

***Apolipoprotein C3* (-455T>C) polymorphism confers susceptibility to nonalcoholic fatty liver disease in the Southern Han Chinese population**

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Abstract

AIM: To investigate the relationship between *Apolipoprotein C3* (*APOC3*) (-455T>C) polymorphism and nonalcoholic fatty liver disease (NAFLD) in the Southern Chinese Han population.

METHODS: In this prospective case-control study, we recruited 300 NAFLD patients and 300 healthy controls to a cohort representing Southern Chinese Han population at The First Affiliated Hospital, Sun Yat-sen University, from January to December 2012. Polymerase chain reaction-restriction fragment length polymorphism and DNA sequencing were used to genotype the *APOC3* (-455T>C) variants.

RESULTS: After adjusting for age, gender, and body-

mass index, TC and CC genotypes were found to increase the susceptibility to NAFLD compared to the TT genotype, with adjusted odds ratios (ORs) of 1.77 (95%CI: 1.16-2.72) and 2.80 (95%CI: 1.64-4.79), respectively. Further stratification analysis indicated that carriers of the CC genotype was more susceptible to insulin resistance (IR) than those of the TT genotype, with an OR of 3.24 (95%CI: 1.52-6.92). The CC genotype also was associated with a significantly higher risk of hypertension, hypertriglyceridemia, and low levels of high-density lipoprotein cholesterol (HDL) ($P < 0.05$). No association was found between the *APOC3* (-455T>C) polymorphism and obesity, impaired glucose tolerance, hyperuricemia, hypercholesterolemia, or high levels of low-density lipoprotein cholesterol (LDL) ($P > 0.05$).

CONCLUSION: *APOC3* (-455T>C) genetic variation is involved in the susceptibility to developing NAFLD, IR, hypertension, hypertriglyceridemia, and low HDL in the Southern Chinese Han population.

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Key words: *Apolipoprotein C3*; Nonalcoholic fatty liver disease; Insulin resistance; Metabolic disorder; Polymorphism

Core tip: This study represents the first study to investigate the relationship between the *Apolipoprotein C3* (*APOC3*) (-455T>C) polymorphism and nonalcoholic fatty liver disease (NAFLD) susceptibility in the Southern Chinese Han population. After adjusting for age, gender, and body-mass index, we found that *APOC3* (-455T>C) genetic variation was involved in the susceptibility to developing NAFLD, insulin resistance, hypertension, hypertriglyceridemia, and low high-density lipoprotein cholesterol. In the additive genetic model, carriers of variant-type homozygote CC showed the highest susceptibility to the above disorders, followed

by carriers of heterozygote TC and wild-type homozygote TT.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is an escalating public health problem, affecting up to 35% of adults in the United States and 15% in relatively affluent regions in China^[1,2]. NAFLD includes a spectrum of hepatic disorders extending from nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis (NASH), fibrosis/cirrhosis, and hepatocellular carcinoma (HCC). It was estimated that 10%-20% of patients with NAFL would develop NASH, and 10%-29% of patients with NASH would progress to cirrhosis within 10 years^[3,4]. The hallmark of NAFLD is an imbalance between triglyceride (TG) acquisition and removal in the liver, and steatosis is defined as TG accumulation to levels that are more than 5% of the total liver weight. Therefore, genetic factors that promote TG acquisition in the liver and inhibit TG removal from the liver would cause steatosis^[5]. The major pathway for TG export from the liver is secretion into the blood as very-low-density lipoprotein (VLDL). Mutations in the major components involved in VLDL pathways may be additional causes for hepatic steatosis^[5].

Apolipoprotein C3 (APOC3) is a major constituent of VLDL, and inhibits the hydrolysis of TG-rich particles by lipoprotein lipase^[6]. Transgenic mice overexpressing human *APOC3* were predisposed to hepatic steatosis, which indicated that *APOC3* might play an important role in the development of NAFLD^[7,8]. Recently, a single-nucleotide polymorphism [SNP; rs2854116, *APOC3* (-455T>C)] in the promoter region of *APOC3* has been reported to be associated with the susceptibility to developing NAFLD and insulin resistance (IR)^[9]. However, conclusions drew from other studies were not in accordance with that finding^[10-12]. This inconsistency might be due to factors such as gender, geographical region, and ethnicity. Until now, no data about this field have been reported in the Southern Chinese Han population. In this prospective case-control study, we explored the genotype frequency of *APOC3* (-455T>C) in the Southern Chinese Han population and analyzed its association with NAFLD and metabolic disorders.

MATERIALS AND METHODS

Ethics statement

This study protocol was approved by the Human Ethics Committee of The First Affiliated Hospital, Sun Yat-sen

University. In addition, written consent was given by the patients for their information to be stored in the hospital database and used for research.

Subjects

In this prospective, case-control study, 600 unrelated individuals, including 300 NAFLD patients and 300 age- and gender-matched healthy controls, were enrolled between January and December 2012. The study was conducted in the health examination center of The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China. The male-to-female ratio was 2. For the NAFLD group, subjects were enrolled after a diagnosis of fatty liver defined by the presence of at least two of the following three abnormal findings on abdominal ultrasonography: diffusely increased liver near field ultrasound echo ("bright liver"); liver echo greater than kidney echo; vascular blurring and the gradual attenuation of far field ultrasound echo^[13]. Subjects with secondary diagnoses of hepatic steatosis, such as that caused by alcohol abuse (alcohol consumption > 140 g/wk in men or > 70 g/wk in women), hepatitis B, hepatitis C, or other cryptogenic liver diseases, were excluded from the study. For the healthy control group, subjects who were free of elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and had no liver steatosis examined by abdominal ultrasonography, and lacked any sign of metabolic disorders such as hypertension, overweight, obesity, hyperuricemia, and dyslipidemia were enrolled. All subjects were of Southern Chinese Han ethnicity.

Clinical data collection

Demographic and clinical data were collected by structured interview using a questionnaire. Body mass, height, and blood pressure were measured by trained nurses. Body-mass index [BMI (kg/m²)] was calculated. BMI ≥ 25 kg/m² was considered as obesity. Abdominal ultrasonography and blood biochemical analysis were routinely conducted. TG, high-density lipoprotein cholesterol (HDL), total cholesterol, low-density lipoprotein cholesterol (LDL), fasting plasma glucose (FPG), fasting plasma insulin (FPI), serum uric acid (UA), ALT, and AST were measured with an Abbott i2000 Automatic Biochemistry Analyzer (Abbott, United States). The homeostasis model for assessment of insulin resistance (HOMA-IR) was calculated as described in a previous study^[14]. IR was defined as HOMA-IR > 3. Hypertension, low HDL, hypertriglyceridemia, and impaired glucose tolerance (IGT) were diagnosed using the International Diabetes Federation consensus worldwide definition of metabolic syndrome^[15]. Hyperuricemia, hypercholesterolemia, and increased LDL were diagnosed according to American College of Rheumatology guidelines for management of gout and ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults^[16,17].

DNA extraction

Genomic DNA was extracted from 200 uL of peripheral blood using the Blood DNA Extraction Kit (Qiagen, Ger-

Table 1 Clinical characteristics between the control group and the nonalcoholic fatty liver disease group *n* (%)

Item	Control	NAFLD	<i>P</i> value	Frequency of disorders in NAFLD group
Gender (<i>n</i>)				--
Male	200	200	1.0	--
Female	100	100	1.0	--
Age (yr)	39.5 ± 9.1	40.7 ± 9.7	0.11	--
BMI (kg/m ²)	22.1 ± 2.8	26.3 ± 4.2	< 0.001	186 (62.0)
Blood pressure (mmHg)	--	--	--	64 (21.3)
Systolic pressure	119 ± 13	127 ± 13	< 0.001	--
Diastolic pressure	75 ± 10	82 ± 9	< 0.001	--
HDL (mmol/L)	--	--	--	128 (42.7)
Male	1.4 ± 0.2	1.1 ± 0.2	< 0.001	107 (53.5)
Female	1.4 ± 0.2	1.3 ± 0.3	< 0.001	21 (21.0)
Total cholesterol (mmol/L)	4.7 ± 0.5	5.4 ± 1.0	< 0.001	122 (40.7)
TG (mmol/L)	1.0 ± 0.5	2.5 ± 2.5	< 0.001	119 (39.7)
UA (μmol/L)	--	--	--	104 (34.7)
Male	335.3 ± 51.2	415.5 ± 97.2	< 0.001	89 (44.5)
Female	241.3 ± 39.5	324.0 ± 83.6	< 0.001	15 (15.0)
LDL (mmol/L)	2.7 ± 0.5	3.3 ± 0.9	< 0.001	90 (30.0)
FPI (μU/mL)	6.0 ± 3.1	10.62 ± 4.5	< 0.001	--
HOMA-IR	1.2 ± 0.7	2.4 ± 1.2	< 0.001	74 (24.7)
FPG (mmol/L)	4.8 ± 0.5	5.3 ± 1.1	< 0.001	67 (22.3)
ALT (U/L)	19.0 ± 8.7	42.1 ± 32.6	< 0.001	114 (38.0)
AST (U/L)	24.1 ± 4.9	33.1 ± 14.8	< 0.001	148 (49.3)

NAFLD: Nonalcoholic fatty liver disease; BMI: Body mass index; HDL: High-density lipoprotein cholesterol; TG: Triglyceride; UA: Uric acid; LDL: Low-density lipoprotein cholesterol; FPI: Fasting plasma insulin; HOMA-IR: Homeostasis model of assessment for insulin resistance index; FPG: Fasting plasma glucose; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

many). DNA concentration and quality were measured and assessed by Nanodrop 2000C (Thermo Fisher, United States) and 2% agarose gel electrophoresis (Sigma, Germany).

Genotype analysis

Genotypes of *APOC3* (-455T>C) were examined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. The primers were described previously^[18], and were used for PCR with an annealing temperature of 68 °C to amplify a 194-bp fragment containing the *APOC3* target site (-455T>C). PCR products were separated by 8% polyacrylamide gel electrophoresis (PAGE) after digestion with BtsCI (New England BioLabs, United States) in a water bath at 50 °C overnight. To confirm the genotyping results, randomly selected PCR samples were examined by DNA sequencing using ABI 3730 XL (ABI, United States) by the chain termination method (Shenzhen BGI, China).

Statistical analysis

Data were analyzed using SPSS software (version 19.0, IBM, United States). Hardy-Weinberg equilibrium in the control group was tested using Pearson's χ^2 -test. Logistic regression analyses were performed to obtain the odds ratios (ORs) and their 95% CIs. Age (> 40 years), gender, and BMI were considered as potential confounders for NAFLD and metabolic disorders including hypertension, IR, IGT, dyslipidemia, and hyperuricemia. Therefore, they were included in the multivariate analyses with a forward conditional model when we explored the associa-

tions between the *APOC3* (-455T>C) polymorphism and NAFLD susceptibility and the above metabolic disorders. Independent *t*-tests or nonparametric Mann-Whitney *U* tests were used as appropriate to evaluate the difference between the control and NAFLD groups. A two-tailed *P* < 0.05 was considered statistically significant.

RESULTS

Clinical characteristics

There was no significant difference between the NAFLD group and the control group with respect to the age and gender distribution (Table 1). Measurement values of BMI, blood pressure, UA, FPG, FPI, and lipid profile were significantly different between the control group and NAFLD group (*P* < 0.001, Table 1). Obesity, elevated AST, low HDL, hypercholesterolemia, and hypertriglyceridemia were the most common characteristics in the NAFLD group (Table 1).

APOC3 (-455T>C) polymorphism

Genotypes of all 600 subjects including 300 NAFLD patients and 300 controls were successfully assessed by PCR-RFLP. The PCR-RFLP products were separated into three genotypes, including wild-type homozygote (TT), heterozygote (TC), and variant-type homozygote (CC). These genotypes were resolved as fragments of 122 and 72 bp (TT); 122, 72, and 194 bp (TC); and 194 bp (CC) (Figure 1). These genotypes were confirmed by DNA sequencing in randomly selected samples (Figure 2). Among healthy controls, the *APOC3* genotype distri-

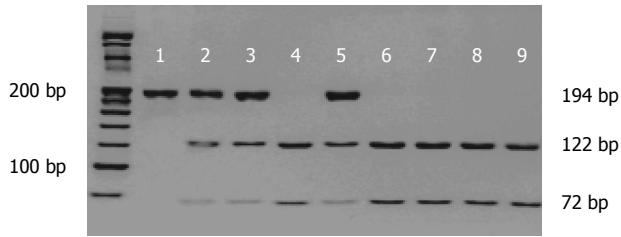


Figure 1 Genotyping of the *Apolipoprotein C3* (-455T>C) polymorphism. Polymerase chain reaction-restriction fragment length polymorphism and polyacrylamide gel (8%) electrophoresis of the *Apolipoprotein C3* (-455T>C) polymorphism illustrated the wild-type homozygote TT (122 and 72 bp), heterozygote TC (194, 122, and 72 bp), and variant-type homozygote CC (194 bp) genotypes. TT: 4, 6-9; TC: 2, 3, 5; CC: 1.

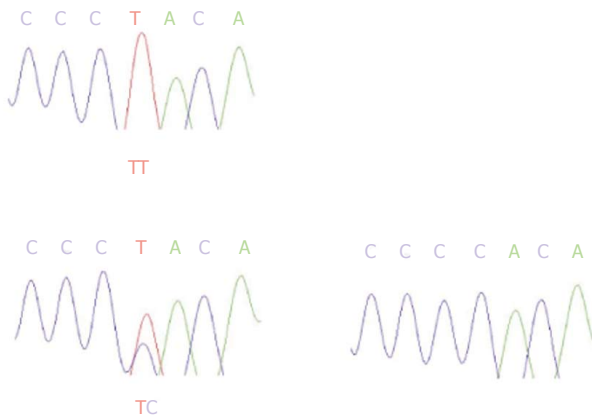


Figure 2 Sequencing analysis for genotypes of the *Apolipoprotein C3* (-455T>C) polymorphism. A: TT genotype; B: TC genotype; C: CC genotype.

Table 2 Association between *Apolipoprotein C3* (-455T>C) polymorphism and nonalcoholic fatty liver disease susceptibility *n* (%)

Genotype	Group		<i>P</i> value	Adjusted OR (95%CI)
	Control group	NAFLD group		
TT	134 (44.7)	94 (31.3)	-	1
TC	123 (41.0)	131 (43.7)	0.009 ¹	1.77 (1.16-2.72) ¹
CC	43 (14.3)	75 (25.0)	< 0.001 ²	2.80 (1.64-4.79) ²

¹TC *vs* TT; ²CC *vs* TT. NAFLD: Nonalcoholic fatty liver disease.

bution was in Hardy-Weinberg equilibrium ($P > 0.05$).

Association between the *APOC3* (-455T>C) polymorphism and NAFLD susceptibility

The frequencies of *APOC3* (-455T>C) genotypes in the control group (44.7% TT, 41.0% TC, and 14.3% CC) were significantly different from those in the NAFLD group (31.3% TT, 43.7% TC, and 25.0% CC) ($P < 0.001$) (Table 2). After adjusting for age, gender, and BMI, the TC and CC genotypes showed an increased risk of NAFLD, with adjusted ORs of 1.77 (95%CI: 1.16-2.72) and 2.80 (95%CI: 1.64-4.79), respectively (Table 2).

Table 3 Correlation between *Apolipoprotein C3* (-455T>C) polymorphism and insulin resistance, impaired glucose tolerance in the nonalcoholic fatty liver disease group *n* (%)

Genotype	Metabolic component		<i>P</i> value	Adjusted OR (95%CI)
	Non-IR	IR		
TT	78 (34.5)	16 (21.7)	-	1
TC	99 (43.8)	32 (43.2)	0.06 ¹	1.96 (0.97-3.96) ¹
CC	49 (21.7)	26 (35.1)	0.00 ²	3.24 (1.52-6.92) ²
	Non-IGT	IGT		
TT	77 (33.0)	17 (25.4)	-	1
TC	98 (42.1)	33 (49.2)	0.23 ¹	-
CC	58 (24.9)	17 (25.4)	0.89 ²	-

¹TC *vs* TT; ²CC *vs* TT. IR: Insulin resistance; IGT: Impaired glucose tolerance.

Correlation of the *APOC3* (-455T>C) polymorphism with IR or IGT in the NAFLD group

Compared to the TT genotype, the CC genotype was associated with an increased risk of IR in NAFLD patients, with an adjusted OR of 3.24 (95%CI: 1.52-6.92). The TC genotype shared the similar frequency in both non-IR and IR patients in the NAFLD group (95%CI: 0.97-3.96, $P = 0.06$). In addition, no association was found between the *APOC3* (-455T>C) polymorphism and IGT in the NAFLD group ($P > 0.05$) (Table 3).

Correlation of the *APOC3* (-455T>C) polymorphism with hypertension or dyslipidemia in the NAFLD group

Compared to the TT genotype, the TC and CC genotypes were associated with an increased risk of hypertension in NAFLD patients, with adjusted ORs of 2.16 (95%CI: 1.02-4.57) and 3.42 (95%CI: 1.53-7.60), respectively (Table 4).

Compared to the TT genotype, NAFLD patients with the TC and CC genotypes were more susceptible to hypertriglyceridemia, with adjusted ORs of 2.03 (95%CI: 1.12-3.68) and 3.80 (95%CI: 1.95-7.41), respectively. The CC genotype was associated with a significantly higher risk of low HDL in NAFLD patients, with an adjusted OR of 2.34 (95%CI: 1.21-4.52) compared to the TT genotype. The TC genotype had a similar incidence of low HDL as that of the TT genotype (95%CI: 0.76-2.42, $P = 0.30$). No association between the *APOC3* (-455T>C) polymorphism and hypercholesterolemia or increased LDL was found in the Southern Chinese Han population ($P > 0.05$) (Table 4). No association was found between the *APOC3* (-455T>C) polymorphism and obesity, hyperuricemia, or elevated ALT and AST ($P > 0.05$, data not shown).

DISCUSSION

The present study examined 300 NAFLD patients and 300 healthy controls to investigate the relationship between *APOC3* (-455T>C) polymorphism and NAFLD susceptibility in the Southern Chinese Han population. We found that variation in *APOC3* (-455T>C) conferred

Table 4 Correlation between *Apolipoprotein C3* (-455T>C) polymorphism and hypertension, dyslipidemia in the nonalcoholic fatty liver disease group *n* (%)

Genotype	Metabolic component		P value	Adjusted OR (95%CI)
	Non-hypertension	Hypertension		
TT	82 (34.7)	12 (18.8)	-	1
TC	102 (43.2)	29 (45.3)	0.04 ¹	2.16 (1.02-4.57) ¹
CC	52 (22.1)	23 (35.9)	0.003 ²	3.42 (1.53-7.60) ²
	Non-hypertriglyceridemia	Hypertriglyceridemia		
TT	71 (39.3)	23 (19.3)	-	1
TC	77 (42.5)	54 (45.4)	0.02 ¹	2.03 (1.12-3.68) ¹
CC	33 (18.2)	42 (35.3)	< 0.001 ²	3.80 (1.95-7.41) ²
	Normal HDL	Low HDL		
TT	63 (36.6)	31 (24.2)	-	1
TC	75 (43.6)	56 (43.8)	0.30 ¹	1.36 (0.76-2.42) ¹
CC	34 (19.8)	41 (32.0)	0.01 ²	2.34 (1.21-4.52) ²
	Non-hypercholesterolemia	Hypercholesterolemia		
TT	57 (32.0)	37 (30.3)	-	1
TC	81 (45.5)	50 (41.0)	0.52 ¹	-
CC	40 (22.5)	35 (28.7)	0.20 ²	-
	Normal LDL	Increased LDL		
TT	65 (31.0)	29 (32.2)	-	1
TC	89 (42.3)	42 (46.7)	0.41 ¹	-
CC	56 (26.7)	19 (21.1)	0.33 ²	-

¹TC vs TT; ²CC vs TT. HDL: High-density lipoprotein cholesterol; LDL: Low-density lipoprotein cholesterol.

Table 5 *Apolipoprotein C3* (-455T>C) polymorphism among different ethnicities: data from the literature *n* (%)

Ref.	Ethnicity	Genotype of <i>APOC3</i> (-455T>C)		
		TT	TC	CC
Kozlitina <i>et al</i> ^[10]	African Americans	106 (8.7)	484 (39.9)	623 (51.4)
Kozlitina <i>et al</i> ^[10]	Europeans	316 (38.4)	390 (47.4)	117 (14.2)
Kozlitina <i>et al</i> ^[10]	Hispanics	163 (37.7)	209 (48.4)	60 (13.9)
Hyysalo <i>et al</i> ^[12]	Finns	145 (33.7)	190 (44.2)	95 (22.1)
Niu <i>et al</i> ^[20]	Northern Chinese	206 (25.8)	375 (46.9)	218 (27.3)

susceptibility to NAFLD. These results did not support those of previous studies from Shenyang and Qingdao (the Northern China) that found no association between *APOC3* (-455T>C) and susceptibility to NAFLD^[19,20]. These inconsistencies may be explained by several considerations. First, the previous studies performed in Shenyang and Qingdao enrolled the Northern Chinese population as the study subjects. By contrast, our study was performed in Southern China and enrolled only the Southern Chinese Han population. Second, the two previous studies defined healthy controls as “no hepatic steatosis.” This was different from our definition of healthy controls as “subjects who were free of elevated ALT or AST and had no liver steatosis, and lacked any kind of metabolic disorders such as hypertension, obesity, hyperuricemia, and dyslipidemia”. In fact, similar discrepancies were found in studies of the association between *APOC3* (-455T>C) polymorphism and acute coronary syndrome (ACS) that were conducted in different regions of China^[21,22]. Third, PAGE gel was used to differentiate PCR products after enzyme digestion in the present study. This method had a much higher sensitivity in detecting smaller-sized PCR

products.

Our discovery of the association between *APOC3* (-455T>C) polymorphism and NAFLD was similar to the results from a study of an Asian Indian population^[9], which revealed that C allele carriers showed a markedly higher hepatic TG content (HTGC)^[9]. By contrast, another study enrolling multi-ethnicities showed that the C allele was associated with a significant reduction in HTGC in African Americans^[10], while the association was not found in European Americans and Hispanics. Other two studies enrolling Finns and Caucasians did not find the association either^[12,23].

There is strong evidence for a close association between NAFLD and IR^[24-28]. IR is a key disorder that promotes the development and progression of NAFLD, and it is associated with *APOC3*^[7,8,29,30]. Transgenic mice overexpressing human *APOC3* were predisposed to hepatic steatosis and IR^[7,8]. In our study, NAFLD patients had a greater probability of IR than that of controls, as expected (24.7% vs 1.3%, $P < 0.001$). Subjects carrying the C allele (TC or CC) developed IR more commonly, and it was in agreement with that found in the Indian population^[9]. However, one study recruiting multi-ethnicities including African Americans, European Americans and Hispanics found no association between *APOC3* (-455T>C) polymorphism and the level of HOMA-IR except in Hispanics, and C allele carriers showed a lower level of HOMA-IR^[10]. No association between *APOC3* (-455T>C) polymorphism and IGT was found in our study. These results might be due to the small sample size of NAFLD patients with IGT [67 of 300 (22.3%)].

The association between *APOC3* (-455T>C) polymorphism and metabolic syndrome (MetS) has been reported, in which an *APOC3* variant was associated with

a greater likelihood of MetS compared with that of wild type^[31-33]. Carriers of *APOC3* (-455T>C) variations were more susceptible to metabolic disorders, such as hypertriglyceridemia, low HDL levels^[21,34], and hypertension^[31]. However, no association between *APOC3* (-455T>C) and total cholesterol or LDL was found in our study or in a previous study^[34]. The role of *APOC3* in lipid metabolism was confirmed in a previous study, which showed that a null mutation of *APOC3* leads to lower fasting and postprandial plasma TGs, higher levels of HDL, and lower levels of LDL^[35].

The -455T>C conversion is located in a putative insulin-response element of *APOC3*, which is associated with plasma TG levels^[33,36,37]. *In vitro* promoter studies indicated that insulin bound to this site and inhibited *APOC3* transcription and translation. By contrast, the polymorphic variants prevent insulin binding and promote *APOC3* transcription, thus promoting the synthesis of APOC3. As a result, the level of circulating APOC3 increases and acts as a lipoprotein lipase inhibitor, leading to decreased clearance of TG-rich particles, which ultimately results in hypertriglyceridemia^[38,39]. The circulating TG-rich particles are preferentially taken up by the liver by means of a receptor-mediated process^[40-42], which results in NAFLD and hepatic IR.

The reasons for the different conclusions between our study and previous studies are not clear^[10-12]. One possible explanation might be that alcohol use, obesity, and exercise training confounded the relationship between *APOC3* (-455T>C) polymorphism, NAFLD susceptibility, and IR. Another possibility might be different roles of the *APOC3* (-455T>C) polymorphism in different ethnicities. Previous studies examined *APOC3* polymorphisms in Asian Indians, African Americans, European Americans, Hispanics, Finns, Caucasians, and Northern Chinese Han population^[9-12,19,20] (Table 5), but the association between *APOC3* (-455T>C) polymorphism and NAFLD susceptibility was found only in the Asian Indian and African American populations. We examined the Southern Chinese Han population to assess the correlation between *APOC3* (-455T>C) polymorphism and NAFLD susceptibility, and the result was similar to that found in the Asian Indian population^[9].

In summary, our results indicate that *APOC3* (-455T>C) polymorphism is associated with an increased susceptibility to NAFLD, IR, and metabolic disorders. However, there are several limitations in our study. First, the diagnosis of NAFLD was based primarily on ultrasonographic results, not on the quantitative measurement of hepatic TG content by magnetic resonance imaging. Second, the serum concentration of APOC3 was not obtained in our study. Further studies will be conducted to explore the function of the *APOC3* polymorphism.

COMMENTS

Background

The pathogenesis of nonalcoholic fatty liver disease (NAFLD) has not been elucidated clearly, but it was supposed to be closely related with genetic factors

due to its high prevalence among first-degree relatives or twins. Recently, *Apolipoprotein C3* (*APOC3*) (-455T>C) genetic variation has been reported to be associated with susceptibility to NAFLD and IR in an Indian population. However, the conclusion drew from studies performed in African Americans, European Americans, Hispanics, Caucasians, and Finns were not in accordant with that in the Indian population. Until now, no data about the association between the *APOC3* (-455T>C) polymorphism and NAFLD in the Southern Chinese Han population were reported.

Research frontiers

The relationship between *APOC3* (-455T>C) polymorphism and NAFLD varied in different ethnicities. Therefore, studies performed using the same procedures and methods enrolling more ethnicities were needed. In addition, further studies should be conducted to explore the function of the *APOC3* (-455T>C) polymorphism.

Innovations and breakthroughs

This study represents the first published study to investigate the relationship between the *APOC3* (-455T>C) polymorphism and NAFLD susceptibility in the Southern Chinese Han population.

Applications

Physicians would pay more attention to individuals who were C allele carriers of *APOC3* (-455T>C), and provide early intervention before the NAFLD development.

Peer review

The association between *APOC3* (-455T>C) and NAFLD susceptibility in the Southern Chinese Han population is one of major interests. This manuscript is very interesting from this aspect.

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