Int J Clin Exp Pathol 2017;10(11):11147-11158
www.ijcep.com /ISSN:1936-2625/IJCEP0059891

Original Article

DNAH11 rs12670798 variant and G × E interactions on serum lipid levels, coronary heart disease, ischemic stroke and the lipid-lowering efficacy of atorvastatin

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Received June 20, 2017; Accepted June 25, 2017; Epub November 1, 2017; Published November 15, 2017

Abstract: Previous genome-wide association studies have showed that the rs12670798 variant in the dynein axonemal heavy chain 11 gene (*DNAH11*) is associated with some serum lipid phenotypes. The present study was undertaken to detect the *DNAH11* rs12670798 variant and G × E interactions on serum lipid levels, coronary heart disease (CHD), ischemic stroke (IS), and the lipid-lowering efficacy of atorvastatin in the Chinese Han population. This study included 1,108 unrelated patients (CHD, 568 and IS, 540) and 541 healthy controls. Genotypes of the *DNAH11* rs12670798 were determined by the Snapshot technology. A total of 724 hyperlipidemic patients were treated with atorvastatin calcium tablet 20 mg per day for 8 weeks after genotyping. Serum total cholesterol (TC) levels in controls were different among the three genotypes of the rs12670798 (P = 0.019), the C allele carriers had higher TC levels than the C allele non-carriers. The C allele carriers were associated with an increased risk of CHD (CT genotype: OR = 1.345, 95% CI = 0.975-1.855, P = 0.071; CC genotype: OR = 1.590, 95% CI = 1.109-2.278, P = 0.012). The C allele carriers were also associated with an increased risk of IS (CT genotype: OR = 1.597, 95% CI = 1.153-2.213, P = 0.005; CC genotype: OR = 1.722, 95% CI = 1.192-2.488, P = 0.004). The C allele carriers had lower TC, low-density lipoprotein cholesterol, apolipoprotein (Apo) A1 and ApoB levels than the C allele non-carriers after atorvastatin treatment. Stratified analysis showed that the *DNAH11* rs12670798 may interact with the gender, age, body mass index, cigarette smoking, and alcohol consumption to affect the risk of CHD and IS. The *DNAH11* rs12670798 variant was associated with elevated serum TC levels, and increased risk of CHD and IS in the Chinese Han population. The C allele carriers had higher serum TC levels and the risk of CHD and IS than the C allele non-carriers, but they had lower TC, LDL-C, ApoA1 and ApoB levels than the C allele non-carriers after atorvastatin treatment.

Keywords: Coronary heart disease, ischemic stroke, dynein axonemal heavy chain 11, single nucleotide polymorphism, lipids, atorvastatin

Introduction

Coronary heart disease (CHD) and ischemic stroke (IS), two of the most common cardiovascular diseases, are associated with high morbidity and mortality and remain the most common causes of death globally [1]. A main underlying pathology of CHD and IS is atherosclerosis, a process of cumulative deposition of lipoproteins in the arteries. Thus, CHD and IS may share some common aspects of their underlying pathogenesis, as well as risk factors, such as dyslipidemia, obesity, hypertension, diabetes, chronic kidney disease and cigarette smoking [1, 2]. Studies of siblings, twins, and families suggest a heritable genetic contribution to CHD and IS, with the heritability ranging from 30% to 60% in population based samples [3-8]. Previous genome-wide association studies (GWASs) and meta-analyses have identified various genes and loci in the predisposition to CHD [9] or IS [10] in different populations. Furthermore, certain genetic variants originally shown to influence the risk of CHD were also subsequently found to be associated with IS [11, 12], suggestive of a shared genetic architecture for the two conditions.

The dynein axonemal heavy chain 11 gene (*DNAH11*, also known as *CILD7*, *DNHBL*, *DPL11*,

DNAHBL, or *DNAHC11*) maps on chromosome 7p21 [13]. *DNAH11* encodes a ciliary outer dynein arm protein and is a member of the dynein heavy chain family. It is a microtubule-dependent motor ATPase and has been reported to be involved in the movement of respiratory cilia. Mutations in this gene have been implicated in causing Kartagener Syndrome (a combination of situs inversus totalis and Primary Ciliary Dyskinesia, also called Immotile Cilia Syndrome 1) and male sterility [14-18]. Recently, a single nucleotide polymorphism (SNP) of rs12670798 (G > A at 7p21.38 or T > C at 7p21.57) in the *DNAH11* has been found to be associated with modifications of plasma total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels in some GWASs [19-21] but not in others [22-24]. Therefore, the purpose of the present study was to detect the association of the *DNAH11* rs12670798 (T > C at 7p21.57) and serum lipid traits and the risk of CHD and IS in the Han Chinese population.

Materials and methods

Study patients

A total of 1,108 unrelated patients with CHD (n = 568) and IS (n = 540) were recruited in this association study. All of them were hospitalized patients in the First Affiliated Hospital, Guangxi Medical University. The diagnosis of CHD was based on typical clinical symptoms, electrocardiographic findings, as well as increased serum markers such as creatinine kinase-MB and troponin T. Coronary angiography was also performed in these patients. The selected CHD patients were subject to significant coronary stenosis (≥ 50%) in at least either one of the three main coronary arteries or their major branches (branch diameter ≥ 2 mm). Additionally, angiographic severity of disease was defined as single or multi-vessel disease based on the number of involved artery (luminal narrowing ≥ 50%) in the three major coronary arteries [25, 26]. The classification of IS was made according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria [27]. The selected IS patients in this study included individuals who were eligible for one of the two subtypes of TOAST criteria: large-artery atherosclerosis and small-vessel occlusion. Subjects with a history of hematologic, neoplastic, renal, liver, thyroid, autoimmune diseases and type 1 diabetes were excluded. The selected IS patients who had a past history of CHD, or the selected CHD patients who had a past history of IS were excluded from the study.

Control subjects

A total of 541 control subjects matched by age, gender, and ethnic group were consecutively recruited from the Physical Examination Center of the First Affiliated Hospital, Guangxi Medical University during the same period when CHD and IS patients were recruited. The controls were free of CHD and IS by questionnaires, history taking and clinical examination. All enrolled individuals were Han Chinese from Guangxi, the People's Republic of China. A standard questionnaire was used to collect the clinical data of the participants. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University (No. Lunshen 2009-Guike-018; Jan. 7, 2009). Informed consent was obtained from all subjects after they received a full explanation of the study.

Atorvastatin treatment

A total of 724 hyperlipidemic patients [TC > 5.17 mmol/L, and/or triglyceride (TG) > 1.70 mmol/L; 253 from control, 248 from CHD and 223 from IS] were treated with atorvastatin calcium tablet (Lipitor, Pfizer Wuxi Pharmaceutical Co., Ltd.) 20 mg per day for 8 weeks after genotyping and the first biochemical measurements. After 8 weeks of atorvastatin treatment, blood samples were collected again, and the second biochemistry analyses for serum lipid profiles were performed.

Biochemical measurements

Venous blood samples were obtained from all subjects after at least 12 hours of fasting. The levels of serum TC, TG, high-density lipoprotein cholesterol (HDL-C), and LDL-C in samples were determined by enzymatic methods with commercially available kits. Serum apolipoprotein (Apo) A1 and ApoB levels were detected by the immunoturbidimetric immunoassay. The normal values of serum TC, TG, HDL-C, LDL-C, ApoA1, ApoB levels, and the ratio of ApoA1 to ApoB in our Clinical Science Experiment Center were 3.10-5.17, 0.56-1.70, 0.91-1.81, 2.70-3.20 mmol/L, 1.00-1.78, 0.63-1.14 g/L, and 1.00-2.50; respectively [28, 29]. The individuals with TC > 5.17 mmol/L, and/or TG > 1.70 mmol/L were defined as hyperlipidemic [28, 29]. Type 2 diabetes was diagnosed

Table 1. General characteristics and serum lipid levels between the controls and patients

Characteristic	Control	CHD	IS	P_{CHD}	P_{IS}
Number	541	568	540		
Male/Female	394/147	422/146	390/150	0.580	0.823
Age, years	61.89±10.43	62.20±10.51	62.18±12.36	0.624	0.184
Height	158.59±8.30	164.20±6.89	163.75±7.18	0.000	0.000
Weight	56.51±9.16	64.59±10.71	62.97±11.20	0.000	0.000
Body mass index, kg/m^2	22.42±2.83	22.88±3.24	23.42±3.51	0.000	0.000
Systolic blood pressure, mmHg	127.69±18.98	132.84±23.13	147.54±22.98	0.000	0.000
Diastolic blood pressure, mmHg	80.44±11.48	79.07±13.38	83.71±12.92	0.067	0.000
Pulse pressure, mmHg	47.25±14.04	53.77±16.85	63.84±17.76	0.000	0.000
Cigarette smoking, n (%)					
Nonsmoker	335 (61.9)	298 (52.5)	314 (58.1)		
< 20 cigarettes/day	107 (19.8)	132 (23.2)	157 (29.1)		
≥ 20 cigarettes/day	99 (18.3)	138 (24.3)	69 (12.8)	0.005	0.000
Alcohol consumption, n (%)					
Nondrinker	307 (56.7)	421 (74.1)	381 (70.6)		
< 25 g/day	160 (29.6)	102 (18.0)	126 (22.3)		
≥ 25 g/day	74 (13.7)	45 (7.9)	33 (6.1)	0.000	0.000
Total cholesterol, mmol/L	4.90±0.86	4.53±1.20	4.52±1.15	0.000	0.000
Triglyceride, mmol/L	1.01 (0.69)	1.35 (0.96)	1.36 (0.93)	0.000	0.000
HDL-C, mmol/L	1.88±0.46	1.14±.034	1.23±0.40	0.000	0.000
LDL-C, mmol/L	2.73±0.70	2.71±1.00	2.68±0.90	0.707	0.296
Apolipoprotein (Apo) A1, g/L	1.42±0.26	1.04±0.52	1.03±0.22	0.000	0.000
ApoB, g/L	0.90±0.23	0.90±0.27	0.89±0.25	0.293	0.753
ApoA1/ApoB	1.91±1.91	1.37±2.47	1.19±0.60	0.000	0.000
Type 2 diabetes, n (%)	21 (3.9)	90 (15.8)	120 (22.2)	0.000	0.000
Hypertension, n (%)	178 (32.9)	297 (52.3)	323 (59.8)	0.000	0.000
Hyperlipidemia, n (%)	180 (33.3)	260 (45.8)	246 (45.6)	0.000	0.000

CHD, coronary heart disease; IS, ischemic stroke; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; P_{CHD}, CHD *vs*. controls; P_{IS}, IS *vs*. controls. The value of triglyceride was presented as median (interquartile range), the difference between CHD/IS patients and controls was determined by the Wilcoxon-Mann-Whitney test. The remaining characteristics between patients and controls were tested by the Student's unpaired *t*-test.

according to the WHO diagnostic criteria for diabetes: (1) fasting glucose (FPG) ≥ 7.0 mmol/L; (2) 2 h postprandial glucose ≥ 11.1 mmol/L; or (3) self-reported diagnosis of diabetes or use of anti-diabetic medications [30, 31]. Hypertension was defined according to the criteria outlined by the 1999 World Health Organization-International Society of Hypertension Guidelines for the management of hypertension [32, 33]. Normal weight, overweight and obesity were defined as a body mass index (BMI) < 24, 24-28, and > 28 kg/m^2; respectively [34].

Genotyping

Genomic deoxyribonucleic acid (DNA) was extracted from leucocytes of venous blood using the phenol-chloroform method. Genotyping of the *DNAH11* rs12670798 SNP was performed by the Snapshot technology platform [35-42]. All experimental manipulations were completed in the Center for Human Genetics Research, Shanghai Genesky Bio-Tech Co. Ltd., China. Primers were designed with online Primer 3 software (http://frodo.wi.mit.edu/). The sense and antisense primers were 5'-GCCAGCATTTCAGCCTCCACT-3' and 5'-AGCACGCAAGGTGAACAGGTG-3', respectively.

Statistical analyses

The statistical analyses were performed using the statistical software package SPSS 21.0 (SPSS Inc., Chicago, Illinois). Quantitative variables were expressed as mean ± standard

Table 2. Genotypic and allelic frequencies of the *DNAH11* rs12670798 SNP and the risk of coronary heart disease and ischemic stroke [n (%)]

Genotype/allele	Control n = 541	CHD n = 568	IS n = 540	OR (95% CI)$_{CHD}$	P_{CHD}	OR (95% CI)$_{IS}$	P_{IS}
TT	145 (26.8)	116 (20.4)	100 (18.5)	1		1	
CT	264 (48.8)	277 (48.8)	285 (52.8)	1.35 (0.98-1.86)	0.071	1.60 (1.15-2.21)	0.005
CC	132 (24.4)	175 (30.8)	155 (28.7)	1.59 (1.10-2.28)	0.012	1.72 (1.19-2.49)	0.004
X^2		8.905	10.911				
P		0.012	0.004				
TT	145 (26.8)	116 (20.4)	100 (18.5)	1		1	
CT+CC	396 (73.2)	452 (79.6)	440 (81.5)	1.43 (1.06-1.93)	0.021	1.64 (1.20-2.23)	0.02
X^2		6.267	10.580				
P		0.012	0.001				
T	554 (51.2)	509 (44.8)	485 (44.9)	1		1	
C	528 (48.8)	627 (55.2)	595 (55.1)	1.29 (1.09-1.52)	0.02	1.29 (1.09-1.52)	0.01
X^2		9.081	8.578				
P		0.003	0.003				
P_{HWE}	0.862	0.946	0.302				

Adjusted for sex, age, smoking, drinking, body mass index, diabetes, hypertension, hyperlipidemia. CHD, coronary heart disease; IS, ischemic stroke; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; CI, confidence interval.

deviation (serum TG levels were presented as medians and interquartile ranges). Qualitative variables were expressed as percentages. The Handy-Weinberg equilibrium was determined using the standard goodness-of-fit test. A chi-square analysis was used to evaluate the difference in genotype distribution between the groups. The general characteristics between patient and control groups were tested by the Student's unpaired *t*-test. The association of genotypes and serum lipid parameters in control group was tested by analysis of covariance (ANCOVA). Unconditional logistic regression analysis was used to assess the correlation between the risk of CHD and IS and genotypes. Age, gender, BMI, cigarette smoking and alcohol consumption were adjusted for the statistical analysis. Odds ratio (OR) and 95% confidence interval (95% CI) were calculated using unconditional logistic regression analysis. Results were considered to be statistically significant if bilateral *P*-values were less than 0.05.

Results

General characteristics of the participants

The general characteristics of the patient and control groups are shown in **Table 1**. The values of height, weight, BMI, systolic blood pressure, pulse pressure, serum TG; the percentages of subjects who smoked cigarettes; and the prevalence of type 2 diabetes, hypertension and hyperlipidemia were higher in both CHD and IS than in control groups ($P < 0.01$ for all), whereas the levels of serum TC, HDL-C, ApoA1; the ratio of ApoA1 to ApoB; and the percentages of subjects who consumed alcohol were lower in both CHD and IS than in control groups ($P < 0.001$ for all). There were no differences in the values of age, gender, diastolic blood pressure, serum LDL-C and ApoB between the control and patient groups ($P > 0.05$ for all).

Genotypic and allelic frequencies

The genotypic and allelic frequencies of the *DNAH11* rs12670798 SNP are presented in **Table 2**. The genotype distribution of the SNP was concordant with the Hardy-Weinberg equilibrium in both cases and controls. The frequency of the C allele was 48.8% in controls, 55.2% in CHD group ($P < 0.01$ vs. controls), and 55.1% in IS group ($P < 0.01$ vs. controls); respectively. The frequencies of the TT, CT and CC genotypes were 26.8%, 48.8% and 24.4% in controls; 20.4%, 48.8% and 30.8% in CHD patients ($P = 0.012$ vs. controls); and 18.5%, 52.8% and 28.7% in IS patients ($P < 0.01$ vs. controls); respectively.

DNAH11 rs12670798 SNP and the risk of CHD and IS

As shown in **Table 2**, the rs12670798 C allele carriers were still associated with an increased

Table 3. Genotypes of the *DNAH11* rs12670798 SNP and serum lipid levels in controls

Genotype	N	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	ApoA1 (g/L)	ApoB (g/L)	ApoA1/ApoB
TT	145	4.74±0.87	1.01 (0.56)	1.90±0.43	2.63±0.68	1.41±0.20	0.88±0.23	1.80±1.27
CT	262	4.92±0.83	1.03 (0.71)	1.86±0.46	2.76±0.69	1.42±0.30	0.90±0.21	1.85±1.82
CC	132	5.02±0.89	0.95 (0.62)	1.91±0.49	2.79±0.73	1.42±0.23	0.87±0.25	2.17±2.50
F		3.994	3.438	1.273	1.955	0.188	1.079	1.560
P		0.019	0.179	0.281	0.143	0.829	0.341	0.211

TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B. The value of triglyceride was presented as median (interquartile range), and the difference among the genotypes was determined by the Kruskal-Wallis test. The association of genotypes and the remaining serum lipid parameters was tested by analysis of covariance (ANCOVA).

risk of CHD (CT genotype: OR = 1.345, 95% CI = 0.975-1.855, *P* = 0.071; CC genotype: OR = 1.590, 95% CI = 1.109-2.278, *P* = 0.012) after adjustment for age, gender, BMI, cigarette smoking, alcohol consumption and hypertension. The C allele carriers were also associated with an increased risk of IS (CT genotype: OR = 1.597, 95% CI = 1.153-2.213, *P* = 0.005; CC genotype: OR = 1.722, 95% CI = 1.192-2.488, *P* = 0.004) after adjustment for those potential confounders.

DNAH11 rs12670798 SNP and serum lipid levels

The association of the *DNAH11* rs12670798 SNP and serum lipid levels in control group is summarized in **Table 3**. Serum TC levels were different among the three genotypes of the *DNAH11* rs12670798 SNP (*P* = 0.019), the C allele carriers had higher serum TC levels than the C allele non-carriers.

DNAH11 rs12670798 SNP and the stratified risk factors of CHD or IS

Table 4 lists the results of the *DNAH11* rs12670798 SNP and the stratified risk factors of CHD or IS including gender, age, BMI, cigarette smoking, alcohol consumption, and hypertension. The results showed that the subjects with CC genotype had higher risk of CHD in age ≤ 60 years, BMI ≤ 24 kg/m^2, non-smoking, non-drinking subgroups, and higher risk of IS in male, BMI ≤ 24 kg/m^2 and smoking subgroups than in the corresponding subgroups (*P* < 0.05-0.01); respectively.

The lipid-lowering efficacy of atorvastatin in different genotype individuals

After 8-week treatment of atorvastatin, the levels of TC, TG, LDL-C and ApoB were significantly decreased in the hyperlipidemic patients (*P* < 0.001 for all; **Figure 1**). There was no significant difference in serum HDL-C and ApoA1 levels. We also found that the *DNAH11* rs12670798 SNP changed the effects of atorvastatin treatment on some serum lipid parameters. The rs12670798 C allele carriers had lower TC, LDL-C and ApoB levels than the rs12670798 C allele non-carriers after atorvastatin treatment. However, the rs12670798 C allele carriers also decreased serum ApoA1 levels after atorvastatin treatment, especially in the subjects with CT genotype (*P* < 0.01 for all; **Figure 1**).

Discussion

The association between the *DNAH11* rs12670798 SNP and the risk of CHD is inconsistent. Two previous studies failed to find a significant genetic association between the *DNAH11* rs12670798 SNP and the risk of CHD [20, 24]. The association between the *DNAH11* rs12670798 SNP and the risk of IS has not been reported previously. In an exome array analysis for IS, Söderholm *et al.* [43] showed that no exome variant or gene was significantly associated with all IS after Bonferroni correction (all $P > 1.8 \times 10^{-6}$ for single-variant and $> 4.15 \times 10^{-6}$ for gene-based analysis). The strongest association in single-variant analysis was found for a missense variant in the *DNAH11* (rs143362381; $P = 5.01 \times 10^{-6}$). In the present study, however, we showed that the genotypic and allelic frequencies of the *DNAH11* rs12670798 SNP were different between CHD/IS patients and healthy controls. The frequency of the C allele was higher in CHD (55.2%) and IS (55.1%) patients than in controls (48.8%, *P* < 0.01 for each). The frequency of the CC genotype was also higher in CHD (30.8%, *P* < 0.01) and IS (28.7%, *P* < 0.05) patients than in con-

Table 4. Stratified analyses of the *DNAH11* rs12670798 SNP and the risk of coronary heart disease and ischemic stroke

Factor	OR (95% CI)$_{TT}$	OR (95% CI)$_{CT}$	OR (95% CI)$_{CC}$	P_{CT}	P_{CC}
CHD					
Gender/male	1	1.208 (0.833-1.752)	1.508 (0.994-2.287)	0.320	0.053
Gender/female	1	1.995 (1.021-3.895)	2.067 (0.982-4.351)	0.043	0.056
Age/≤ 60 years	1	1.116 (0.679-1.832)	2.013 (1.157-3.504)	0.666	0.013
Age > 60 years	1	1.359 (0.841-2.199)	1.519 (0.987-2.338)	0.211	0.057
BMI/≤ 24 kg/m^2	1	1.466 (0.980-2.195)	1.641 (1.040-2.590)	0.063	0.033
BMI/> 24 kg/m^2	1	1.148 (0.661-1.993)	1.534 (0.837-2.811)	0.624	0.166
Smoking/No	1	1.606 (1.029-2.505)	1.813 (1.107 -2.970)	0.037	0.018
Smoking/Yes	1	1.085 (0.671-1.755)	1.471 (0.851-2.542)	0.740	0.167
Drinking/No	1	1.447 (0.979-2.140)	1.643 (1.056-2.555)	0.064	0.028
Drinking/Yes	1	1.189 (0.673-2.102)	1.418 (0.758-2.653)	0.551	0.275
Hypertension/No	1	1.135 (0.744-1.731)	1.764 (1.094-2.844)	0.558	0.020
Hypertension/Yes	1	1.476 (0.855-2.548)	1.722 (1.042 -2.845)	0.162	0.034
IS					
Gender/male	1	1.642 (1.114-2.420)	1.867 (1.211-2.879)	0.012	0.005
Gender/female	1	1.308 (0.622-2.749)	1.453 (0.766-2.756)	0.479	0.253
Age/≤ 60 years	1	1.496 (0.905-2.473)	2.002 (1.132 -3.539)	0.116	0.017
Age > 60 years	1	1.492 (0.905-2.459)	1.596 (1.025-2.487)	0.117	0.039
BMI/≤ 24 kg/m^2	1	1.739 (1.162-2.604)	1.869 (1.183-2.952)	0.007	0.007
BMI/> 24 kg/m^2	1	1.351 (0.760-2.401)	1.515 (0.794-2.891)	0.306	0.207
Smoking/No	1	1.555 (1.002-2.365)	1.609 (0.999-2.592)	0.039	0.051
Smoking/Yes	1	1.679 (0.983-2.869)	2.047 (1.123-3.733)	0.058	0.019
Drinking/No	1	1.549 (0.979-2.450)	1.578 (1.055 -2.361)	0.061	0.026
Drinking/Yes	1	1.759 (0.981-3.154)	2.278 (1.196 -4.339)	0.058	0.012
Hypertension/No	1	1.416 (0.905-2.216)	2.037 (1.232 -3.369)	0.128	0.006
Hypertension/Yes	1	1.496 (0.870-2.572)	1.898 (1.164 -3.092)	0.145	0.010

OR, odds ratio; CI, confidence interval. OR and 95% CI were obtained from unconditional Logistic regression model after adjusted for age, gender, body mass index, smoking status, alcohol consumption, hypertension.

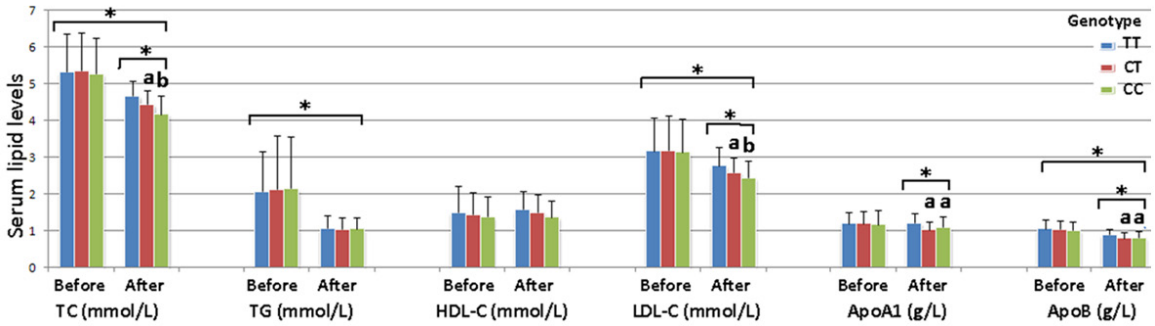

Figure 1. The lipid-lowering response to atorvastatin treatment in different *DNAH11* rs12670798 genotype individuals in hyperlipidemia. TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B. Genotype and atorvastatin interaction decreases serum lipid levels ($^aP < 0.01$ in comparison with TT genotype; $^bP < 0.01$ in comparison with CT genotype; $^*P < 0.001$.

trols (24.4%). Unconditional logistic regression analysis also showed that the C allele carriers were associated with an increased risk of CHD and IS after adjustment for age, gender, BMI, cigarette smoking, alcohol consumption and hypertension. To the best of our knowledge,

this is the first investigation demonstrating that the *DNAH11* rs12670798 SNP was significantly associated with the risk of CHD and IS in the Chinese Han population. These findings suggest that the *DNAH11* rs12670798 SNP may be a susceptibility locus for CHD and IS in our study populations.

The minor allele frequency (MAF) of the *DNAH11* rs12670798 SNP was different in diverse racial/ethnic groups. In a total of 17,797-22,562 persons, aged 18-104 years and from geographic regions spanning from the Nordic countries to Southern Europe, Aulchenko *et al.* [19] showed that the MAF of A allele (G > A at 7p21.38) was 24%. But the MAF of A allele was 33% in 1,466 individuals of African ancestry from Spanish Town, Jamaica [21]. The MAF of C allele was 23% in individuals of European descent (ascertained in the United States, Europe, or Australia) [20], 50% in 1,360 non-type 2 diabetes controls, 1,173 type 2 diabetes patients, and a community-based prospective epidemiologic cohort of 2,105 subjects from Chinese Shanghai [23], 48.5% in controls and 49.5% in CHD patients recruited from Zhongnan Hospital of Wuhan University and Asia Heart Hospital, China [24]. In the present study, we showed that the MAF of C allele was 55.2% in CHD, 55.1% in IS patients and 48.8% in controls. These findings suggest that the prevalence of the *DNAH11* rs12670798 SNP might have a racial/ethnic specificity.

The potential association between the *DNAH11* rs12670798 SNP and serum lipid profiles in humans has been evaluated in several GWASs. However, previous findings on the association of this polymorphism with the changes in plasma lipid levels are inconsistent. Several GWASs have reported significant association between the *DNAH11* rs12670798 SNP and TC and LDL-C levels [19-21], whereas several reports failed to find a significant genetic effect on TC and LDL-C concentrations [22-24]. In a previous GWAS in 16 European population cohorts, Aulchenko *et al.* [19] showed that the *DNAH11* rs12670798 SNP was associated with TC ($P = 9.2 \times 10^{-7}$) and LDL-C ($P = 6.1 \times 10^{-9}$) [21]. The *DNAH11* rs12670798 SNP was also previously reported for association with TC and LDL-C in Utah residents with ancestry from northern and western Europe ($P = 9 \times 10^{-10}$) [20]. In individuals of African ancestry from Spanish Town, Jamaica ($n = 1128$), however, Gupta *et al.* [22] showed no association between the *DNAH11* rs12670798 SNP and serum LDL-C levels ($P = 0.24$). No association between the *DNAH11* rs12670798 SNP and serum TC, log (TG), HDL-C, LDL-C, log (TC/HDL-C) was observed in Chinese non-type 2 diabetes controls, type 2 diabetes patients, and CHD patients [23, 24]. In the current study, we found that serum TC levels in control group were different among the three genotypes of the *DNAH11* rs12670798 SNP, the C allele carriers had higher serum TC levels than the C allele non-carriers. These results suggest that the *DNAH11* rs12670798 SNP may also be a genetic marker associated with dyslipidemia in our study populations. The reasons for these differences in different studies remain unclear. In addition to different genetic background, sample size and different statistical method may also contribute to the discrepancies among our study and other studies in different populations.

The interactions of the *DNAH11* rs12670798 SNP and environmental factors on the risk of CHD and IS are not known. In the present study, stratified analyses according to gender, age, BMI, smoking, drinking, and hypertension showed that the *DNAH11* rs12670798 CC genotype was associated with an increased risk of CHD in patients with age ≤ 60 years, BMI ≤ 24 kg/m^2, non-smoking, non-drinking subgroups, and higher risk of IS in male, BMI ≤ 24 kg/m^2 and smoking subgroups than in the corresponding subgroups; respectively. These findings suggest that the *DNAH11* rs12670798 SNP may interact with these parameters to influence the risk of CHD and IS. But these interactions still need to be determined.

Statins are the most prescribed class of hypolipidemic drugs, which have shown high effectiveness in reducing the risk of myocardial infarction, stroke, and other cardiovascular events by lowering plasma concentration of LDL-C for more than 20 years [44]. A recent report indicates that 25% of Americans over age 45 takes a statin, and it is predicted the number will grow as the populations of Westernized countries continue to age and maintain unhealthy lifestyles [45]. However, there are wide interindividual differences in both serum lipid levels and the magnitude of response to statin therapy. The lipid-lowering

response to atorvastatin therapy in different *DNAH11* rs12670798 genotype individuals has not been reported previously. In the present study, we for the first time showed that the lipid-lowering response to atorvastatin treatment in different *DNAH11* rs12670798 genotype individuals was different in the hyperlipidemic patients. The rs12670798 C allele carriers had lower serum TC, LDL-C and ApoB levels than the rs12670798 C allele non-carriers after atorvastatin treatment. However, the rs12670798 C allele carriers also decreased serum ApoA1 levels after atorvastatin treatment, especially in the CT genotype individuals. Thus, the results of the present study suggest that the *DNAH11* rs12670798 C allele carriers benefited more from atorvastatin therapy than the *DNAH11* rs12670798 C allele non-carriers in decreasing serum TC, LDL-C and ApoB levels. But the C allele carriers may have a detrimental effect by decreasing serum ApoA1 levels after atorvastatin treatment.

Limitations

There were several potential limitations in this study. Firstly, the sample size was relatively small compared to many GWASs and replication studies. Therefore, further studies with larger sample sizes are needed to confirm our results. Secondly, there were significant differences in the general characteristics between the control and patient groups. Although age, gender, BMI, cigarette smoking and alcohol consumption have been adjusted for the statistical analysis, we could not completely eliminate the potential effects of these factors on serum lipid levels and the risk of CHD and IS. Thirdly, the association of the SNP and serum lipid levels in the CHD and IS groups was not analyzed because of the extensive use of lipid lowering therapy in these participants with various pharmacological agents, dosing, and medication compliance. Finally, it is well known that both CHD and IS are the complex multifactorial disorders that are believed to result from an interaction between the genetic background of an individual and various environmental factors. Although we have detected the association between the *DNAH11* rs12670798 SNP and the risk of CHD and IS, there are still many undiscovered environmental factors and genetic variants and their interactions.

Conclusions

The present study showed that the genotypic and allelic frequencies of the *DNAH11* rs12670798 SNP were different between CHD/IS patients and healthy controls. The frequency of the C allele was higher in CHD and IS patients than in controls. The C allele carriers were associated with an increased risk of CHD and IS after controlling for potential confounders. The C allele carriers also had higher serum TC levels than the C allele non-carriers. These results suggest that the *DNAH11* rs12670798 SNP may be a susceptibility gene for CHD and IS in our study populations. The response to atorvastatin treatment in different *DNAH11* rs12670798 genotype individuals was different in the hyperlipidemic patients. The *DNAH11* rs12670798 C allele carriers benefited more from atorvastatin therapy than the *DNAH11* rs12670798 C allele non-carriers in decreasing serum TC, LDL-C and ApoB levels. The observed associations in this study suggest that the detection of *DNAH11* rs12670798 SNP may have a potential role on the genetic diagnosis and pharmacogenomics therapy of dyslipidemia and atherosclerosis-related diseases such as CHD and IS in the Chinese populations. However, large studies of populations with different ethnic origins are required to confirm these observations.

Acknowledgements

This study was supported by the Science Foundation of Guangxi Returned Oversea Scholars (No: 0991004) and the National Natural Science Foundation of China (No: 30960130).

Disclosure of conflict of interest

None.

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