD, Li YM, et al. Guidelines for the diagnosis and management of nonalcoholic fatty liver disease: update 2010: (published in Chinese on Chinese Journal of Hepatology 2010; 18: 163-166). *J Dig Dis* 2011; 12: 38–44. doi: 10.1111/j.1751-2980.2010.00476.x.

22. Byrne CD and Targher G. NAFLD: a multisystem disease. *J Hepatol* 2015; 62: S47–S64. doi: 10.1016/j.jhep.2014.12.012.

23. Lee JE, Lee YJ, Chung SY, et al. Severity of nonalcoholic fatty liver disease is associated with subclinical cerebro-cardiovascular atherosclerosis risk in Korean men. *PLoS One* 2018; 13: e0193191. doi: 10.1371/journal.pone.0193191.

24. Liu Q, Liu SS, Zhao ZZ, et al. TRIB1 rs17321515 gene polymorphism increases the risk of coronary heart disease in general population and non-alcoholic fatty liver disease patients in Chinese Han population. *Lipids Health Dis* 2019; 18: 165. doi: 10.1186/s12944-019-1108-2.

25. Cheng Y, An B, Jiang M, et al. Association of Tumor Necrosis Factor-alpha Polymorphisms and Risk of Coronary Artery Disease in Patients With Nonalcoholic Fatty Liver Disease. *Hepat Mon* 2015; 15: e26818. doi: 10.5812/hepatmon.26818.

26. Liu DJ, Peloso GM, Yu H, et al. Exome-wide association study of plasma lipids in >300,000 individuals. *Nat Genet* 2017; 49: 1758–1766. doi: 10.1038/ng.3977.

27. Chen S, Zhang L, Wang HW, et al. The M235T polymorphism in the angiotensinogen gene and heart failure: a meta-analysis. *J Renin Angiotensin Aldosterone Syst* 2014; 15: 190–195. doi: 10.1177/1470320312465455.

28. Al-Najai M, Muiya P, Tahir AI, et al. Association of the angiotensinogen gene polymorphism with atherosclerosis and its risk traits in the Saudi population. *BMC Cardiovasc Disord* 2013; 13: 17. doi: 10.1186/1471-2261-13-17.

29. Brouwers MC, Cantor RM, Kono N, et al. Heritability and genetic loci of fatty liver in familial combined hyperlipidemia. *J Lipid Res* 2006; 47: 2799–2807. doi: 10.1194/jlr.M600312-JLR200.

30. DeFilippis AP, Blaha MJ, Martin SS, et al. Nonalcoholic fatty liver disease and serum lipoproteins: the Multi-Ethnic Study of Atherosclerosis. *Atherosclerosis* 2013; 227:

429–436.   doi: 10.1016/j.
atherosclerosis.2013.01.022.

31. Do R, Willer CJ, Schmidt EM, et al.
Common variants associated with plasma
triglycerides and risk for coronary artery dis-
ease. *Nat Genet* 2013; 45: 1345–1352. doi:
10.1038/ng.2795.

32. Chapman MJ, Guérin M and Bruckert E.
Atherogenic, dense low-density lipoproteins.
Pathophysiology and new therapeutic
approaches. *Eur Heart J* 1998; 19: A24–A30.

33. Mato JM, Alonso C, Noureddin M, et al.
Biomarkers and subtypes of deranged lipid
metabolism in non-alcoholic fatty liver dis-
ease. *World J Gastroenterol* 2019; 25:
3009–3020. doi: 10.3748/wjg.v25.i24.3009.

34. Vilar CP, Cotrim HP, Florentino GS, et al.
Nonalcoholic fatty liver disease in patients
with coronary disease from a Brazil northeast

area. *Arq Gastroenterol* 2015; 52: 111–116.
doi: 10.1590/s0004-28032015000200007.

35. Liu H, Yao Y, Wang Y, et al. Ideal cardio-
vascular health metrics and the risk of
non-alcoholic fatty liver disease: A cross-
sectional study in northern China. *Liver Int*
2019; 39: 950–955. doi: 10.1111/liv.14060.

36. Dongiovanni P, Petta S, Maglio C, et al.
Transmembrane 6 superfamily member 2
gene variant disentangles nonalcoholic stea-
tohepatitis from cardiovascular disease.
Hepatology 2015; 61: 506–514. doi:
10.1002/hep.27490.

37. Brouwers M, Simons N, Stehouwer CDA,
et al. Relationship Between Nonalcoholic
Fatty Liver Disease Susceptibility Genes
and Coronary Artery Disease. *Hepatol
Commun* 2019; 3: 587–596. doi: 10.1002/
hep4.1319.