



Association Between Four *ABCA1* gene Polymorphisms and Risk of Non-Alcoholic Fatty Liver Disease in a Chinese Han Population

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Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) as a severe health problem is the leading cause of morbidity and mortality from the chronic liver disease worldwide. NAFLD is tightly associated with dyslipidemia although the etiology is still unclear. ATP binding cassette subfamily A member 1 (*ABCA1*) is involved in cholesterol efflux, fatty acid oxidation, and inflammation. Although some reports show that the *ABCA1* polymorphisms affect the lipids metabolism and severity of clinical liver diseases, the effects of *ABCA1* polymorphisms on the development of NAFLD are unknown.

Objectives: The current study was performed to investigate the association between the *ABCA1* polymorphisms and the development of NAFLD and the effect of the four *ABCA1* SNPs on the serum lipid levels.

Methods: The *ABCA1* polymorphisms (rs1800977, rs2066714, rs2066715, and rs2230808) were determined in 265 NAFLD patients and 126 healthy controls using the sequencing and polymerase chain reaction analysis. Serum lipid profiles and liver enzymes were examined using standard clinical laboratory methods.

Results: There was a significant difference ($P < 0.05$) in the genotype of the *ABCA1* rs1800977 G/A polymorphisms between NAFLD patients and healthy controls. No significant differences were found in genotypes and allele frequencies of the *ABCA1* rs2066714T/C, rs2066715T/C, and rs2230808C/T between NAFLD patients and healthy controls. The *ABCA1* rs1800977 A was independently associated with NAFLD after adjusting for the effects of age, gender, and BMI. Compared to the noncarriers in NAFLD patients, the carriers of *ABCA1* rs2066714 C showed a significantly higher level of LDL ($P = 0.045$) and the carriers of *ABCA1* rs2230808 T showed a significantly lower level of HDL ($P = 0.039$).

Conclusions: We first demonstrated the association between the *ABCA1* polymorphisms and the risk of NAFLD in a Chinese Han population. The *ABCA1* rs1800977 may be a protective factor against the development of NAFLD. The *ABCA1* rs2066714 C allele could increase the serum LDL cholesterol level, and the *ABCA1* rs2230808 T allele could decrease the serum HDL cholesterol level in NAFLD patients.

Keywords: Single Nucleotide Polymorphism, Non-Alcoholic Fatty Liver Disease, Polymorphism

1. Background

Non-alcoholic fatty liver disease (NAFLD) comprises a spectrum ranging from simple steatosis, non-alcoholic steatohepatitis (NASH), and fibrosis to cirrhosis. NAFLD is defined as the increased hepatic triglyceride content (HTGC) without the consumption of alcohol (1). Simple steatosis is usually benign and can progress to steatohepatitis, fibrosis, and cirrhosis, ultimately leading to

the development of hepatocellular carcinoma (HCC) (2-4). NAFLD is commonly associated with obesity, insulin resistance, and hyperlipidemia, all of which are the typical metabolism syndromes (5, 6). Several population-based epidemiological studies have shown that the incidence of NAFLD in China is approximately 15% in adults and 1.3% in children and adolescents (7) while the incidence of NAFLD is 20% - 30% in Western countries (8). NAFLD is becoming a major public health problem worldwide, and is associated

with numerous pathogenesis, such as metabolic, genetic, environmental, and gut microbial factors (9).

Although abundant studies have been conducted in the fundamental and clinical features of NAFLD, the detailed pathogenic mechanism of NAFLD is still unclear. The classical “two hits” theory has been put forward as a pathogenesis of NAFLD (10). The first “hit” results in hepatic steatosis, mainly caused by obesity, type 2 diabetes mellitus, dyslipidemia, and other metabolic factors. The second “hit” leads to hepatocellular injury and liver inflammation by oxidative stress and proinflammatory cytokines (11). In recent years, a “multi-parallel hit” model has been proposed as a new pathogenesis of NAFLD due to many factors suggesting that gut-derived and adipose tissue-derived factors may react in parallel, finally resulting in the liver inflammation (12). Meanwhile, accumulated evidence suggests a link between cholesterol homeostasis and accumulation of hepatic cholesterol in the pathogenesis of steatohepatitis (13-16). Cholesterol consumption has been shown to significantly associate with the risk of cirrhosis or liver cancer in the national health and nutrition examination survey, but serum cholesterol level was not associated with the risk of cirrhosis or liver cancer (17). Furthermore, epidemiological data prove that the increased cholesterol intake contributes to the risk and severity of NAFLD (17). Caballero et al. also reported that the accumulation of hepatic free cholesterol (FC) increases the development of NASH and fibrosis in patients with NAFLD (15, 18). Although accumulated evidence suggests that the increased cholesterol level is associated with the NAFLD, the detailed mechanism of cholesterol in the development of NAFLD remains unclear. Therefore, the significant role of cholesterol in the pathogenesis of NAFLD needs further study.

The ATP binding cassette subfamily A member 1 (*ABCA1*) gene is located on chromosome 9 (9q31) and contains 58 exons (19, 20) (<https://www.ncbi.nlm.nih.gov/gene/19>). *ABCA1* is a 2261-amino acid membrane protein that contains 12 transmembrane domains and mediates cholesterol efflux from cell to lipid-free apolipoprotein A-I (apoA-I) and apolipoprotein E (apoE) (21). Besides, *ABCA1* is an essential regulator of high-density lipoproteins (HDL) and reverse cholesterol transport (22). In 2002, Hong et al. reported that the C254T variant in *ABCA1* was associated with a reduced HDL cholesterol and familial hypoalphalipoproteinemia (FHA), suggesting that the *ABCA1* polymorphism may affect the lipids metabolism (23). Recent studies have shown that the SNPs in *ABCA1* might modulate plasma triglyceride and HDL cholesterol levels in HIV-infected patients (24). Although *ABCA1* polymorphism is a risk factor for the lipids metabolism, a few studies have been conducted in the NAFLD patients, especially in the Chinese Han population.

2. Objectives

The aim of this study was to investigate the association between four *ABCA1* SNPs and the development of NAFLD in a Chinese Han population and the effect of the four *ABCA1* SNPs on the serum lipid levels.

3. Methods

3.1. Study Subjects

The study was performed in accordance with the principles of the declaration of Helsinki and its appendices (25) and approved by the ethics committee of the Qingdao municipal hospital. A written informed consent form was obtained from each patient before participation in the study.

A total of 391 unrelated Chinese Han adult subjects of both sexes, including 265 NAFLD patients diagnosed with B-type ultrasonography and 126 healthy control subjects were enrolled in this study. All subjects were recruited from the department of gastroenterology and the Medical Center of Qingdao Municipal hospital from November 2015 to August 2017. A standard study questionnaire was used to obtain the basic clinicopathological information (name, age, gender, and so on).

The diagnosis of NAFLD was performed under standard clinical evaluation conditions according to the American association for the study of liver diseases (AASLD) criteria. Hepatic steatosis was observed by B-type ultrasonography in NAFLD patients. Other causes of liver disease were excluded, including high alcohol intake (> 210/140 g/w for males/females), as confirmed by at least one family member or friend and carboxy-desialylated transferrin determination, viral and autoimmune hepatitis, hereditary hemochromatosis, and alpha-antitrypsin deficiency. Subjects with other related diseases such as type 1 diabetes mellitus and coronary atherosclerotic disease (CAD) were also excluded. The controls were confirmed as healthy by medical history, general examinations, and laboratory examinations at the same hospital.

3.2. Biochemical Analyses

Blood samples of each subject for biochemical analyses were collected into sterile tubes containing ethylene diamine tetraacetic acid after a 12-hour overnight fast. The height and body mass of each subject were obtained to calculate body mass index (BMI) expressed as mass (kg)/height (m)². Environmental factors, such as diet and physical activity, were not recorded in this study. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using a blood pressure gauge. Alanine aminotransferase (ALT), aspartate aminotransferase

(AST), γ -glutamyl transferase (GGT), and alkaline phosphatase (ALP) were measured using the standard clinical laboratory methods in the central laboratory of Qingdao municipal hospital. Glucose (Glu), triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were measured using routine enzymatic methods.

3.3. Genetic Analysis

Genomic DNA was extracted from peripheral blood samples using the Genomic DNA Purification Kit (BioTeke, Biotechnology, Beijing, China) following the manufacturer's instructions and stored at -20°C until use. Genotyping for the *ABCA1* rs1800977G/A, rs2066714T/C, rs2066715T/C, and rs2230808C/T was performed by polymerase chain reaction (PCR) analysis using the primers shown in Table 1. Amplification was performed in a total volume of 25 μ L containing 12.5 μ L Taq PCR MasterMix (TaKaRa), 2 μ L genomic DNA, 1 μ L forward primer, 1 μ L reverse primer, and 8 μ L purified water on PCR amplification equipment (Labnet, United States) as follows: initial step of 95°C for 10 minutes, followed by 35 cycles: denaturation at 94°C for 1 minute, annealing at 60°C for 1 minute, and elongation at 70°C for 1 minute. All PCR products were resolved using 2% agarose gel electrophoresis at 110 V for 30 minutes and stained with ethidium bromide. The genotypes of the four sites underwent direct sequencing using the ABI Prism sequence detection system ABI3730 (Foster City, CA, USA). The genotyping success rate was > 95%.

Table 1. Primers Used for Genotyping Analysis

SNPs	Primer Sequences (5' - 3')	Amplicon Size, bp
rs1800977	ACGTTGGATGTCACCTCTCCTCGCAATTAC	109
	ACGTTGGATGTGACCGATAGTAACCTCTGC	
rs2066714	ACGTTGGATGAAAGAGCAGGAGGTCAACAG	100
	ACGTTGGATGGGAAAGTGATGAGAAGAGCC	
rs2066715	ACGTTGGATGAGAGGAAGGTGTCAACAGC	101
	ACGTTGGATGACCTGTTGAGAGTCTCTGTG	
rs2230808	ACGTTGGATGGCAGATCGATTCTCAACAG	100
	ACGTTGGATGAGACAGCGTTTACCTTGAC	

3.4. Statistical Analysis

Statistical analyses were performed using SPSS version 22.0 statistical software for Windows. Baseline characteristics are shown as mean \pm SD. Differences in the characteristics of the study population between different groups were examined using the Student's t-test and the χ^2 test.

Hardy-Weinberg equilibrium between expected and observed genotype distributions was estimated by the χ^2 test. Genotypes and alleles were estimated by the chi-square test and DNA distributions between NAFLD patients and controls were analyzed by Pearson's χ^2 test or Fisher's exact test when appropriate. The strength of the association between the polymorphisms and NAFLD was assessed by the logistic regression analysis, adjusted for confounders (age, gender, and BMI), estimated by the odds ratio (OR) with 95% confidence interval (CI). Levels of significance were defined as $P < 0.05$.

4. Results

4.1. Characteristics of the Study Population

Patients in the NAFLD group (199 males, 66 females, mean age 39.73 \pm 6.38 years) and control group (88 males, 38 females, mean age 38.46 \pm 6.94 years) had no statistical differences in sex ($P = 0.273$) and age ($P = 0.075$). The clinical characteristics of the study participants are shown in Table 2. The patients with NAFLD had higher BMI, SBP, and DBP, higher serum levels of ALT, GGT, ALP, Glu, TG, TC, LDL, and lower serum levels of HDL than the healthy controls.

Table 2. Demographic and Clinical Characteristics of NAFLD Patients and Healthy Controls^a

	NAFLD Patients (n = 265)	Controls (n = 126)	P Value
BMI, kg/m ²	26.43 \pm 3.05	23.16 \pm 2.86	< 0.001
SBP, mmHg	124.66 \pm 14.18	117.37 \pm 12.24	< 0.001
DBP, mmHg	85.00 \pm 9.89	79.71 \pm 9.84	< 0.001
ALT, U/L	34.09 \pm 20.67	23.95 \pm 32.17	< 0.001
AST, U/L	24.22 \pm 9.25	22.82 \pm 16.55	0.279
GGT, U/L	43.95 \pm 32.84	28.47 \pm 23.27	< 0.001
ALP, U/L	70.98 \pm 16.76	66.98 \pm 18.58	0.034
Glu, mmol/L	5.05 \pm 1.20	4.50 \pm 0.88	< 0.001
TG, mmol/L	1.98 \pm 1.23	1.29 \pm 1.05	< 0.001
TC, mmol/L	5.64 \pm 1.06	5.20 \pm 0.85	< 0.001
HDL, mmol/L	1.25 \pm 0.35	1.40 \pm 0.29	< 0.001
LDL, mmol/L	3.39 \pm 0.75	2.98 \pm 0.64	< 0.001

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; GGT, γ -glutamyl transferase; Glu, Glucose; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; TG, triglyceride; TC, total cholesterol.

^aValues are expressed as mean \pm SD and compared by Student's t-test.

4.2. Genotypes and Allele distribution of rs1800977G/A, rs2066714T/C, rs2066715T/C, and rs2230808C/T

The genotypes distribution of each *ABCA1* SNP site was in accordance with the Hardy-Weinberg equilibrium in

NAFLD patients and healthy controls as shown in Table 3. To ensure the accuracy of genotypes, we randomly selected 100 subjects for reverse sequencing. The success rate of duplicated genotyping was > 100%. As shown in Table 4, there was a significant difference in the genotype of *ABCA1* rs1800977 between the NAFLD patients group and control group ($P = 0.009$). A strong link of the *ABCA1* rs1800977 allele with NAFLD is shown in Table 5; the frequency of *ABCA1* rs1800977 GA + AA genotypes was significantly lower in the NAFLD patients than in the control group (OR: 0.751; 95%CI: 0.606 - 0.931). After adjusting for gender, age, and BMI, the frequency of *ABCA1* rs1800977 GA + AA genotypes was significantly lower in the NAFLD patients than in the control group (OR: 0.678; 95%CI: 0.525 - 0.876).

Table 3. Results of the Hardy-Weinberg Equilibrium^a

Gene Locus Groups	χ^2	P Value
rs1800977		
NAFLD	3.16	0.075
Controls	3.54	0.060
rs2066714		
NAFLD	2.01	0.16
Controls	0.44	0.51
rs2066715		
NAFLD	0.85	0.36
Controls	0.0043	0.98
rs2230808		
NAFLD	0.11	0.735
Controls	3.65	0.059

^aData were compared by chi-square test.

4.3. Association of the Genetic Variants in the *ABCA1* Gene with Clinical Parameters in Non-Alcoholic Fatty Liver Disease Patients

To estimate whether gene polymorphisms are correlated with clinical parameters, we compared the clinical characteristics in carriers and non-carriers of the four alleles in overall series, NAFLD patients, and healthy controls. As shown in the results, in the NAFLD patients group, the carriers of rs2066714 C showed a higher serum level of LDL compared to the noncarriers (Table 6), and the carriers of 2230808 T showed a lower serum level of HDL compared to the noncarriers (Table 7). In addition, no significant difference was observed in the lipid variables (TC, TG, LDL, and HDL levels) and indices of liver damage severity (serum ALT and AST levels) between the carriers and noncarriers of rs1800977 A and rs2066715 C in overall series, NAFLD patients, and healthy controls (Data not shown).

5. Discussion

In this study, we investigated the relationship between the genetic variants of *ABCA1* rs1800977, rs2066714, rs2066715, and rs2230808 and the risk of NAFLD for the first time. Previous studies have proposed that multiple factors were related to the development and progression of NAFLD such as insulin resistance, obesity, oxidative stress, and so on (26). In this study, we diagnosed the NAFLD by routine blood testing and liver ultrasonography instead of biopsy, which is regarded as the gold standard of NAFLD diagnosis. In the majority of cases, patients with NAFLD possessed elevated levels of serum lipid profiles and liver enzymes, especially ALT and GGT, which had been used as the marker of liver injury (27-29). In our study, there was a significantly higher level of serum TG, TC, LDL, ALT, GGT, and ALP in NAFLD patients compared to the healthy controls. In addition, the serum concentration of HDL was significantly lower in NAFLD patients than in healthy controls.

Accumulated evidence has shown that the *ABCA1* variants were tightly associated with the abnormal metabolism of serum lipid profiles and liver enzymes (30). Recently, a report showed that the pathogenic mutations of *ABCA1* (including rs9282543, rs137854496, rs374190304, rs141021096, and rs150125857) were observed in Canadians, which increased the risk of atherosclerosis compared to the patients without such mutations (31). Kolovou et al. conducted a study to investigate the association between *ABCA1* gene polymorphism and the plasma lipid variables in Greek nurses; the results indicated that the R1578K variant (rs2230808) of *ABCA1* gene tightly affected the lipid variables; this may be helpful in assessing the risk of premature coronary heart disease and atherosclerosis (30). The association of *ABCA1* R230C variant (rs9282541) with high triglyceride levels was observed in the Mexican school-age children, which confers *ABCA1* R230C as a potential risk factor for the high TG syndrome (32). Although many studies of *ABCA1* polymorphism have been conducted in patients with dyslipidemia, coronary heart disease, and atherosclerosis, which were tightly associated with the development of NAFLD, the effects of *ABCA1* variants on NAFLD have not been reported. Our study first presented the evidence that there is a significant difference in the genotype of *ABCA1* rs1800977 between the NAFLD patients and healthy controls in a Chinese Han population. However, the frequency of *ABCA1* rs1800977 GA + AA genotypes was significantly lower in the NAFLD patients than in the control group. After adjusting for gender, age, and BMI, the frequency of *ABCA1* rs1800977 GA+AA genotypes was significantly lower in the NAFLD patients than in the control group. These results suggest that *ABCA1* rs1800977 was associated with the development

Table 4. Distribution of Genotypes and Allele Frequencies of *ABCA1* in NAFLD Patients and Controls^{a,b}

	NAFLD	Controls	χ^2	P Value
rs1800977				
Genotypes			6.892	0.009
GG	147 (55.5)	52 (41.3)		
GA	93 (35.1)	65 (51.6)		
AA	25 (9.4)	9 (7.1)		
Alleles			2.986	0.086
G	387 (73.0)	169 (67.1)		
A	143 (27.0)	83 (32.9)		
rs2066714				
Genotypes			2.134	0.144
TT	139 (52.5)	76 (60.3)		
TC	112 (42.3)	42 (33.3)		
CC	14 (5.2)	8 (6.4)		
Alleles			1.044	0.307
T	390 (73.6)	194 (77.0)		
C	140 (26.4)	58 (23.0)		
rs2066715				
Genotypes			0.418	0.518
TT	90 (34.0)	47 (37.3)		
TC	135 (50.9)	60 (47.6)		
CC	40 (15.1)	19 (15.1)		
Alleles			0.200	0.655
T	315 (59.4)	154 (61.1)		
C	215 (40.6)	98 (38.9)		
rs2230808				
Genotypes			1.049	0.306
CC	105 (39.6)	57 (45.2)		
CT	125 (47.2)	48 (38.1)		
TT	35 (13.2)	21 (16.7)		
Alleles			0.052	0.820
C	335 (63.2)	162 (64.3)		
T	195 (36.8)	90 (35.7)		

^aData were compared by chi-square test.

^bValues are expressed as No. (%).

of NAFLD, and the allele A of *ABCA1* rs1800977 may possess a protective role against NAFLD. The potential mechanism may be that the variant of rs1800977 A allele inhibits the reverse cholesterol transport that in turn reduces the cholesterol accumulation in the liver and increases the serum cholesterol concentration. However, the detailed mechanism still needs further study.

In addition to hepatic triglyceride accumulation, abnormal cholesterol efflux plays a critical role in advanced NAFLD (NASH and fibrosis) (15, 18, 33, 34). Previous studies have indicated that *ABCA1* is involved in the cellular export of several groups of molecules, including cholesterol and phospholipids (35). These lipid molecules were removed from the cells by apolipoprotein A-I and other

Table 5. Association of Genotypes with NAFLD in the Study Group^a

	Unadjusted		Adjusted	
	OR (95% CI)	P Value	OR (95% CI)	P Value
rs1800977				
GG	1		1	
GA + AA	0.751 (0.606 - 0.931)	0.009	0.678 (0.525 - 0.876)	0.003
rs2066714				
TT	1		1	
TC + CC	1.174 (0.946 - 1.456)	0.145	1.246 (0.936 - 1.657)	0.131
rs2066715				
TT	1		1	
TC + CC	1.076 (0.862 - 1.341)	0.518	1.044 (0.786 - 1.386)	0.767
rs2230808				
CC	1		1	
CT + TT	1.122 (0.906 - 1.390)	0.293	1.109 (0.849 - 1.448)	0.450

^aThe multiple-logistic regression model was adjusted for gender, age, and BMI.

Table 6. Clinical Characteristics of *ABCA1* rs2066714 C Carriers and Non-Carriers in the Study Population^a

	Overall Series			NAFLD Patients			Controls		
	Carriers (n = 229)	Non-Carriers (n = 162)	P Value	Carriers (n = 160)	Non-carriers (n = 105)	P value	Carriers (n = 69)	Non-Carriers (n = 57)	P Value
Female/Male	42/134	62/153	0.268	25/101	41/98	0.070	17/33	21/55	0.446
Age, y	39.17 ± 6.61	39.50 ± 6.56	0.625	40.11 ± 6.54	39.38 ± 6.23	0.353	37.24 ± 6.85	38.32 ± 7.45	0.415
BMI, kg/m²	25.26 ± 3.43	25.52 ± 3.27	0.457	26.55 ± 2.76	26.32 ± 3.30	0.532	22.90 ± 3.01	23.33 ± 2.77	0.414
SBP, mmHg	121.69 ± 13.13	123.06 ± 14.97	0.342	125.47 ± 14.96	123.92 ± 13.43	0.376	117.00 ± 13.29	117.62 ± 11.58	0.783
DBP, mmHg	82.96 ± 9.96	83.70 ± 10.43	0.471	85.25 ± 1.24	84.78 ± 9.59	0.700	79.82 ± 9.97	79.63 ± 9.82	0.917
ALT, U/L	31.81 ± 29.78	29.62 ± 18.62	0.395	33.56 ± 19.82	34.57 ± 21.47	0.694	19.67 ± 9.72	26.77 ± 40.52	0.227
AST, U/L	24.48 ± 15.14	22.92 ± 6.64	0.205	23.99 ± 7.05	24.46 ± 10.89	0.684	20.23 ± 4.49	24.53 ± 20.88	0.154
GGT, U/L	37.33 ± 28.37	40.94 ± 33.75	0.251	47.63 ± 37.09	40.61 ± 28.16	0.082	24.09 ± 12.34	31.35 ± 27.96	0.087
ALP, U/L	68.81 ± 17.72	70.77 ± 17.08	0.270	71.78 ± 16.28	70.27 ± 17.20	0.465	68.23 ± 18.88	66.15 ± 18.46	0.541
Glu, mmol/L	4.80 ± 0.95	4.95 ± 1.33	0.232	5.10 ± 1.34	5.00 ± 1.07	0.506	4.56 ± 1.25	4.46 ± 0.51	0.514
TG, mmol/L	1.72 ± 1.19	1.79 ± 1.25	0.567	2.07 ± 1.27	1.90 ± 1.18	0.261	1.11 ± 0.89	1.41 ± 1.14	0.120
TC, mmol/L	5.47 ± 0.97	5.52 ± 1.07	0.608	5.72 ± 1.14	5.56 ± 0.98	0.209	5.03 ± 0.66	5.32 ± 0.95	0.066
HDL, mmol/L	1.30 ± 0.30	1.29 ± 0.38	0.701	1.25 ± 0.42	1.24 ± 0.28	0.742	1.38 ± 0.24	1.42 ± 0.31	0.457
LDL, mmol/L	3.21 ± 0.70	3.32 ± 0.78	0.156	3.49 ± 0.80	3.31 ± 0.69	0.045	2.89 ± 0.54	3.04 ± 0.70	0.190

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; GGT, γ -glutamyl transferase; Glu, Glucose; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

^aValues are expressed as mean ± SD and compared by Student's t-test.

apolipoproteins of nascent HDL cholesterol in a process that contributes to the initial step of reverse cholesterol transport (36). *ABCA1* polymorphisms affecting the serum levels of HDL cholesterol have been reported in many studies. Hong et al. investigated the relationship of the *ABCA1* C254T variant with the serum HDL cholesterol levels in the patients with premature coronary artery disease. The results showed that the single defective allele in *ABCA1* may be associated with reduced HDL cholesterol levels (23). Similar results were observed in the Mexican school-age

children with a high prevalence of obesity; the study reported a tight association of R230C variant of the *ABCA1* gene with the low HDL cholesterol levels (32). In this study, we observed that the serum HDL cholesterol levels were significantly lower in the NAFLD patients with *ABCA1* rs2230808 T allele than in the NAFLD patients without the variant, which is in accordance with previous studies. However, there was no significant difference in the serum HDL cholesterol levels between the carriers and noncarriers of the *ABCA1* rs1800977 A, rs2066714 C, and rs2066715 C

Table 7. Clinical Characteristics of *ABCA1* rs2230808 T Carriers and Non-Carriers in the Study Population^a

	Overall Series			NAFLD Patients			Controls		
	Carriers (n = 229)	Non-Carriers (n = 162)	P Value	Carriers (n = 160)	Non-Carriers (n = 105)	P Value	Carriers (n = 69)	Non-Carriers (n = 57)	P Value
Female/Male	60/169	44/118	0.832	38/122	28/77	0.591	22/47	16/41	0.642
Age, y	39.21 ± 6.37	39.40 ± 6.74	0.782	39.86 ± 6.57	39.53 ± 6.10	0.688	38.33 ± 7.05	37.35 ± 7.42	0.449
BMI, kg/m ²	25.21 ± 3.30	25.49 ± 3.40	0.412	26.36 ± 3.26	26.54 ± 2.77	0.640	23.49 ± 2.92	22.77 ± 2.76	0.159
SBP, mmHg	122.82 ± 15.14	121.95 ± 13.13	0.544	123.94 ± 13.47	125.74 ± 15.19	0.313	117.32 ± 11.07	117.44 ± 13.62	0.957
DBP, mmHg	84.06 ± 11.14	82.75 ± 9.41	0.210	84.16 ± 9.51	86.29 ± 10.35	0.086	79.49 ± 8.35	79.96 ± 11.47	0.790
ALT, U/L	32.68 ± 32.90	29.51 ± 18.23	0.268	32.81 ± 19.27	36.04 ± 22.60	0.214	21.86 ± 12.69	26.49 ± 45.85	0.424
AST, U/L	24.46 ± 15.69	23.30 ± 8.70	0.350	23.94 ± 9.64	24.70 ± 8.64	0.515	21.82 ± 5.76	24.03 ± 23.84	0.459
GGT, U/L	41.77 ± 35.49	36.97 ± 27.14	0.148	41.01 ± 28.88	48.42 ± 37.79	0.072	27.59 ± 19.77	29.53 ± 27.06	0.644
ALP, U/L	71.27 ± 18.54	68.57 ± 16.57	0.132	70.11 ± 16.65	72.31 ± 16.91	0.297	65.01 ± 15.94	69.37 ± 21.26	0.191
Glu, mmol/L	4.84 ± 1.17	4.89 ± 1.11	0.625	5.04 ± 1.09	5.05 ± 1.35	0.985	4.54 ± 1.09	4.45 ± 0.53	0.548
TG, mmol/L	1.80 ± 1.19	1.73 ± 1.24	0.555	1.89 ± 1.23	2.11 ± 1.22	0.146	1.35 ± 1.18	1.22 ± 0.88	0.506
TC, mmol/L	5.51 ± 1.19	5.49 ± 0.89	0.827	5.61 ± 0.86	5.69 ± 1.32	0.540	5.22 ± 0.90	5.19 ± 0.81	0.863
HDL, mmol/L	1.34 ± 0.43	1.26 ± 0.25	0.034	1.21 ± 0.20	1.30 ± 0.50	0.039	1.39 ± 0.30	1.42 ± 0.27	0.480
LDL, mmol/L	3.26 ± 0.82	3.27 ± 0.68	0.900	3.39 ± 0.65	3.41 ± 0.89	0.779	2.99 ± 0.70	2.97 ± 0.58	0.889

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; Glu, Glucose; GGT, γ -glutamyl transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglyceride; SBP, systolic blood pressure.

^aValues are expressed as mean ± SD and compared by Student's t-test.

in NAFLD patients. In addition, the increased serum levels of LDL cholesterol were observed in the NAFLD patients with the *ABCA1* rs2066714 C allele. The same result was also reported in Turkish adults indicating that the R219K variant (rs2230806 G > A) of *ABCA1* was associated with the increased serum LDL cholesterol levels (37). No significant difference in the serum LDL cholesterol levels of NAFLD patients was observed between the carriers and noncarriers of the *ABCA1* rs1800977 A, rs2066715 C, and rs2230808 T.

In summary, we investigated the association between the *ABCA1* polymorphisms and NAFLD in a Chinese Han population for the first time. Our results provided preliminary evidence that *ABCA1* rs1800977 is associated with the risk of NAFLD and the rs1800977 A variant may possess a protective role against the development of NAFLD. The rs2066714 C variant of *ABCA1* increased the serum LDL cholesterol level and the rs2230808 T variant decreased the serum HDL cholesterol level in the NAFLD patients. Although rs1800977 showed a significant association with the development of NAFLD, the distribution of homozygote genotypes for the allele A demonstrated a higher frequency in the control group rather than in the NAFLD group. The detailed mechanism of the *ABCA1* polymorphisms in the development of NAFLD and lipids metabolism remains unknown. Therefore, stronger and more validated data of *ABCA1* polymorphisms in NAFLD obtained from studies with different and preferably larger populations are needed.

Footnotes

Authors' Contribution: Cong Wang and Shousheng Liu contributed equally to this work. Study concept and design: Cong Wang and Shousheng Liu; acquisition of data: Cong Wang, Shousheng Liu, and Linlin Lu; analysis and interpretation of data: Cong Wang, Songling Liao, and Haiyan Yue; drafting of the manuscript: Cong Wang, Quanjiang Dong, Yongning Xin; critical revision of the manuscript for important intellectual content: Shiyong Xuan; statistical analysis: Cong Wang and Shousheng Liu; administrative, technical, and material support: Yongning Xin; study supervision: Shiyong Xuan.

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References

- Liu YL, Reeves HL, Burt AD, Tiniakos D, McPherson S, Leathart JB, et al. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. *Nat Commun.* 2014;5:4309. doi: 10.1038/ncomms5309. [PubMed: 24978903]. [PubMed Central: PMC4279183].
- Musso G, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med.* 2011;43(8):617-49. doi: 10.3109/07853890.2010.518623. [PubMed: 21039302].
- Ekstedt M, Franzén LE, Mathiesen UL, Thorelius I, Holmqvist M, Bode-mar G, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology.* 2006;44(4):865-73. doi: 10.1002/hep.21327. [PubMed: 17006923].

4. McCullough AJ. The clinical features, diagnosis and natural history of nonalcoholic fatty liver disease. *Clin Liver Dis*. 2004;**8**(3):521-33. viii. doi: [10.1016/j.cld.2004.04.004](https://doi.org/10.1016/j.cld.2004.04.004). [PubMed: [1531061](https://pubmed.ncbi.nlm.nih.gov/1531061/)].
5. Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, et al. Association of nonalcoholic fatty liver disease with insulin resistance. *Am J Med*. 1999;**107**(5):450-5. [PubMed: [10569299](https://pubmed.ncbi.nlm.nih.gov/10569299/)].
6. Wanless IR, Lentz JS. Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. *Hepatology*. 1990;**12**(5):1106-10. [PubMed: [2227807](https://pubmed.ncbi.nlm.nih.gov/2227807/)].
7. Wang FS, Fan JG, Zhang Z, Gao B, Wang HY. The global burden of liver disease: the major impact of China. *Hepatology*. 2014;**60**(6):2099-108. doi: [10.1002/hep.27406](https://doi.org/10.1002/hep.27406). [PubMed: [25164003](https://pubmed.ncbi.nlm.nih.gov/25164003/)]. [PubMed Central: [PMC4867229](https://pubmed.ncbi.nlm.nih.gov/PMC4867229/)].
8. Benedict M, Zhang X. Non-alcoholic fatty liver disease: An expanded review. *World J Hepatol*. 2017;**9**(16):715-32. doi: [10.4254/wjh.v9.i16.715](https://doi.org/10.4254/wjh.v9.i16.715). [PubMed: [28652891](https://pubmed.ncbi.nlm.nih.gov/28652891/)]. [PubMed Central: [PMC5468341](https://pubmed.ncbi.nlm.nih.gov/PMC5468341/)].
9. Rinella ME. Nonalcoholic fatty liver disease: a systematic review. *JAMA*. 2015;**313**(22):2263-73. doi: [10.1001/jama.2015.5370](https://doi.org/10.1001/jama.2015.5370). [PubMed: [26057287](https://pubmed.ncbi.nlm.nih.gov/26057287/)].
10. Wang JT, Liu YL. Non-alcoholic fatty liver disease: the problems we are facing. *Hepatobiliary Pancreat Dis Int*. 2003;**2**(3):334-7. [PubMed: [14599934](https://pubmed.ncbi.nlm.nih.gov/14599934/)].
11. Musso G, Gambino R, Cassader M. Cholesterol metabolism and the pathogenesis of non-alcoholic steatohepatitis. *Prog Lipid Res*. 2013;**52**(1):175-91. doi: [10.1016/j.plipres.2012.11.002](https://doi.org/10.1016/j.plipres.2012.11.002). [PubMed: [23206728](https://pubmed.ncbi.nlm.nih.gov/23206728/)].
12. Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatology*. 2010;**52**(5):1836-46. doi: [10.1002/hep.24001](https://doi.org/10.1002/hep.24001). [PubMed: [21038418](https://pubmed.ncbi.nlm.nih.gov/21038418/)].
13. Sozen E, Ozer NK. Impact of high cholesterol and endoplasmic reticulum stress on metabolic diseases: An updated mini-review. *Redox Biol*. 2017;**12**:456-61. doi: [10.1016/j.redox.2017.02.025](https://doi.org/10.1016/j.redox.2017.02.025). [PubMed: [28319895](https://pubmed.ncbi.nlm.nih.gov/28319895/)]. [PubMed Central: [PMC5357672](https://pubmed.ncbi.nlm.nih.gov/PMC5357672/)].
14. Puri P, Wiest MM, Cheung O, Mirshahi F, Sargeant C, Min HK, et al. The plasma lipidomic signature of nonalcoholic steatohepatitis. *Hepatology*. 2009;**50**(6):1827-38. doi: [10.1002/hep.23229](https://doi.org/10.1002/hep.23229). [PubMed: [19937697](https://pubmed.ncbi.nlm.nih.gov/19937697/)]. [PubMed Central: [PMC5031239](https://pubmed.ncbi.nlm.nih.gov/PMC5031239/)].
15. Puri P, Baillie RA, Wiest MM, Mirshahi F, Choudhury J, Cheung O, et al. A lipidomic analysis of nonalcoholic fatty liver disease. *Hepatology*. 2007;**46**(4):1081-90. doi: [10.1002/hep.21763](https://doi.org/10.1002/hep.21763). [PubMed: [17654743](https://pubmed.ncbi.nlm.nih.gov/17654743/)].
16. Ioannou GN. The Role of Cholesterol in the Pathogenesis of NASH. *Trends Endocrinol Metab*. 2016;**27**(2):84-95. doi: [10.1016/j.tem.2015.11.008](https://doi.org/10.1016/j.tem.2015.11.008). [PubMed: [26703097](https://pubmed.ncbi.nlm.nih.gov/26703097/)].
17. Ioannou GN, Morrow OB, Connole ML, Lee SP. Association between dietary nutrient composition and the incidence of cirrhosis or liver cancer in the United States population. *Hepatology*. 2009;**50**(1):175-84. doi: [10.1002/hep.22941](https://doi.org/10.1002/hep.22941). [PubMed: [19441103](https://pubmed.ncbi.nlm.nih.gov/19441103/)].
18. Caballero F, Fernandez A, De Lacy AM, Fernandez-Checa JC, Caballeria J, Garcia-Ruiz C. Enhanced free cholesterol, SREBP-2 and StAR expression in human NASH. *J Hepatol*. 2009;**50**(4):789-96. doi: [10.1016/j.jhep.2008.12.016](https://doi.org/10.1016/j.jhep.2008.12.016). [PubMed: [19231010](https://pubmed.ncbi.nlm.nih.gov/19231010/)].
19. Singaraja RR, Brunham LR, Visscher H, Kastelein JJ, Hayden MR. Efflux and atherosclerosis: the clinical and biochemical impact of variations in the ABCA1 gene. *Arterioscler Thromb Vasc Biol*. 2003;**23**(8):1322-32. doi: [10.1161/01.ATV.0000078520.89539.77](https://doi.org/10.1161/01.ATV.0000078520.89539.77). [PubMed: [12763760](https://pubmed.ncbi.nlm.nih.gov/12763760/)].
20. Luciani MF, Denizot F, Savary S, Mattei MG, Chimini G. Cloning of two novel ABC transporters mapping on human chromosome 9. *Genomics*. 1994;**21**(1):150-9. doi: [10.1006/geno.1994.1237](https://doi.org/10.1006/geno.1994.1237). [PubMed: [8088782](https://pubmed.ncbi.nlm.nih.gov/8088782/)].
21. Wang S, Smith JD. ABCA1 and nascent HDL biogenesis. *Biofact*. 2014;**40**(6):547-54. doi: [10.1002/biof.1187](https://doi.org/10.1002/biof.1187). [PubMed: [25359426](https://pubmed.ncbi.nlm.nih.gov/25359426/)]. [PubMed Central: [PMC4294467](https://pubmed.ncbi.nlm.nih.gov/PMC4294467/)].
22. Liu Y, Tang C. Regulation of ABCA1 functions by signaling pathways. *Biochim Biophys Acta*. 2012;**1821**(3):522-9. doi: [10.1016/j.bbali.2011.08.015](https://doi.org/10.1016/j.bbali.2011.08.015). [PubMed: [21920460](https://pubmed.ncbi.nlm.nih.gov/21920460/)]. [PubMed Central: [PMC3243790](https://pubmed.ncbi.nlm.nih.gov/PMC3243790/)].
23. Hong SH, Rhyne J, Zeller K, Miller M. ABCA1(Alabama): a novel variant associated with HDL deficiency and premature coronary artery disease. *Atherosclerosis*. 2002;**164**(2):245-50. [PubMed: [12204794](https://pubmed.ncbi.nlm.nih.gov/12204794/)].
24. Tarr PE, Telenti A. Toxicogenetics of antiretroviral therapy: genetic factors that contribute to metabolic complications. *Antivir Ther*. 2007;**12**(7):999-1013. [PubMed: [18018758](https://pubmed.ncbi.nlm.nih.gov/18018758/)].
25. Rickham PP. Human Experimentation. Code of Ethics of the World Medical Association. Declaration of Helsinki. *Br Med J*. 1964;**2**(5402):177. [PubMed: [14150898](https://pubmed.ncbi.nlm.nih.gov/14150898/)]. [PubMed Central: [PMC1816102](https://pubmed.ncbi.nlm.nih.gov/PMC1816102/)].
26. Malhi H, Gores GJ. Molecular mechanisms of lipotoxicity in non-alcoholic fatty liver disease. *Semin Liver Dis*. 2008;**28**(4):360-9. doi: [10.1055/s-0028-1091980](https://doi.org/10.1055/s-0028-1091980). [PubMed: [18956292](https://pubmed.ncbi.nlm.nih.gov/18956292/)]. [PubMed Central: [PMC2908270](https://pubmed.ncbi.nlm.nih.gov/PMC2908270/)].
27. Schindhelm RK, Diamant M, Dekker JM, Tushuizen ME, Teerlink T, Heine RJ. Alanine aminotransferase as a marker of non-alcoholic fatty liver disease in relation to type 2 diabetes mellitus and cardiovascular disease. *Diabetes Metab Res Rev*. 2006;**22**(6):437-43. doi: [10.1002/dmrr.666](https://doi.org/10.1002/dmrr.666). [PubMed: [16832839](https://pubmed.ncbi.nlm.nih.gov/16832839/)].
28. Westerbacka J, Corner A, Tiikkainen M, Tamminen M, Vehkavaara S, Hakkinen AM, et al. Women and men have similar amounts of liver and intra-abdominal fat, despite more subcutaneous fat in women: implications for sex differences in markers of cardiovascular risk. *Diabetologia*. 2004;**47**(8):1360-9. doi: [10.1007/s00125-004-1460-1](https://doi.org/10.1007/s00125-004-1460-1). [PubMed: [15309287](https://pubmed.ncbi.nlm.nih.gov/15309287/)].
29. Manne V, Handa P, Kowdley KV. Pathophysiology of Nonalcoholic Fatty Liver Disease/Nonalcoholic Steatohepatitis. *Clin Liver Dis*. 2018;**22**(1):23-37. doi: [10.1016/j.cld.2017.08.007](https://doi.org/10.1016/j.cld.2017.08.007). [PubMed: [29128059](https://pubmed.ncbi.nlm.nih.gov/29128059/)].
30. Kolovou V, Marvaki A, Boutsikou M, Vasilopoulos G, Degiannis D, Marvaki C, et al. Effect of ATP-binding Cassette Transporter A1 (ABCA1) Gene Polymorphisms on Plasma Lipid Variables and Common Demographic Parameters in Greek Nurses. *Open Cardiovasc Med J*. 2016;**10**:233-9. doi: [10.2174/1874192401610010233](https://doi.org/10.2174/1874192401610010233). [PubMed: [27990182](https://pubmed.ncbi.nlm.nih.gov/27990182/)]. [PubMed Central: [PMC5120382](https://pubmed.ncbi.nlm.nih.gov/PMC5120382/)].
31. Abdel-Razek O, Sadananda SN, Li X, Cermakova L, Frohlich J, Brunham LR. Increased prevalence of clinical and subclinical atherosclerosis in patients with damaging mutations in ABCA1 or APOA1. *J Clin Lipidol*. 2018;**12**(1):116-21. doi: [10.1016/j.jacl.2017.10.010](https://doi.org/10.1016/j.jacl.2017.10.010). [PubMed: [29150341](https://pubmed.ncbi.nlm.nih.gov/29150341/)].
32. Gamboa-Melendez MA, Galindo-Gomez C, Juarez-Martinez L, Gomez FE, Diaz-Diaz E, Avila-Arcos MA, et al. Novel association of the R230C variant of the ABCA1 gene with high triglyceride levels and low high-density lipoprotein cholesterol levels in Mexican school-age children with high prevalence of obesity. *Arch Med Res*. 2015;**46**(6):495-501. doi: [10.1016/j.arcmed.2015.07.008](https://doi.org/10.1016/j.arcmed.2015.07.008). [PubMed: [26256050](https://pubmed.ncbi.nlm.nih.gov/26256050/)].
33. Min HK, Kapoor A, Fuchs M, Mirshahi F, Zhou H, Maher J, et al. Increased hepatic synthesis and dysregulation of cholesterol metabolism is associated with the severity of nonalcoholic fatty liver disease. *Cell Metab*. 2012;**15**(5):665-74. doi: [10.1016/j.cmet.2012.04.004](https://doi.org/10.1016/j.cmet.2012.04.004). [PubMed: [22560219](https://pubmed.ncbi.nlm.nih.gov/22560219/)]. [PubMed Central: [PMC3361911](https://pubmed.ncbi.nlm.nih.gov/PMC3361911/)].
34. Tomita K, Teratani T, Suzuki T, Shimizu M, Sato H, Narimatsu K, et al. Free cholesterol accumulation in hepatic stellate cells: mechanism of liver fibrosis aggravation in nonalcoholic steatohepatitis in mice. *Hepatology*. 2014;**59**(1):154-69. doi: [10.1002/hep.26604](https://doi.org/10.1002/hep.26604). [PubMed: [23832448](https://pubmed.ncbi.nlm.nih.gov/23832448/)].
35. Wilkens S. Structure and mechanism of ABC transporters. *F1000Prime Rep*. 2015;**7**:14. doi: [10.12703/P7-14](https://doi.org/10.12703/P7-14). [PubMed: [25750732](https://pubmed.ncbi.nlm.nih.gov/25750732/)]. [PubMed Central: [PMC4338842](https://pubmed.ncbi.nlm.nih.gov/PMC4338842/)].
36. Oram JF, Heinecke JW. ATP-binding cassette transporter A1: a cell cholesterol exporter that protects against cardiovascular disease. *Physiol Rev*. 2005;**85**(4):1343-72. doi: [10.1152/physrev.00005.2005](https://doi.org/10.1152/physrev.00005.2005). [PubMed: [16183915](https://pubmed.ncbi.nlm.nih.gov/16183915/)].
37. Coban N, Onat A, Komurcu Bayrak E, Gulec C, Can G, Erginel Unaltuna N. Gender specific association of ABCA1 gene R219K variant in coronary disease risk through interactions with serum triglyceride elevation in Turkish adults. *Anadolu Kardiyol Derg*. 2014;**14**(1):18-25. doi: [10.5152/akd.2013.234](https://doi.org/10.5152/akd.2013.234). [PubMed: [24084154](https://pubmed.ncbi.nlm.nih.gov/24084154/)].