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Original Article

Effect of *APC* variant in coronary artery disease and response to atorvastatin therapy

Lifang Li[1*], Li Zhang[1*], Zhenfeng Cheng[1], Mengting Chen[2]

[1]Department of Cardiology, Huzhou Central Hospital, Huzhou, Zhejiang, China; [2]Department of Respiratory Medicine, Huzhou Central Hospital, Huzhou, Zhejiang, China. [*]Co-first authors.

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Abstract: Genome-wide association studies (GWAS) have recently been used to demonstrate that the single nucleotide polymorphism (SNP) of the adenomatous polyposis coli (*APC)* gene is a susceptible biomarker for coronary artery disease (CAD). In light of this finding, we aimed to explore the association between rs449650 and the risk of CAD in Han Chinese. A total of 666 CAD patients and 434 controls were involved in the current association study. The results showed that the *APC* rs449650 was significantly associated with CAD in males (χ^2 = 8.14, df = 1, P = 0.004, OR = 1.45, 95% CI = 1.12-1.86). Moreover, the association was stronger under the recessive model in males (χ^2 = 12.71, df = 1, P = 0.0004, OR = 3.07, 95% CI = 1.61-5.82). A further analysis by age showed that there was a 79% increased risk of CAD for males younger than 65 years (genotype: χ^2 = 11.53, df = 2, P = 0.003; allele: χ^2 = 9.94, df = 1, P = 0.002, OR = 1.79, 95% CI = 1.24-2.57). The group of rs449650-AAcarriers was more responsive to atorvastatin therapy than other groups based on a decrease in triglyceride (TG) levels (CC: P > 0.05; AC: P > 0.05; AA: P < 0.001). Additionally, there were no significant differences in the concentrations of high-density lipoprotein cholesterol (HDL-C) (P > 0.05) and apolipoprotein A-I (ApoA-I) (P > 0.05) before and after four weeks of atorvastatin therapy in rs449650-AAcarriers. Our case-control study shows that the *APC* polymorphism rs449650 is significantly associated with CAD risk in young Han Chinese males. In addition, the genotype rs449650-AA could affect the response to atorvastatin therapy by affecting the plasma concentrations of TG, HDL-C and ApoA-I.

Keywords: Adenomatous polyposis coli, rs449650, coronary artery disease, atorvastatin

Introduction

Coronary artery disease (CAD) is characterized by occlusive epicardial coronary artery stenosis. CAD is the leading cause of death in the United States and worldwide [1]. The incidence of CAD is also increasing rapidly in developed countries [2]. As a complex disease, CAD is related to multiple genetic and environmental factors. The risk factors for CAD include unhealthy diet [3], smoking, and intemperance [4]. The environmental factors may lead to CAD through their impacts on the epigenetic changes of CAD-related genes [5]. In addition, recent studies have indicated that mutations in CAD-related gene were the main risk factors for CAD [5].

Adenomatous polyposis coli (*APC*) is classified as a tumor suppressor gene. The protein APC plays a critical role in several cellular processes that determine whether a cell may develop into a tumor [6]. *APC* is involved in various important processes, including the development of more effective strategies for chemoprevention, prognosis, and chemotherapy of certain types of tumors [7]. In a previous study, Palacio-Rúa et al. suggested that the *APC* single nucleotide polymorphism (SNP) rs41115 was significantly associated with stomach and colorectal cancers [8]. Feng et al. [9] also reported that the *APC* polymorphism rs459552 was a susceptible locus for colorectal cancer. There was a significant correlation between the *APC* polymorphism rs1801155 and colorectal neoplasia in average risk Ashkenazi Jews [10]. Mostowska et al. [11] revealed significantly increased *APC* rs11954856 and rs351771 frequencies in Polish women with ovarian cancer. Genome-wide association studies (GWAS) have discovered that the polymorphism rs383830 of *APC*

is a CAD susceptible biomarker in Europeans [12]. And this locus was also connected with the risk of CAD in Han Chinese [13]. The SNPs rs383830 and rs449650 of *APC* gene were about 36bp away from each other, and the D' and R-square values indicated high linkage disequilibrium relationship. However, there were no studies about the relation between the rs449650 and CAD risk.

In this work, we recruited 666 patients with angiography-proven CAD and 434 controls, and performed a case-control test to validate the contribution of *APC* rs449650 to CAD in Han Chinese. Blood lipid levels of 419 CAD patients were collected before and after four weeks of atorvastatin therapy and analyzed if they carried the APC rs449650 genotype.

Materials and methods

Sample collection

A total of 1100 unrelated Han Chinese originated from the Huzhou city (Zhejiang, China) were involved in the current study. Among the participants, 666 were CAD patients who had a history of angioplasty or coronary artery bypass surgery, or showed diameter stenosis of greater than 50% in any of the main coronary arteries based on angiographic evidence. For the control group, 434 residents from the Huzhou city were chosen. These participants did not have a history or electrocardiographic signs of CAD. Samples were collected between May 2012 and October 2015 at the Huzhou Central Hospital. Patients were examined through standardized coronary angiography [14] and the results were judged by at least two independent cardiologists. Blood lipid levels of 419 CAD patients were collected before and after four weeks of atorvastatin therapy and analyzed if they carried the *APC* rs449650 genotype. The study protocol was approved by the ethic committees of Huzhou Central Hospital, and informed consents were obtained from all patients.

Analysis of biochemical variables

Blood samples were collected from the participants in a fasting state by the same investigator. Information related to CAD risk factors, including hypertension, diabetes, smoking, and serum cholesterol, was collected from all participants. Blood samples were stored at -80°C in 3.2% citrate sodium-treated tubes until further analysis. The lipid profiles of different patients before and after four weeks of atorvastatin therapy were compared. Plasma levels of triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels were measured using an enzymatic endpoint assay. The levels of apolipoprotein A-I (ApoA-I), apolipoprotein B (ApoB), and apolipoprotein E (ApoE) were measured by the transmission turbidimetric method [5].

SNP genotyping

Genomic DNAs were extracted from peripheral blood samples using a conventional phenol/chloroform method. DNA concentrations were quantified using the NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA). Polymerase Chain Reaction (PCR) was performed for genotyping using ABI Geneamp® PCR System 9700 (Applied Biosystems, Foster City, CA, USA). PCR cycle was as follows: an initial denaturation at 94°C for 15 sec, followed by 45 amplification cycles (94°C for 20 sec, 56°C for 30 sec, and a final extension stage at 72°C for 3 min. Primer extension for genotyping was performed on the Sequenom® Mass-ARRAY iPLEX® platform according to the manufacturer's instructions [15].

Statistical analyses

Consistency of the genotype frequencies with Hardy-Weinberg equilibrium (HWE) was performed using Arlequin program (version 3.5). Means and standard deviations (SD) were used for the description of the continuous variables. All data were analyzed with the SPSS statistical software (version 16.0). The Pearson's χ^2-test was used to compare the genotype distribution, allele frequencies, and other categorical phenotypes between cases and controls. Differences in continuous variables of biochemical indicators between cases and controls were compared for significance by Student's t-test and for nonparametric indicators by Kruskal-Wallis test. All the *P* values were adjusted for the history of smoking, diabetes, and hypertension. Power analysis was performed by the Power and Sample Size Calculation Software (version 3.0.43). A two-sided *P* value of < 0.05

Table 1. Distribution comparison of *APC* gene rs449650polymorphism between CAD and control groups[a]

Gender	Group	Genotype	χ^2	P (df = 2)	Allele	χ^2	P (df = 1)	OR (95% CI)
		CC/AC/AA			C/A			
All	Control (N = 434)	222/178/34			622/246			
	CAD case (N = 666)	292/296/78	7.60	0.022	880/452	7.59	0.006	1.30 (1.08-1.56)
Female	Control (N = 141)	74/47/20			195/87			
	CAD case (N = 396)	175/179/42	6.17	0.046	529/263	0.53	0.467	1.11 (0.83-1.49)
Male	Control (N = 293)	148/131/14			427/159			
	CAD case (N = 270)	117/117/36	13.18	0.001	351/189	8.14	0.004	1.45 (1.12-1.86)

a: All the *P* values we readjusted for the history of smoking, diabetes and hypertension.

Table 2. Comparison of the dominant model and recessive model between cases and controls in different gender[a]

Gender	Group	Dominant		χ^2	P (df = 2)	OR (95% CI)	Recessive		χ^2	P (df = 1)	OR (95% CI)
		CC	AC+AA				CC+AC	AA			
All	Control	222	212				400	34			
	CAD case	292	374	5.64	0.017	1.34 (1.05-1.71)	588	78	4.32	0.038	1.56 (1.02-2.38)
Female	Control	74	67				121	20			
	CAD case	175	221	2.87	0.09	1.39 (0.95-2.05)	354	42	1.30	0.254	0.72 (0.41-1.27)
Male	Control	148	145				279	14			
	CAD case	117	153	2.91	0.088	1.33 (0.96-1.86)	234	36	12.71	0.0004	3.07 (1.61-5.82)

a: All the *P* values were adjusted for the history of smoking, diabetes and hypertension.

was considered to indicate a statistically significant result.

Results

Genotypic and allelic comparison of *APC* rs449650 between CAD cases and controls are shown in **Table 1**. No departure of HWE was observed for rs449650 ($P > 0.05$). The results showed that SNP rs449650 was association with CAD both at the genotype level ($\chi^2 = 7.60$, df = 2, $P = 0.022$) and allele level ($\chi^2 = 7.59$, df = 1, $P = 0.006$). The rs449650-Aallele frequency was significantly higher in cases than in controls (33.9% versus 28.3%; $P = 0.006$; OR = 1.30, 95% CI = 1.08-1.56). Furthermore, when we stratified the data analysis into each sex group with respect to allele and genotype frequencies, we found a significant difference in the genotype distributions in males ($\chi^2 = 6.17$, df = 2, $P = 0.046$) and females ($\chi^2 = 13.18$, df = 2, $P = 0.001$). Between male groups, the Aallele frequency of rs449650 was significantly higher in cases than in controls (35.0% versus 27.1%; $\chi^2 = 8.14$, df = 1, $P = 0.004$; OR = 1.45, 95% CI = 1.12-1.86).

As shown in **Table 2**, significant results were found in both the dominant model (CC versus AC+AA: $\chi^2 = 5.64$, $P = 0.017$, OR = 1.34, 95% CI = 1.05-1.71) and recessive model (CC+AC versus AA: $\chi^2 = 4.32$, $P = 0.038$, OR = 1.56, 95% CI = 1.02-2.38). In the sex analysis, a positive association was observed between rs449650 and CAD under the recessive model in males ($\chi^2 = 12.71$, df = 1, $P = 0.0004$, OR = 3.07, 95% CI = 1.61-5.82). However, no significant differences were observed between cases and controls in the female group ($P > 0.05$).

Because aging is a risk factor of CAD, we performed an age-stratified analysis. As shown in **Table 3**, a strong association was found between rs449650 and CAD in all subjects (genotype: $\chi^2 = 7.51$, df = 2, $P = 0.023$; allele: $\chi^2 = 7.40$, df = 1, $P = 0.007$, OR = 1.41, 95% CI = 1.10-1.81) and in males aged under 65 (genotype: $\chi^2 = 11.53$, df = 2, $P = 0.003$; allele: $\chi^2 = 9.94$, df = 1, $P = 0.002$, OR = 1.79, 95% CI = 1.24-2.57). No significant difference was observed for subjects above 65 ($P > 0.05$).

Patients were treated with atorvastatin for four weeks. The lipid profiles of different patients

Table 3. Post hoc analysis of *APC* rs449650with the risk of CAD in different age subgroups[a]

Age	Group	Genotype	χ^2	P (df = 2)	Allele	χ^2	P (df = 1)	OR (95% CI)
		CC/AC/AA			C/A			
All < 65	Cases (N = 338)	147/150/41			444/232			
	Controls (N = 263)	139/106/18	7.51	0.023	384/142	7.40	0.007	1.41 (1.10-1.81)
All ≥ 65	Cases (N = 328)	145/146/37			436/220			
	Controls (N = 171)	83/72/16	1.00	0.606	238/104	1.00	0.317	1.15 (0.87-1.53)
Female < 65	Cases (N = 229)	109/89/31			307/151			
	Controls (N = 80)	40/29/11	0.18	0.914	109/51	0.06	0.806	1.05 (0.72-1.55)
Female ≥ 65	Cases (N = 167)	66/90/11			222/112			
	Controls (N = 61)	34/18/9	11.69	0.003	86/36	0.66	0.417	1.21 (0.77-1.89)
Male < 65	Cases (N = 109)	38/61/10			137/81			
	Controls (N = 183)	99/77/7	11.53	0.003	275/91	9.94	0.002	1.79 (1.24-2.57)
Male ≥ 65	Cases (N = 161)	79/56/26			214/108			
	Controls (N = 110)	49/54/7	8.72	0.013	152/68	0.41	0.522	1.13 (0.78-1.63)

a: All the *P* values were adjusted for the history of smoking, diabetes and hypertension.

Table 4. Comparison of blood lipid levels before and after treatment in different genotypes of *APC* rs449650[a]

Genotype (N)	T (week)	TC (mmol/L)	TG (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)	ApoA-I (g/L)	ApoB (g/L)
CC	0	6.37±0.14	1.70±1.29	3.19±0.53	1.24±1.05	0.97±0.33	1.20±0.42
N = 182	4	4.33±0.39	1.54±0.24	2.25±0.31	1.76±1.32	1.09±0.38	0.79±0.69
	P	< 0.001	> 0.05	< 0.001	< 0.001	< 0.001	< 0.001
AC	0	6.29±1.77	3.40±2.40	3.03±1.14	1.53±0.62	0.93±0.29	1.33±0.43
N = 193	4	4.24±1.37	3.11±3.06	2.59±1.17	1.84±0.57	1.04±0.38	0.80±0.41
	P	< 0.001	> 0.05	< 0.001	< 0.001	< 0.001	< 0.001
AA	0	5.49±1.20	4.03±2.84	2.44±1.11	1.53±0.38	0.89±0.22	1.39±0.89
N = 44	4	4.27±0.87	2.73±1.77	1.96±0.88	1.46±0.27	0.99±0.26	0.87±0.51
	P	< 0.001	< 0.01	< 0.05	> 0.05	> 0.05	< 0.001

a: All the *P* values were adjusted for the history of smoking, diabetes and hypertension.

before and after atorvastatin therapy were compared (**Table 4**). The group of the rs449650-AAcarriers was more responsive than other groups in terms of TG levels (CC: P > 0.05; AC: P > 0.05; AA: P < 0.001). Additionally, there were no significant differences in the concentrations of HDL-C (P > 0.05) and ApoA-I (P > 0.05) in the rs449650-AAcarriers before and after atorvastatin therapy for four weeks.

Discussion

In the present case-control study, we aimed to explore the significance of *APC* rs449650. Our results showed that *APC* rs449650 presented a strong relation with the risk of CAD in Han Chinese. In addition, through gender-stratified comparison, rs449650 was found to associate with CAD risk in young males. Patients with rs449650-AAalso showed better response to atorvastatin therapy.

Age is known as a predictor of CAD risk [16]. Genetic polymorphisms may play an important role in the pathogenesis of early-onset CAD [17]. Accumulating evidence has associated a variant in chromosome 9p21 with early-onset CAD in different populations [18, 19]. SNP rs4977574 of *CDKN2BAS* gene showed an 87% increased risk of CAD for females younger than 65 years [19]. Sexual dimorphism is frequently observed in the prevalence and severity of cardiovascular diseases. Previous studies have revealed different genetic risks of CAD between male and female Han Chinese. Peng et al. [20] reported a significant association between the

KIF6 variant and CAD in women but not in men. Post hoc analysis revealed a stronger association of rs1746048 with the risk of CAD for subjects aged 65 years or older [21]. *PDGFD* rs974819 with a sex-dependent genetic effect was also associated with an increased risk of CAD in Han Chinese [22]. Our results showed that rs449650 was associated with CAD risk in males aged fewer than 65. Our findings may provide new clues to predict the risk of cardiac events in younger males.

Atorvastatin is used primarily for lowering blood cholesterol and for prevention of events associated with cardiovascular diseases. It works by inhibiting HMG-CoA reductase, an enzyme found in liver tissues that plays a key role in the production of cholesterol. Several genetic polymorphisms have been found to be associated with a higher incidence of undesirable side effects of atorvastatin. Pasanen et al. [23] have confirmed that the *SLCO1B1* polymorphism has a larger effect on the AUC of atorvastatin than the more hydrophilic rosuvastatin. Li et al. [24] reported that the carriers of rs20455-T were at a greater risk for primary and secondary CAD events, and statin therapy significantly reduced coronary events in rs20455-T carriers but not in noncarriers. The study by Alzoubi et al. [25] showed that the *MDR1* gene polymorphisms G2677T and C3435T are associated with the lipid lowering effect of atorvastatin among Jordanians. In this work, our results suggest that rs449650-AAcarriers are more responsive than other genotype groups in terms of TG level after atorvastatin therapy for four weeks. However, atorvastatin therapy did not change the concentrations of HDL-C and ApoA-I in rs449650-AAcarriers. We suspect that this phenomenon is related to increased plasma levels of pharmacologically active metabolites, such as atorvastatin lactone and *p*-hydroxy atorvastatin.

APC SNPs are located on 5q21.1, which have been shown to associate with CAD risk [12, 26]. Our results found a significant association between rs449650 with CAD in young males. The allele frequencies of rs449650-A are 0.339 and 0.283 in CAD cases and non-CAD controls, respectively. These are similar to the allele frequencies reported by HapMap in Asian populations (0.200 in HapMap-CHD) and European populations (0.208 in HapMap-CEU). Although, 1100 individuals were recruited in the present study, an association power of only 79.0% were registered between rs449650 and CAD risk, suggesting that may be our sample size was not enough. We cannot exclude the possibility that the negative findings may be due to a lack of statistical power. Therefore, studies with a larger sample size are necessary to verify potential associations between other polymorphisms of *APC* and CAD risk in various populations.

In conclusion, our case-control study shows that the *APC* polymorphism rs449650 is significantly associated with CAD risk in young Han Chinese males. In addition, the genotype rs449650-AA could affect the response to atorvastatin therapy by affecting the concentrations of TG, HDL-C and ApoA-I.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Li Zhang, Department of Cardiology, Huzhou Central Hospital, 198 Hongqi Road, Huzhou 313003, Zhejiang, China. Tel: 86-572-2555537; Fax: 86-572-2555537; E-mail: zhanglinbu2010@163.com

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