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RESEARCH REPORT

Lack of association between apolipoprotein C3 gene polymorphisms and risk of nonalcoholic fatty liver disease in a Chinese Han population

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Abstract

AIM: To investigate the association between two polymorphisms of apolipoprotein C3 (*APOC3*) and risk of nonalcoholic fatty liver disease (NAFLD) in a Chinese Han population.

METHODS: Genotypes for rs2854116 and rs2854117 in *APOC3* and the known rs738409 in patatin-like phospholipase domain-containing protein 3 (PNPLA3) in 390 patients with NAFLD and 409 control subjects were determined by sequencing and polymerase chain reaction analysis. Serum lipid profiles were determined using biochemical methods, and an index of insulin resistance (IR, HOMA-IR), serum *APOC3* concentrations and total antioxidant status (TAS) were also assessed.

RESULTS: No significant differences in genotype and allele frequencies of rs2854116 and rs2854117 were found between the NAFLD population and the controls ($P > 0.05$). The OR for the association between -455C and -482T allele carriers and the risk of NAFLD were 1.06 (95%CI: 0.72-1.57, $P > 0.05$) and 1.00 (95%CI: 0.68-1.48, $P > 0.05$), respectively. The variant carriers did not have a significantly increased risk of NAFLD or elevated clinical and biochemical parameters such as *APOC3* concentrations, IR (1.42 ± 0.43 *vs* 1.48 ± 0.52, $P > 0.05$), liver enzymes and TAS (13.94 ± 2.01 *vs* 14.38 ± 1.92, $P > 0.05$) compared with the controls. Moreover, the results were similar when testing was carried out independent of the genetic variation in PNPLA3.

CONCLUSION: The two polymorphisms of the *APOC3* gene are not associated with a risk of NAFLD, or with lipid profiles, IR and oxidative stress in the Chinese Han population.

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Key words: Polymorphism; Single nucleotide; Nonalcoholic fatty liver disease; Apolipoprotein C3; Insulin resistance; Oxidative stress

Core tip: In this study, we determined the relationship between two polymorphisms in apolipoprotein C3 (*APOC3*) and susceptibility to nonalcoholic fatty liver disease (NAFLD) in a Han population in China. We also examined the influence of *APOC3* genotypes on insulin resistance, obesity, fasting TG levels and total antioxidant status. We found that the two polymorphisms of the *APOC3* gene are not associated with a risk of NAFLD, or with lipid profiles, IR and oxidative stress in the Chinese Han population.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease and is now recognized as a major public health problem worldwide[1,2]. It is estimated that the prevalence of NAFLD ranges from 6.3% to 33% with a median of 20% in the general population, based on a variety of assessment methods[3]. It is not a 'Western disease', it is recognized as the main cause of chronic liver disease in the developing world, and the prevalence of NAFLD in the Chinese population is approximately 15%[4-6]. NAFLD encompasses a wide spectrum of hepatic disorders, ranging from simple fatty liver NAFL to nonalcoholic steatohepatitis and hepatic cirrhosis, which may progress to hepatocellular carcinoma.

NAFLD is a multifactorial disorder arising from the interplay between genetic and environmental influences. Genetic association is a powerful tool for determining the mechanism of NAFLD[7,8], in particular, patatin-like phospholipase-3 (PNPLA3) and apolipoprotein C3 (*APOC3*) have been suggested as potential genes related to NAFLD susceptibility or disease progression. The association between the *PNPLA3* gene I148M variant and NAFLD has been demonstrated in a number of studies, confirming that this gene is an important genetic determinant of disease development[9-11]. *APOC3* is involved in triacylglycerol metabolism, which inhibits lipoprotein lipase (LPL) and mediates lipoprotein uptake by the liver[12]. Recently, Petersen *et al*[13] carried out a candidate gene study and reported that two variants (rs2854117 and rs2854116) of the promoter region in the *APOC3* gene located in an insulin response element (IRE)[14] led to increased uptake of chylomicron remnants by the liver, and this resulted in NAFLD and hepatic insulin resistance in Asian-Indian men, however, subsequent studies obtained different results in American or European subjects[15-18]. No research has been carried out on the association between the two polymorphisms of *APOC3* and NAFLD in the Chinese population.

In this study, we determined the relationship between the two polymorphisms in *APOC3* and susceptibility to NAFLD in a Han population in China. In addition, we examined the influence of *APOC3* genotypes on insulin resistance, obesity, fasting TG levels and total antioxidant status.

MATERIALS AND METHODS

Subjects

We selected a total of 799 unrelated adult subjects of both sexes, including 390 unrelated Chinese patients (175 males, 215 females, mean age 49.76 ± 16.17 years) diagnosed with NAFLD by B-type ultrasonography between April 2012 and April 2013, and 409 healthy controls matched for sex and age (198 males, 211 females, mean age 47.69 ± 15.86 years). The volunteers were recruited from the Department of Gastroenterology and the Medical Center of Qingdao Municipal Hospital. All subjects were Northern Han Chinese in origin. The diagnosis of NAFLD was performed under standard clinical evaluation conditions according to the AASID criteria. Other causes of liver disease were excluded, including increased alcohol intake (> 210/140 g/wk for males/females), as confirmed by at least one family member or friend and carboxydesialylated transferrin determination, viral and autoimmune hepatitis, hereditary hemochromatosis, and alphal-antitrypsin deficiency. We also excluded subjects with type 1 diabetes mellitus. The controls were confirmed as healthy by medical history, general examinations and laboratory examinations at the same hospital. We excluded subjects as previously described[19]. Basic information (names, ages, *etc.*) was obtained by questionnaire. All subjects were of Chinese Han ethnicity.

Informed consent was obtained from subjects who agreed to participate in the study, and the study protocol was approved by the Ethical Committee of Qingdao Municipal Hospital.

Biochemical analyses

Blood samples for biochemical analyses were obtained after an overnight fast. Glucose, insulin, cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides (TG) were measured using routine enzymatic methods. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ-glutamyltransferase (GGT) concentrations were measured as previously described[20]. Plasma *APOC3* concentrations were measured enzymatically using an Assay Max human Apolipoprotein C-Ⅲ enzyme linked immunosorbent assay (ELISA) Kit (n = 180) (BlueGene, China). Insulin sensitivity was assessed using the homeostasis model assessment of insulin resistance (HOMA-IR) and a 2-h oral glucose-tolerance test (OGTT). The HOMA-IR was calculated as fasting insulin concentration (μU/mL) × fasting glucose concentration (mmol/L)/22.5[21]. Ninety-eight subjects were selected to complete a standard 75 g OGTT and measurements of glucose and insulin were recorded at baseline and after 30, 60, 90, and 120 min. Total antioxidant status (TAS), independent of the genetic variation in PNPLA3 (n = 231), was measured colorimetrically using the Total Antioxidant Capacity Assay Kit (Sciencell, United States) and the ABTS method. The color change was measured using a spectrophotometer. The reaction was calibrated with Trolox (a water-soluble analogue of vitamin E).

Genetic analysis

Genomic DNA was extracted from peripheral blood using the Genomic DNA Purification Kit (BioTeke, Biotechnology, Beijing, China) following the supplier's instructions. The success rate for DNA extraction was 100% and the DNA samples were stored in a freezer at -20 ℃ until

Table 1 Demographic and clinical features of patients with nonalcoholic fatty liver disease and controls *n* (%)

	NAFLD patients (*n* = 390)	Controls (*n* = 409)	*P* value
BMI (kg/m^2)	24.83 ± 2.82	22.18 ± 4.02	0.000[a]
Waist (cm)	84.18 ± 8.19	75.78 ± 9.13	0.000[a]
Hypertension	89 (22.8)	42 (10.3)	0.000[c]
T2DM	87 (22.3)	36 (8.8)	0.000[c]
ALT (U/L)	44.86 ± 40.16	34.32 ± 22.27	0.000[a]
AST (U/L)	33.40 ± 32.60	32.60 ± 36.42	0.762
GGT (U/L)	43.82 ± 42.17	34.00 ± 26.92	0.000[a]
FPG (mmol/L)	6.03 ± 1.77	5.18 ± 0.82	0.357
UA (mmol/L)	326.03 ± 105.24	332.88 ± 104.74	0.000[a]
TG (mmol/L)	2.91 ± 1.43	2.26 ± 1.55	0.000[a]
TC (mmol/L)	5.17 ± 1.00	4.77 ± 0.85	0.000[a]
HDL (mmol/L)	1.71 ± 0.99	2.38 ± 2.36	0.000[a]
LDL (mmol/L)	4.28 ± 3.44	3.17 ± 1.78	0.000[a]
FINS (μU/mL)	6.27 ± 1.34	5.51 ± 1.01	0.000[a]
HOMA-IR (U)	1.67 ± 0.62	1.27 ± 0.32	0.000[a]

Data are expressed as mean ± SD, [a]$P < 0.05$ *vs* controls, by unpaired Student's *t* test; [c]$P < 0.05$ *vs* controls, by χ^2 test. Body mass index (BMI) was calculated as body weight (kg)/height (m^2); ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: γ-glutamyltransferase; FPG: Fasting blood glucose; UA: uric Acid; TC: Total cholesterol; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; FINS: Fasting blood insulin; NAFLD: Nonalcoholic fatty liver disease; TG: Triglyceride; HOMA-IR: Homeostatic model assessment of insulin resistance.

use. The primers for rs2854116 and rs2854117 used in the reaction were: 5'-GAAGGTGAACGAGAGAATCAGTCCTG-3' (forward); 5'-GCCTCGGGCCCATCTCAGCCTTTCACACTG-3' (reverse). Amplification was performed in a total volume of 25 μL containing 12.5 μL Taq polymerase chain reaction (PCR) MasterMix, 2 μL genomic DNA, 1 μL forward primer, 1 μL reverse primer and 8 μL purified water, on PCR amplification equipment (Labnet, United States) as follows: initial step of 95 ℃ for 10 min, followed by 35 cycles: denaturation at 95 ℃ for 40 s, annealing at 56 ℃ for 40 s, and elongation at 72 ℃ for 50 s. Amplicon size was 511 bp. The primers for rs738408 were 5'-CTGAAGTCCGAGGGTGTATG-3' (forward); 5'-AGCTGTGGCTACTCTGTCTG-3' (reverse). The PCR amplification profile was as follows: initial step of 94 ℃ for 5 min, followed by 30 cycles: denaturation at 94 ℃ for 30 s, annealing at 57.5 ℃ for 30 s, and elongation at 72 ℃ for 1 min. Amplicon size was 396 bp. All PCR products were resolved using 2% agarose gel electrophoresis at 110 V for 30 min and stained with ethidium bromide. The genotypes of the two genes underwent direct sequencing using the ABI Prism Sequence Detection System ABI3730 (Genscript). The genotyping success rate was > 95%.

The two single nucleotide polymorphisms (SNPs) in *APOC3* are in strong linkage disequilibrium[13-22], thus, we divided the study subjects into two groups for analysis: no variant allele expression (noncarriers) at both SNPs and one or more alleles expressed (Carriers) at either SNP.

Statistical analysis

Statistical analyses were performed using SPSS statistical software, version 17.0 for Windows. Genotypes and alleles were estimated by counting and the distributions between NAFLD patients and controls were analyzed by Pearson's χ^2 test and Fisher's exact test when appropriate. Hardy-Weinberg equilibrium between expected and observed genotype distributions was assessed using the χ^2 test. Baseline characteristics are shown as mean ± SD. Differences in the characteristics of the study population between different groups were examined using the Student's *t* test, paired samples *t* test or the χ^2 test. The strength of the association between the polymorphisms and NAFLD was evaluated by logistic regression analysis adjusted for confounders [gender, age, body mass index (BMI), weight and waistline, which were considered as continuous variables], estimated by the OR with 95%CI. Linear regression analysis was performed to determine the correlation between plasma *APOC3* concentrations and serum TG levels. Linkage disequilibrium between the rs2854116 and rs2854117 SNPs was calculated using Haploview software and was shown as D'. The present sample size of the study revealed 90% power to detect a significant association ($P < 0.05$) with an effect size index of 0.1. Levels of significance were defined as $P < 0.05$.

RESULTS

Patients in the NAFLD group and control group were matched for sex ($P = 0.316$) and age ($P = 0.068$). The clinical characteristics of the two groups are shown in Table 1, and patients with NAFLD had higher levels of ALT, GGT, FPG, TG, TC and LDL than controls. As NAFLD is a metabolic disease, it showed a strong interaction with hypertension and diabetes (Table 1).

Distributions of the genotypes of these two polymorphisms were in accordance with the Hardy-Weinberg equilibrium in the NAFLD and control groups (P_{NAFLD} = 0.130, 0.054; $P_{control}$ = 0.350, 0.882, respectively). The variant allele frequencies (-455C and -482T) were 0.508 and 0.500 in NAFLD patients, and 0.512 and 0.498 in controls, respectively. To ensure genotyping accuracy, we randomly selected 150 subjects for reverse sequencing. The success rate of duplicated genotyping was > 100%. There were no significant differences in the genotype and allele distribution between the two groups ($P > 0.05$) (Table 2). Presence of the -455C allele and the -482T allele did not increase the risk of developing NAFLD (OR = 1.00, 95%CI: 0.82-1.22, and OR = 0.99, 95%CI: 0.81-1.21, respectively). In contrast, the rs738409G allele of PNPLA3 was associated with a significantly increased risk of NAFLD in the study population (OR = 2.00, 95%CI: 1.80-2.23) (Table 3). There was strong linkage disequilibrium between T-455C and C-482T with D' = 0.943.

Compared with the noncarriers, none of the lipid variables (total cholesterol, HDL, LDL, and circulating TG) and indices of liver damage severity (serum ALT and AST levels) showed statistically relevant differences according to the variant *APOC3* promoters in both the overall series and in the subgroups (Table 4). In the same

Table 2 Distribution of genotype and allele frequencies of apolipoprotein C3 (-455, -482) and patatin-like phospholipase domain-containing protein 3 (rs738409) in nonalcoholic fatty liver disease patients and controls *n* (%)

	NAFLD	Controls	χ^2	*P* value
-455 genotype				
TT	102 (26.2)	104 (25.4)		
TC	180 (46.2)	195 (47.7)		
CC	108 (27.7)	110 (26.9)	0.186	0.911
Allele T	384 (49.2)	403 (49.3)		
C	396 (50.8)	415 (51.2)	0.000	1.000
-482 genotype				
CC	107 (27.4)	104 (25.4)		
CT	176 (45.1)	203 (49.6)		
TT	107 (27.4)	102 (51.2)	1.635	0.442
Allele C	390 (50.0)	411 (50.2)		
T	390 (50.0)	407 (49.8)	0.010	0.960
rs738409 genotype				
CC	48 (12.3)	183 (44.7)		
CG	153 (39.2)	176 (43.0)		
GG	189 (48.5)	50 (12.2)	160.98	0.000
Allele C	249 (31.9)	542 (66.3)		
G	531 (68.1)	276 (33.7)	188.31	0.000

NAFLD: Nonalcoholic fatty liver disease.

Table 3 Odds ratios for nonalcoholic fatty liver disease according to apolipoprotein C3 and patatin-like phospholipase domain-containing protein 3 genotypes and alleles in the study population

	Unadjusted model		Adjusted model	
	OR (95%CI)	*P* value	OR (95%CI)	*P* value
APOC3 gene				
-455TT	1		1	
-455TC + CC	0.96 (0.70-1.32)	0.82	1.06 (0.72-1.57)	0.76
-482CC	1		1	
-482CT + TT	0.90 (0.66-1.24)	0.52	1.00 (0.68-1.48)	1.00
PNPLA3 gene				
CC	1		1	
CG + GG	5.77 (4.03-8.27)	0.00	3.70 (2.44-5.60)	0.00

-455C *vs* T: OR (95%CI) = 1.00 (0.82-1.22): -482C *vs* T: OR (95%CI) = 0.99 (0.81-1.21); PNPLA3G *vs* C: OR (95%CI) = 2.00 (1.80-2.23). The multiple logistic regression model was adjusted for age, gender, body mass index, waist, weight and presence of hypertension and T2DM.

study subjects, the PNPLA3 genotype was shown to predispose to NAFLD. We next evaluated whether these results were similar when controlling for the PNPLA3 mutation. Similar results were obtained (Table 5).

We assessed the relationship between *APOC3* variants and fasting blood insulin (FINS), HOMA-IR and plasma TG. No differences between wild-type homozygotes and variant allele carriers were observed (Tables 4 and 5). *APOC3* concentrations showed a consistent relationship with plasma TG concentrations ($r = 0.706$, $P = 0.000$) (Figure 1), as indicated by linear regression analysis. No associations between the variants and fasting levels of plasma glucose, insulin or HOMA-IR were found. Glucose and insulin levels were similar in the groups throughout the OGTT (Figure 2).

The control group had significantly higher TAS than the NAFLD group ($P = 0.000$). However, mean TAS in the noncarrier and carrier groups was similar at admission ($P = 0.098$) (Figure 3).

DISCUSSION

We read with great interest the article by Petersen who reported a higher prevalence of NAFLD in *APOC3* variant allele carriers compared with wild-type carriers in Indian men[13]. In apparent disagreement with these results, recent findings in the Dallas Heart Study demonstrated different results in the three major ethnic groups included in that study[15]. To highlight the role of *APOC3* in NAFLD progression, we enrolled a total of 799 unrelated Chinese adults in a case-control study to investigate the relationship between two *APOC3* polymorphisms (rs2854116 and rs2854117) and the risk of NAFLD. We did not find significant associations between these polymorphisms and the risk of NAFLD in the Chinese Han population. PNPLA3 gene I148M variant plays a role in NAFLD, which has been demonstrated in a number of studies[9-11]. Our research also demonstrated similar results.

In the Petersen's study, *APOC3* genotypes were found to be associated with hepatic triglyceride content measured by H-MRS[13], while different results were obtained in the Dallas Heart Study[15]. The mechanisms associated with the accumulation of TG in the liver and subsequent hepatocellular damage are not fully understood[23]. We evaluated the changes in ALT and AST, which were used as markers of liver fat accumulation[24-26] and are commonly used in clinical practice[27]. We did not observe significant differences in plasma concentrations of these transaminases between carriers of the variants and noncarrier subjects, even when independent of the genetic variation in PNPLA3. Similar findings were reported in a Caucasian population[17]. Although the pathogenesis of NAFLD is unknown, it is conceivable that a network of interactions between multiple factors is involved in both the development and progression of the disease, such as insulin resistance, obesity and oxidative stress[28].

Insulin resistance is recognized as an essential pathophysiological factor or "first hit" in the development of NAFLD, and may play a fundamental role in the pathogenesis of this disorder[29,30]. We are in agreement with other researchers who have reported that NAFLD has an important role in the etiology of hepatic insulin resistance[31,32]. Our data were consistent with previous findings that genetic variation in *APOC3* expression has little effect on the insulin sensitivity index (HOMA-IR) in humans[15,16,33]. In addition, insulin sensitivity did not change the results of oral glucose-tolerance testing in carriers compared to non-carriers. *APOC3* expression in the liver is physiologically inhibited by insulin, and no obvious increase in serum insulin or decrease in plasma *APOC3* concentration was observed. However, plasma TG concentrations were significantly positively correlated with plasma *APOC3* concentrations ($r = 0.706$), which was consistent with earlier observations[34,35]. *APOC3* was the first lipid-associated gene to be linked to hy-

Table 4 Clinical characteristics of apolipoprotein C3 wild-type homozygotes (rs2854117 C/C and rs2854116 T/T) and carriers of one or more variant alleles (rs2854117 T and rs2854116 C) in the study population

Characteristic	Overall series			NAFLD patients			Controls		
	Noncarriers	Carriers	*P* value	Noncarriers	Carriers	*P* value	Noncarriers	Carriers	*P* value
n	197	602		96	294		101	308	
Female/male	110/87	306/296	0.250	48/48	157/137	0.638	62/39	149/159	0.903
Age (yr)	47.95 ± 16.86	48.95 ± 15.76	0.447	49.16 ± 17.23	49.96 ± 15.83	0.672	46.80 ± 16.51	47.98 ± 15.65	0.516
BMI (kg/m^2)	23.69 ± 4.08	23.42 ± 3.73	0.387	25.21 ± 2.48	24.70 ± 2.91	0.123	22.14 ± 4.07	22.19 ± 4.10	0.910
Waist (cm)	78.97 ± 9.56	80.17 ± 9.66	0.130	85.36 ± 7.11	83.79 ± 8.49	0.074	72.90 ± 7.40	76.72 ± 9.45	0.000
ALT (U/L)	39.47 ± 34.79	39.46 ± 31.98	0.999	48.60 ± 44.85	43.64 ± 38.51	0.294	30.79 ± 17.50	35.48 ± 23.54	0.066
AST (U/L)	32.71 ± 36.37	33.08 ± 37.72	0.904	36.35 ± 42.00	32.44 ± 37.14	0.386	29.25 ± 29.86	33.70 ± 38.31	0.287
FPG (U/L)	5.46 ± 1.26	5.63 ± 1.49	0.135	5.82 ± 1.46	6.09 ± 1.86	0.201	5.12 ± 0.91	5.20 ± 0.79	0.435
TG (mmol/L)	2.67 ± 1.68	2.54 ± 1.47	0.341	2.88 ± 1.29	2.92 ± 1.48	0.840	2.46 ± 1.97	2.20 ± 1.39	0.134
TC (mmol/L)	4.97 ± 0.98	4.96 ± 0.94	0.887	5.18 ± 0.96	5.17 ± 1.01	0.903	4.77 ± 0.96	4.76 ± 0.81	0.935
HDL (mmol/L)	2.25 ± 2.21	1.99 ± 1.72	0.134	1.51 ± 0.46	1.78 ± 1.10	0.001	2.96 ± 2.89	2.19 ± 2.14	0.015
LDL (mmol/L)	3.84 ± 3.09	3.68 ± 2.66	0.477	4.43 ± 3.71	4.24 ± 3.35	0.659	3.28 ± 2.24	3.14 ± 1.61	0.564
FINS (μU/mL)	5.83 ± 1.20	5.90 ± 1.24	0.545	6.22 ± 1.24	6.28 ± 1.36	0.712	5.46 ± 1.03	5.53 ± 1.00	0.554
HOMA-IR (U)	1.42 ± 0.43	1.48 ± 0.52	0.111	1.62 ± 0.57	1.69 ± 0.61	0.309	1.25 ± 0.34	1.28 ± 0.31	0.376
APOC3 (mg/dL)	13.94 ± 2.01	14.38 ± 1.92	0.231	13.60 ± 1.90	14.37 ± 1.98	0.231	15.22 ± 1.79	14.20 ± 1.89	0.059
	(*n* = 34)	(*n* = 146)		(*n* = 19)	(*n* = 80)		(*n* = 15)	(*n* = 66)	

Data are shown as mean ± SD. Statistically significant differences were tested using the Student's unpaired *t* test or by χ^2 test when appropriate. A *P* value < 0.05 was considered significant. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: γ-glutamyltransferase; FPG: Fasting blood glucose; UA: uric Acid; TC: Total cholesterol; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; FINS: Fasting blood insulin; NAFLD: Nonalcoholic fatty liver disease; TG: Triglyceride; HOMA-IR: Homeostatic model assessment of insulin resistance; BMI: Body mass index.

Table 5 Clinical characteristics of patatin-like phospholipase domain-containing protein 3 and apolipoprotein C3 (independently of patatin-like phospholipase domain-containing protein 3 I148M) wild-type homozygotes and carriers of one or more variant alleles in the study population

Characteristic	PNPLA3			APOC3 (independently of PNPLA3 I148M)		
	Noncarriers	Carriers	*P* value	Noncarriers	Carriers	*P* value
n	231	568		82	149	
Female/male	124/107	292/276	0.560	12/70	109/40	0.000
Age (yr)	46.97 ± 16.42	49.41 ± 15.83	0.052	48.89 ± 16.91	45.92 ± 16.11	0.189
BMI (kg/m^2)	22.61 ± 4.08	23.84 ± 3.65	0.000	22.04 ± 4.17	22.92 ± 4.00	0.118
Waist (cm)	75.67 ± 9.00	81.59 ± 9.37	0.000	72.96 ± 7.64	77.16 ± 9.37	0.000
ALT (U/L)	36.03 ± 24.46	40.86 ± 35.40	0.028	34.67 ± 20.30	36.77 ± 26.51	0.501
AST (U/L)	34.07 ± 37.80	32.55 ± 37.21	0.601	32.55 ± 37.21	34.07 ± 37.80	0.662
FPG (U/L)	5.30 ± 1.09	5.71 ± 1.54	0.000	5.26 ± 0.82	5.33 ± 1.21	0.618
TG (mmol/L)	2.34 ± 1.54	2.67 ± 1.51	0.005	2.17 ± 1.09	2.43 ± 1.73	0.216
TC (mmol/L)	4.79 ± 0.90	5.03 ± 0.95	0.001	4.72 ± 0.76	4.83 ± 0.97	0.343
HDL (mmol/L)	2.64 ± 2.54	1.82 ± 1.43	0.000	2.88 ± 2.63	2.50 ± 2.48	0.281
LDL (mmol/L)	3.28 ± 1.98	3.90 ± 3.02	0.001	3.44 ± 1.92	3.19 ± 2.01	0.367
FINS (μU/mL)	5.67 ± 1.13	5.97 ± 1.26	0.001	5.58 ± 1.02	5.72 ± 1.20	0.335
HOMA-IR (U)	1.34 ± 0.38	1.52 ± 0.53	0.000	1.31 ± 0.33	1.35 ± 0.41	0.354

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: γ-glutamyltransferase; FPG: Fasting blood glucose; UA: uric Acid; TC: Total cholesterol; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; FINS: Fasting blood insulin; NAFLD: Nonalcoholic fatty liver disease; TG: Triglyceride; HOMA-IR: Homeostatic model assessment of insulin resistance; PNPLA3: Patatin-like phospholipase domain-containing protein 3; APOC3: Apolipoprotein C3.

pertriglyceridemia[36]. It was reported that the *APOC3* nonsense allele (R19X) caused a 45% reduction in plasma TG levels[37]. However, results for the polymorphic SstI site in the 3' untranslated region of *APOC3* varied. Most studies found a strong interaction between them[38], but the research by Russo resulted in a different conclusion[39]. We confirmed the lack of association between *APOC3* promoter polymorphisms and plasma TG levels in NAFLD patients, whereas Petersen *et al*[13] found that fasting plasma TG levels were approximately 60% higher in male Asian Indian carriers of the variant alleles. These differences may be due to linkage disequilibrium with causal variants in different races.

Obesity is a common and well documented risk factor for NAFLD. The prevalence of NAFLD can exceed 90% in patients with severe obesity undergoing surgery[40]. The classification of obesity is based on BMI, and for Chinese subjects a BMI of 28 kg/m^2 or more is an index of obesity, whereas a BMI greater than 24 kg/m^2 is an index of overweight. In this study, BMI was higher in the NAFLD group (24.83 ± 2.82 kg/m^2) than in controls (22.18 ± 4.02 kg/m^2). However, the two *APOC3* polymorphisms did not influ-

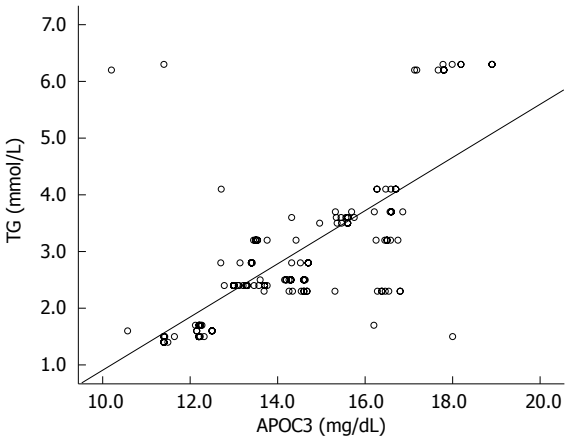

Figure 1 Relationship between serum triglycerides level and plasma apolipoprotein C3 concentration. TG: Triglycerides. *r* and *P* values were calculated using a linear regression model ($r = 0.706$, $P = 0.000$).

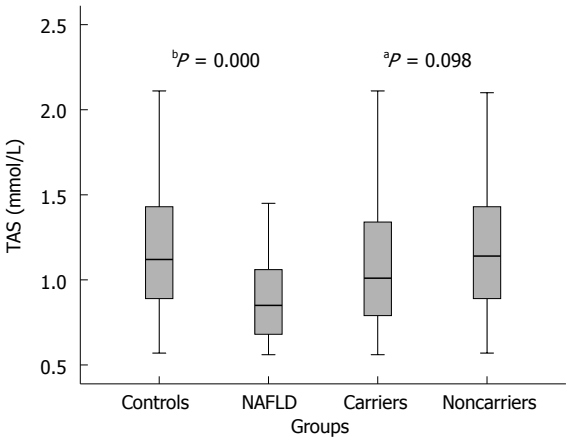

Figure 3 Comparison of total antioxidant status in different groups. [a]*P* and [b]*P* were tested by Student's unpaired *t* test, [a]$P = 0.098$ *vs* carriers, [b]$P < 0.01$ *vs* controls, respectively. TAS: Total antioxidant status.

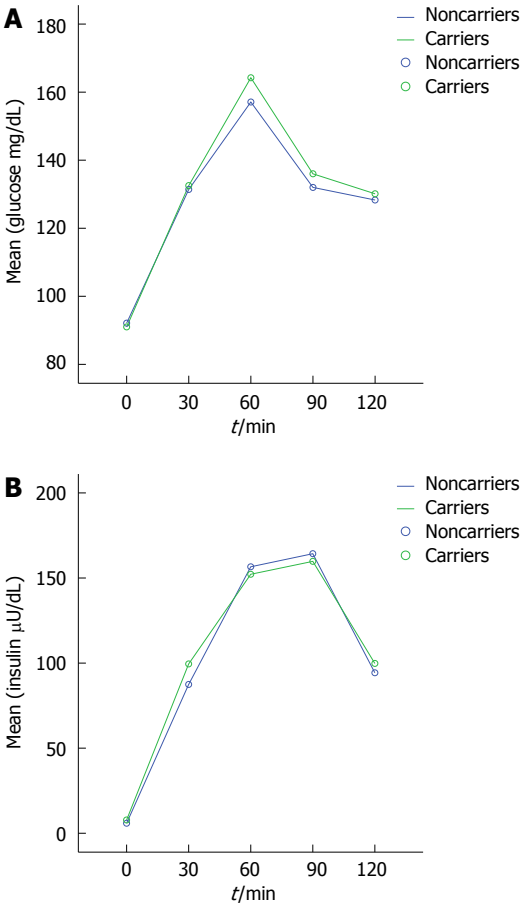

Figure 2 Comparison of glucose (A) and insulin (B) levels between noncarrier and carrier groups in the oral glucose tolerance test. *P* values were calculated by paired samples *t* test ($P = 0.137$, 0.537).

ence BMI ($P > 0.05$). The first metabolic abnormality leading to liver steatosis is a lipotoxic reaction with an oxidative stress component[41-43]. Antioxidants in the body can interrupt oxidative stress-induced reactive oxygen species (ROS). Therefore, plasma TAS should be ranked on the basis of methods currently used to evaluate oxidative stress[43]. We found that TAS was lower in NAFLD patients[41], however, TAS was not reduced in the variant allele expression group.

Limitations of our study include the lack of direct measurement of hepatic fat content by liver biopsy. Liver biopsy or elastography (Fibroscan) is ideal for measuring hepatic fat, but is probably not achievable in a study of this size. We diagnosed NAFLD using routine blood testing and liver ultrasonography, which can reduce the related cost and risk, but are not very accurate. The number of subjects in our study may have been too small to detect a significant association. We think that ethnic origin influences the prevalence of NAFLD, and our study was carried out on non-Asian patients. To resolve this issue, larger studies which should include Asian Indian populations across multiple ethnic groups are required. We need genome-wide association studies to determine the association between the two variant alleles of *APOC3* and NAFLD, similar to the detection of PNPLA3I148M which strongly influences NAFLD[9].

In summary, compared with PNPLA3, the two genetic variants (T-455C at rs2854116 and C-482T at rs2854117) in the *APOC3* gene were not associated with NAFLD risk. Even when independent of the PNPLA3 I148M genotype, the two gene polymorphisms of *APOC3* did not contribute to the inter-individual differences in lipid profiles, insulin resistance, obesity, oxidative stress and susceptibility to NAFLD in the Chinese Han population.

COMMENTS

Background

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease and is now recognized as a major public health problem worldwide. It is a multifactorial disorder arising from the interplay between genetic and environmental influences. Genetic association is a powerful tool for determining the mechanism of NAFLD. Patatin-like phospholipase domain-containing protein 3 (PNPLA3) and apolipoprotein C3 (*APOC3*) have been suggested as potential genes related to NAFLD susceptibility or disease progression. However, since China is the most populated country in the world, a study of the Han population is insufficient to determine the association between these polymorphisms and NAFLD.

Research frontiers

The association between the *PNPLA3* gene I148M variant and NAFLD has been demonstrated in a number of studies. A recent study suggested that pro-

moter region polymorphisms in *APOC3* (T-455C and C-482T) may contribute to NAFLD, TG concentrations and IR in Indian subjects. However, subsequent studies obtained different results in American or European populations. Further studies are required to confirm the association between the two polymorphisms of *APOC3* and NAFLD in different ethnic groups.

Innovations and breakthroughs

This is the first study to systemically determine the relationship between the two polymorphisms (T-455C and C-482T) in *APOC3* and susceptibility to NAFLD in the Han population in China. The findings suggested that the two genetic variants in the *APOC3* gene were not associated with a risk of NAFLD, even when independent of PNPLA3 I148M genotypes. As the pathogenesis of NAFLD is unknown, the authors studied, for the first time, the "two-hit" theory of NAFLD to support their results.

Applications

NAFLD is genetically influenced and the number of common genetic variants associated with this disease is expanding. This study confirmed that PNPLA3I148M plays an important role in NAFLD and related diseases, providing new insights into the biology and genetics of NAFLD and opening up avenues for biological, diagnostic, and therapeutic research into this condition in humans. Although this research found that the two polymorphisms of *APOC3* were not associated with NAFLD risk in Chinese patients, it provides a new direction for further studies on the genetics of NAFLD.

Peer review

The authors collected a total of 799 unrelated Chinese adults in a case-control study to investigate the relationship between the two *APOC3* polymorphisms and the risk of NAFLD, and demonstrated that the two polymorphisms of *APOC3* gene were not associated with risk of NAFLD, or with the lipid profiles, insulin resistance and oxidative stress in the Han population in China. This study is interesting and worthy to be published.

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