Original Article

Carotenoid metabolic (BCO1) polymorphisms and personal behaviors modify the risk of coronary atherosclerosis: a nested case-control study in Han Chinese with dyslipidaemia (2013-2016)

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Background and Objectives: β-Carotene-15,15'-oxygenase (BCO1) is a key enzyme involved in carotenoid metabolism and has been linked with the development of coronary atherosclerosis. This study investigated the association between BCO1 polymorphisms and the risk of coronary atherosclerosis in dyslipidemia participants, and analyzed the influence of personal behaviors on coronary atherosclerosis. **Methods and Study Design:** A nested case-control study was conducted from 2013 to 2016 in which 1359 dyslipidemia participants were recruited. Personal lifestyle parameters, mainly physical activities and diet, were obtained by questionnaires and the genotypes of rs11641677, rs11646692, rs12934922, rs6564851 and rs7501331 in BCO1 were analyzed by ligase detection reaction. In 2016, 166 participants were diagnosed with coronary atherosclerosis and 498 age-and gender-matched controls were recruited. The association between BCO1 polymorphisms and risk of coronary atherosclerosis were analyzed with logistic regression, and the effect of gene-behaviors interaction on the risk of coronary atherosclerosis were determined with crossover analysis. **Results:** After adjustment for potential confounders, logistic regression analysis showed that fried food intake (OR=1.637, 95% CI: 1.127~2.378; *p*=0.010), dessert intake (OR=1.733, 95% CI: 1.158~2.595; *p*=0.008), and physical activity (OR=0.511, 95% CI: 0.309~0.846; *p*=0.009) were risk factors for coronary atherosclerosis. Rs12934922 and rs11646692 reflected high susceptibility to coronary atherosclerosis. Crossover analysis indicated that rs12934922 and rs11646692 interacted with physical activity (Inter-OR=8.82; Inter-OR=3.69), fried food intake (Inter-OR=2.95; Inter-OR=2.36) and dessert intake (Inter-OR=3.95; Inter-OR=2.39) to influence the risk of coronary atherosclerosis. **Conclusions:** In dyslipidemia patients, rs12934922 and rs11646692 may influence the development of coronary atherosclerosis. A combination of BCO1 polymorphisms and several behavioral factors may affect the development of coronary atherosclerosis.

Key Words: β-carotene-15,15'-monooxygenase (BCO1), single nucleotide polymorphisms (SNP), coronary atherosclerosis, personal behaviors, interaction

INTRODUCTION

Cardiovascular disease (CVD) remains a major cause of morbidity and mortality worldwide, accounting for one out of every three deaths.[1,2] Atherosclerosis, a pathological basis of CVD, is a complex chronic progressive inflammatory disorder caused by dyslipidemia, unhealthy diets, physical inactivity and personal hereditary traits.[3-7] Dietary carotenoids are generally proposed to act as free radical quenchers and potent antioxidants in human physiology and have a protective function against dyslipidemia and coronary atherosclerosis.[8,9] A number of studies have reported the relationship between genetic variances and atherosclerosis,[10-12] of which several genes are predicted to be involved in lipid metabolism.

Studies have systematically established that there is a large individual variability in concentrations of dietary derived carotenoids absorbed in the blood.[13] Further exploration demonstrated that, metabolism difference is an intrinsic characteristic which may be due to genetic features of the individuals.[13,14] The β,β-carotene-15,15'-oxygenase (BCO1), which catalyzes the central cleavage of dietary provitamin A carotenoids to retinoids, has been proved to be the key enzyme in carotenoid metabolism,[15,16] and single nucleotide polymorphisms (SNP) variances on BCO1 gene have been shown to influence plasma carotenoid levels and the body's ability to resistoxidative stress. Study from animals has revealed that BCO1 knocked-out mice developed a fatty liver and displayed altered serum lipids with elevated serum unesterified fatty acids. Additionally, this mouse mutant was

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Manuscript received 10 September 2018. Initial review completed 14 October 2018. Revision accepted 26 November 2018.
doi:

more susceptible to high-fat-diet-induced impairments in fatty acid metabolism.[17] Previous report from Clifford A. J[18] also showed that SNP rs6564851on BCO1 gene was associated with plasma HDL-c level among Caucasian populations.

In a cross-sectional study involving 2043 individuals from communities in China, it was reported in 2013 that,rs11646692 and rs7501331 on BCO1 gene might be involved in the regulation of lipoprotein metabolism.[19] After a three-year-follow-up, more than 200 participants who had dyslipidemia were diagnosed with coronary atherosclerosis. Therefore, a nested case-control study was conducted to investigate the association between genetic variations of BCO1 gene and coronary atherosclerosis, as well as the substantial effects of interactions between SNP variants and personal behaviors on coronary atherosclerosis.

METHODS

Study population

The cross-sectional study was conducted in Southern China. Subjects were community residents who subscribed to the New Rural Cooperative Medical Insurance and came to Community Health Centers for biennial routine health examination between April and July 2013. The included subjects met the following criteria: (1) aged 40–80 years, (2) ethnic of Chinese Han, and (3) never diagnosed with severe liver diseases, cancer and without a history of stroke and myocardial infarction. Dyslipidemia was defined according to the Chinese Guidelines on Prevention and Treatment of Dyslipidemia 2007:20 serum total cholesterol (TC) >5.18 mmol/L, and/or triglyceride (TG) >1.70 mmol/L, and/or low density lipoprotein cholesterol (LDL-c) >3.37 mmol/L and/or high density lipoprotein cholesterol (HDL-c) <1. 04 mmol/L. A total of 2349 participants, among whom 1359 had dyslipidemia, were recruited in the study and followed up every year. At the time of recruitment, written informed consent was obtained from the participants. Participants were interviewed using questionnaires by clinicians, and venous blood samples were obtained from the antecubital vein for measurement of serum lipids and genomic DNA extraction. In August 2016 (after a three-year follow-up), electronic health records (EHR) gathered from Centers for Disease Control (CDC) showed that 166 newly diagnosed coronary atherosclerosis developed among those with dyslipidemia, and were chosen as cases for current study. For each case, three subjects from the dyslipidemia population who were free of coronary atherosclerosis were used as controls, matched by age and gender. Coronary atherosclerosis was diagnosed by a percutaneous coronary angiogram and defined by presence of 50% or greater diameter narrowing for each of the three main coronary arteries (left anterior descending coronary artery, left circumflex coronary artery and right coronary artery).

Anthropological measurement and serum lipids

At the time of visiting health centers, anthropological measurements (weight, height, waist circumference and blood pressure) were carried out using standardized methods. Body mass index (BMI) was computed as weight (kg) divided by height squared (m^2). Plasma concentrations of TC, TG, LDL-c and HDL-c were measured on a Hitachi 7180 biochemistry automatic analyser (Hitachi High-Technologies Corporation, Minato-ku, Tokyo, Japan) according to the instructions in the manual.

Selection and assessment of SNPs

Five SNPs were used in this study on the basis of previous related studies and its functions. SNP rs12934922 and rs7501331 map to the exon coding region of BCO1. The variations modify amino acid sequence of the protein and ultimately affect enzyme activity.[21] Rs6564851 locates in the binding site for transcription factor intestine-specific homeobox (ISX) in the BCO1 promoter while ISX acts as a transcriptional repressor of BCO1 expression in human.[22,23] Rs11646692 and rs11641677 locate upstream from the transcription start sites of BCO1, a functional cluster region of multiple transcription binding factors. A previous study showed that generic variances may be associated with dyslipidemia.[19] Detailed information for all the selected SNPs were obtained from NCBI dbSNP (http://www.Ncbi.nlm.nih.gov/SNP/). For each blood sample, one milliliter of whole blood with ethylene diamine tetraacetic acid (EDTA) was sent to Shanghai Generay Biotech Company for DNA extraction and genotyping. Genomic DNA was extracted using the phenol-chloroform method, polymerase chain reaction (PCR) was conducted in EDC-810 Amplifier, and genotyping of the SNPs was measured by the ligase detection reaction (ABI 3730XL sequencer).

Assessment of personal behaviors and other potential confounders

The personal behaviors, mainly including physical activities and diet, were interviewed using a brief questionnaire regarding dietary intake, alcohol consumption, smoking and physical activities. Dietary intake was assessed via a brief semi quantitative food frequency questionnaire. Participants were asked the amount and frequency of consumption of 11 major food groups/items during the past 12 months: vegetables, fruit, egg, meat, chicken, fish/seafood, milk, soya milk, fried food, dessert and salt. The intake of salt was self-estimated based on grams per day. Participants who smoked at least one cigarette per week and drank at least once per week were defined as current smokers and current drinkers, respectively. Since only a few people were former smokers (n=19) and former drinkers (n=4), they were counted as non-smokers or non-drinkers. Physical activity levels were classified into four categories according to their occupation characters. “Sedentary” referred to occupations getting almost no daily exercise such as office staff, “light” referred to those that required the least amount of effort such as salesmen, waiters and teachers, “moderate” referred to occupations that required extended periods of walking, pushing or pulling objects such as cleaning services and drivers, while “heavy” referred to those that frequently required strenuous effort and extensive total body movements such as non-mechanized farming, dancers, construction workers, dockworkers and so on.

Table 1. Characteristics of the subjects who participated in our study†

Characteristics	Total (n=664)	Case (n=166)	Control (n=498)	*p*
Gender				1.000
Male	284 (43%)	71 (43%)	213 (43%)	
Female	380 (57%)	95 (57%)	285 (57%)	
Age, y	65.16±10.34	65.54±10.44	65.01±10.34	0.540
Waist, cm	82.85±9.55	83.86±8.61	82.64±9.49	0.144
BMI, kg/m^2	24.20±3.40	24.18±3.25	23.97±3.45	0.474
SBP, mmHg	143±18.1	140±16.5	144±18.5	0.008
DBP, mmHg	85.9±11.5	85.3±11.0	86.2±11.7	0.407
TC, mmol/L	5.27±0.92	5.20±0.98	5.29±0.90	0.286
TG, mmol/L	1.78±0.95	1.69±0.98	1.82±0.94	0.144
HDL-C, mmol/L	1.24±0.31	1.25±0.33	1.24±0.30	0.888
LDL-C, mmol/L	3.38±0.84	3.45±0.96	3.35±0.84	0.188

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; *p*, *p*-value.
†Apart from gender, all values were expressed as mean±SD.

Personal behavior scoring

This study further explored personal behaviors that are simple to assess and have been well-studied previously in relation to coronary atherosclerosis.[24,25] A binary score for each personal behaviors and comprehensive scores was used, these were called overall behavioral score and diet score. For diet score, participants received 1 point if four or more of the following applicable conditions were met: 1. Intake of less than 150 g of vegetables per day; 2. Daily intake of less than 100 g of fresh fruit; 3. Drink less than 200 mL of milk per day; 4. Drink less than 200 mL of soy milk per day; 5. Eat less than 4 eggs a week; 6. Eat fried foods more than 4 times a week; 7. Eat desserts more than 4 times a week; 8. Daily consumption of more than 9 g of salt, otherwise they received 0 points. For overall behavioral score, participants received 1if three or more of the following conditions were met: 1. Current smoker (smoking for more than three days a week, >15 min per day at least one year or quit smoking <10 years ago); 2. Daily alcohol use (at least once a week, >2 mL each time); 3. Overweight or obese (BMI >24); 4. Physical activity ("Sedentary" and "light"); 5. Diet score received 1 point, otherwise they received 0 points for each factor.

Statistic methods

Data analyses were carried out using SPSS 23.0, Haploview 4.2, gPLINK 2.0 and GMDR 0.9 software. OriginPro 9.1 software was used for graphing.

(1) Analysis of general characteristics: Quantitative variables were expressed as mean ± standard deviation (SD), the differences between cases and controls were tested by the Student's unpaired t-test. Qualitative variables were expressed as percentages. A chi-square analysis was used to evaluate the difference between case and control groups. All the analyses were performed using SPSS 23.0.

(2) Analysis for personal behaviors: Chi-square test was applied to evaluate the distribution differences of behaviors between cases and controls. After adjustment for SBP, unconditional logistic regression was used to assess the correlation between the risk of coronary atherosclerosis and behaviors as well as behavioral score. In logistic regression analysis, physical activity levels (sedentary/light/moderate/heavy) were classified into two categories: low (sedentary/light) and high level (moderate/heavy) of physical activity. All the analyses were performed via SPSS 23.0.

(3) Analysis for SNPs: test for linkage disequilibrium was estimated using Haploview software (version 4.2), r2

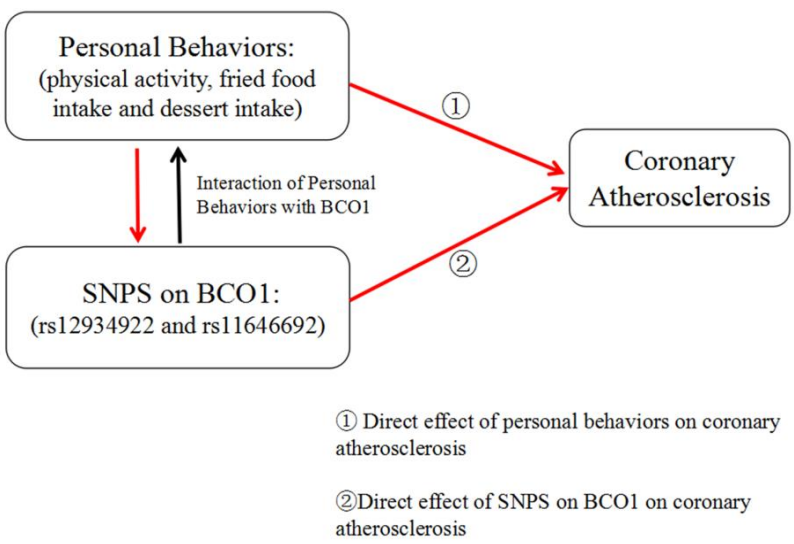

Figure 1. The interaction model of gene-behaviors interactive effects on the risk of coronary atherosclerosis.

were calculated and a likelihood ratio test was performed to determine the significance of departures from linkage equilibrium. gPLINK software (version 2.0) was applied to evaluate the association between SNPs and coronary atherosclerosis risk. The analyses included Hardy-Weinberg Equilibrium test, allele frequency calculation, genotypic association test and logistics regression test. Allele and genotyping frequencies for different genetic models were compared using the chi-square test. After adjustment for SBP, unconditional logistic regression was performed to assess the gene-disease correlation.

(4) Analysis for interactions: The interaction effects of SNPs with multiple personal behaviors were detected by Multifactor Dimensionality Reduction (MDR) and cross-over analysis after controlling for potential confounders (Figure 1). The software used for MDR analysis was GMDR (version 0.9). Crossover analysis was performed via SPSS 23.0.

For logistic regression test and crossover test, odds ratio (OR) and 95% confidence interval (95% CI) were calculated to describe the effect of the factors; $p<0.05$ was considered statistically significant for all the tests.

RESULTS

General characteristics of the subjects

A total of 166 cases (71 males and 95 females) and 498 controls (213 males and 285 females) were recruited in the 1 to 3 matched case-control design. The general characteristic of all the participants were summarized in Table 1. No statistical differences in age, waist circumference, BMI, diastolic blood pressure, TC, TG , HDL-c and LDL-c were observed between the case and control groups ($p>0.05$).Coronary atherosclerosis patients had lower systolic blood pressure levels as compared to the controls ($p<0.05$).

Effect of personal behavioral factors on coronary atherosclerosis

After adjustment for blood pressure, logistic regression indicated that, subjects with a moderate or heavy intensity of physical activity were less likely to have coronary atherosclerosis (OR=0.511, 95% CI: 0.309~0.846; $p=0.009$) (Table 2). Subjects with regular intake of fried food (OR=1.637, 95% CI: 1.127~2.378; $p=0.01$) and dessert (OR=1.733, 95% CI: 1.158~2.595; $p=0.008$) had a higher risk of coronary atherosclerosis. There was no association between coronary atherosclerosis and the remaining behavioral factors as well as the behavioral factor scores (Table 2).

Effect of SNPs on coronary atherosclerosis

The characteristics of five SNPs were listed in Table 3. The genotype distributions of rs11641677, rs11646692, rs129349225 and rs7501331 were concordant with the Hardy–Weinberg equilibrium in the cases and controls ($p>0.05$). Rs6564851 was excluded from the subsequent analyses for failing to pass the HWE test. For the selected SNPs, Linkage disequilibrium test suggested that alleles at loci rs11641677 and rs11646692 were associated and not independent ($r^2=0.98$) (Figure 2).

Three genetic models (additive, dominant and recessive model) were conducted to investigate the relationship between SNPs and coronary atherosclerosis. As shown in Table 4. After adjustment for blood pressure, SNP rs12934922-T was considered to be the protective allele (OR=0.628, 95% CI: 0.413~0.956; $p=0.030$). Compared with the AA carriers, TT or TA carriers were associated with a 40.6% lower risk of coronary atherosclerosis (OR=0.594, 95% CI: 0.375~0.942; $p=0.027$). GG or GC carriers for rs11646692 had a tendency to reduce the risk of coronary incidence (OR=0.702, 95% CI: 0.490~1.006; $p=0.054$). In additional analyses, stepwise adjustment for multiple personal behaviors (model 3 and model 4) were done to evaluate whether the associations of SNPs with the risk of coronary atherosclerosis could be modified through these covariants. After adjustment for blood pressure, BMI, smoking status, alcohol consumption, physical activity and all the dietary factors (Model 4), T allele for rs12934922 remained to be a protective allele (OR=0.637, 95% CI: 0.415~0.976; $p=0.038$) while the OR for individuals with TT or TA genotype was only slightly attenuated from 0.594 to 0.603 (95% CI: 0.377~0.965, $p=0.035$). Likewise, GG or GC genotype carriers of rs11646692 showed a tendency to reduce the risk by 30.03% compared to the CC genotype (OR=0.697, 95% CI: 0.482~1.007; $p=0.054$). For rs11641677, rs6564851 and rs7501331, there was no significant associations observed between SNPs and the risk of coronary atherosclerosis.

Interactive effects of SNPs and behaviors on coronary atherosclerosis

MDR analysis

MDR was performed to analyze the interaction between the 5 selected SNPs and all the personal behaviorsas well as behavior scores for coronary atherosclerosis. After adjustment for blood pressure, no fitted model was

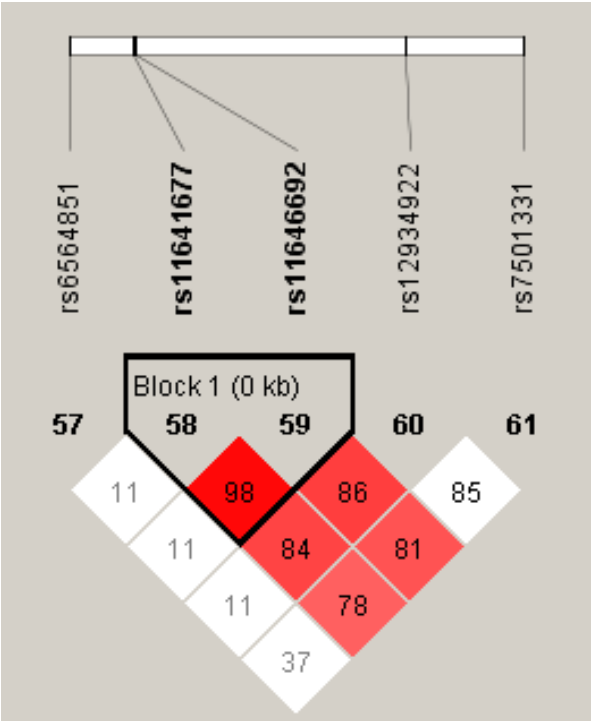

Figure 2. The location and linkage disequilibrium display of BCO1 single-nucleotide polymorphisms (SNPs).

Table 2. Associations of personal behavioral factors with the risk of atherosclerosis

Personal behaviors	Case n (%)	Control n (%)	OR	95% CI	$p^{\dagger}$
Current smoking					
No	129 (77.7)	400 (80.3)	1.000		
Yes	37 (22.3)	98 (19.7)	1.171	0.764~1.794	0.470
Current alcohol consumption					
No	129 (77.7)	363 (72.9)	1.000		
Yes	37 (22.3)	135 (27.1)	0.771	0.509~1.169	0.220
Physical activity					
Sedentary/light	145 (87.3)	388 (77.9)	1.000		
Moderate/heavy	21 (12.7)	110 (22.1)	0.511	0.309~0.846	0.009
Vegetable (g/day)					
<50	27 (16.3)	98 (19.7)	1.000		
50-150	115 (69.3)	312 (62.6)	1.338	0.831~2.155	0.231
>150	24 (14.5)	87 (17.5)	1.001	0.538~1.863	0.997
Fruit (g/day)					
0-150	153 (92.2)	448 (90.0)	1.000		
>150	13 (7.80)	50 (10.0)	0.761	0.403~1.440	0.402
Milk					
None	98 (59.0)	333 (66.9)	1.000		
Regularly	68 (41.0)	165 (33.1)	1.400	0.976~2.010	0.068
Soy milk					
None	118 (71.1)		1.000		
Regularly	48 (28.9)	107 (21.5)	1.484	0.959~2.226	0.078
Egg (/week)					
None	54 (32.5)	185 (37.1)	1.000		
1-4	91 (54.8)	249 (50.0)	1.252	0.850~1.843	0.255
>5	21 (12.7)	64 (12.9)	1.124	0.630~2.005	0.692
Meat (g/week)					
<100	98 (59.0)	317 (63.7)	1.000		
100-200	52 (31.3)	131 (26.3)	1.284	0.867~1.902	0.213
>200	16 (9.6)	50 (10.0)	1.035	0.564~1.899	0.911
Fish/Seafood (g/week)					
<100	91 (54.8)	290 (58.2)	1.000		
100-200	47 (28.3)	135 (27.1)	1.109	0.739~1.667	0.617
>200	28 (16.9)	73 (14.7)	1.222	0.745~2.006	0.427
Poultry (g/week)					
< 50	48 (28.9)	164 (32.9)	1.000		
50-100	104 (62.7)	277 (55.6)	1.192	0.611~2.322	0.607
>100	14 (8.40)	57 (11.4)	1.529	0.817~2.860	0.184
Fried food					
No	52 (31.5)	214 (43.0)	1.000		
Yes	113 (68.5)	284 (57.0)	1.637	1.127~2.378	0.010
Dessert					
None	39 (23.5)	173 (34.7)	1.000		
Regularly	127 (76.5)	325 (65.3)	1.733	1.158~2.595	0.008
Salt (g/day)					
<6	111 (66.9)	304 (61.1)	1.000		
6-9	48 (28.9)	160 (32.1)	0.822	0.557~1.212	0.322
>9	7 (4.20)	34 (6.80)	0.564	0.243~1.309	0.182
Diet score					
0 Score	68 (41.2)	161 (32.3)			
1 Score	97 (58.8)	337 (67.7)	0.72	0.499 ~1.039	0.079
Overall score					
0 Score	85 (51.5)	262 (52.6)			
1 Score	80 (48.5)	236 (47.4)	1.063	0.746 ~1.515	0.737

p: probability for statistic testing. †Adjusted for blood pressure.

successfully detected through MDR analysis (p>0.05).

Crossover analysis

A crossover analysis was carried out to explore the potential interaction between SNPs (rs12934922 TT+TA/AA, rs11646692 GG+GC/CC) and personal behaviors (fried food intake, dessert intake and physical activity) that were significantly associated with coronary atherosclerosis in the previous analysis. In addition, the interaction between SNPs (rs12934922 TT+TA/AA, rs11646692 GG+GC/CC) and overall behavior score as well as overall diet score were performed.

The distributions of rs12934922 genotype and physical activity (p=0.006), fried food intake (p=0.015), dessert intake (p=0.006) were different for the cases and controls (Table 5). Compared with the reference group, AA genotype combined with sedentary or light-intensity physical activity had a 8.82 times higher risk (Figure 3-A), AA carriers with regular intake of fried food had a 2.95 times higher risk (Figure 3-B), while AA genotype combined

Table 3. Characteristics of the 5 SNPs on BCO1

SNP	Position	Allele	Minor allele	MAF	HWE (*p*-value)
rs11641677	81271729	G/A	G	0.340	0.139
rs11646692	81271906	G/C	G	0.334	0.059
rs12934922	81301694	T/A	T	0.127	0.575
rs6564851	81264597	T/C	T	0.215	0.019
rs7501331	81314496	T/G	T	0.164	0.180

SNP: single nucleotide polymorphism; MAF: minor allele frequency; HWE: Hardy–Weinberg equilibrium.

Table 4. Associations of 5 SNPs with the risk of atherosclerosis in different models

SNP	Case (counts)	Control (counts)	Model 1†		Model 2‡		Model 3§		Model 4¶	
			OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
rs11641677										
G/A	99/225	346/640	0.806 (0.611~1.063)	0.127	0.814 (0.616~1.075)	0.147	0.816 (0.617~1.079)	0.153	0.801 (0.601~1.067)	0.129
GG+GA/AA	83/79	293/200	0.717 (0.502~1.025)	0.068	0.721 (0.504~1.032)	0.074	0.726 (0.507~1.040)	0.081	0.722 (0.500~1.044)	0.083
GG/GA+AA	16/146	53/440	0.910 (0.505~1.641)	0.753	0.939 (0.520~1.697)	0.835	0.934 (0.516~1.691)	0.821	0.873 (0.471~1.618)	0.666
rs11646692										
G/C	97/225	341/647	0.808 (0.611~1.069)	0.136	0.814 (0.614~1.080)	0.153	0.818 (0.617~1.085)	0.164	0.796 (0.596~1.063)	0.122
GG+GC/CC	81/80	292/202	0.700 (0.490~1.001)	0.051	0.702 (0.490~1.006)	0.054	0.708 (0.494~1.015)	0.061	0.697 (0.482~1.007)	0.054
GG/GC+CC	16/145	49/445	1.002 (0.553~1.816)	0.995	1.031 (0.567~1.872)	0.921	1.030 (0.566~1.874)	0.923	0.952 (0.512~1.770)	0.875
rs12934922										
T/A	29/293	138/852	0.619 (0.407~0.940)	0.025	0.628 (0.413~0.956)	0.030	0.628 (0.412~0.956)	0.030	0.637 (0.415~0.976)	0.038
TT+TA/AA	27/134	127/368	0.584 (0.369~0.925)	0.022	0.594 (0.375~0.942)	0.027	0.595 (0.374~0.945)	0.028	0.603 (0.377~0.965)	0.035
TT/TA+AA	2/159	11/484	0.554 (0.121~2.524)	0.445	0.563 (0.123~2.579)	0.459	0.551 (0.120~2.531)	0.443	0.566 (0.121~2.642)	0.469
rs7501331										
T/C	58/264	157/831	1.173 (0.834~1.650)	0.360	1.187 (0.842~1.673)	0.327	1.161 (0.823~1.640)	0.395	1.148 (0.806~1.634)	0.445
TT+TC/CC	53/108	149/345	1.136 (0.777~1.663)	0.511	1.151 (0.785~1.687)	0.473	1.128 (0.768~1.657)	0.540	1.114 (0.750~1.653)	0.594
TT/TC+CC	5/156	8/486	1.947 (0.628~6.039)	0.249	1.990 (0.639~6.202)	0.235	1.854 (0.592~5.805)	0.289	1.814 (0.564~5.827)	0.318

p: probability for statistic testing.
†Model 1: Model unadjusted.
‡Model 2: Model adjusted for blood pressure
§Model 3: Model adjusted for blood pressure, BMI, smoking status，alcohol consumption and exercise

Table 5. Distributions of rs12934992 variants and selected behaviors in cases and controls

Behaviors	Status	rs12934922	Case n (%)	Control n (%)	*p*
Physical activity	Moderate/Heavy	TT/AT	1 (0.60)	21 (4.20)	0.006
		AA	20 (12.4)	87 (17.6)	
	Sedentary/Light	TT/AT	26 (16.1)	106 (21.4)	
		AA	114 (70.8)	281 (56.8)	
Fried food intake	None	TT/AT	8 (5.00)	61 (12.3)	0.015
		AA	44 (27.5)	152 (30.7)	
	Regularly	TT/AT	19 (11.9)	66 (13.3)	
		AA	89 (55.6)	216 (43.6)	
Dessert intake	None	TT/AT	5 (3.10)	48 (9.70)	0.006
		AA	32 (20.0)	124 (25.1)	
	Regularly	TT/AT	22 (13.8)	79 (16.0)	
		AA	101 (63.1)	244 (49.3)	
Diet score	0 Score	TT/AT	12 (7.50)	44 (8.90)	0.186
		AA	55 (34.4)	115 (23.2)	
	1 Score	TT/AT	15 (9.40)	83 (16.8)	
		AA	78 (48.8)	253 (51.1)	
Overall score	0 Score	TT/AT	15 (9.40)	73 (14.8)	0.183
		AA	68 (42.5)	187 (37.8)	
	1 Score	TT/AT	12 (7.50)	54 (10.9)	
		AA	65 (40.6)	181 (36.6)	

p: probability for statistic testing.

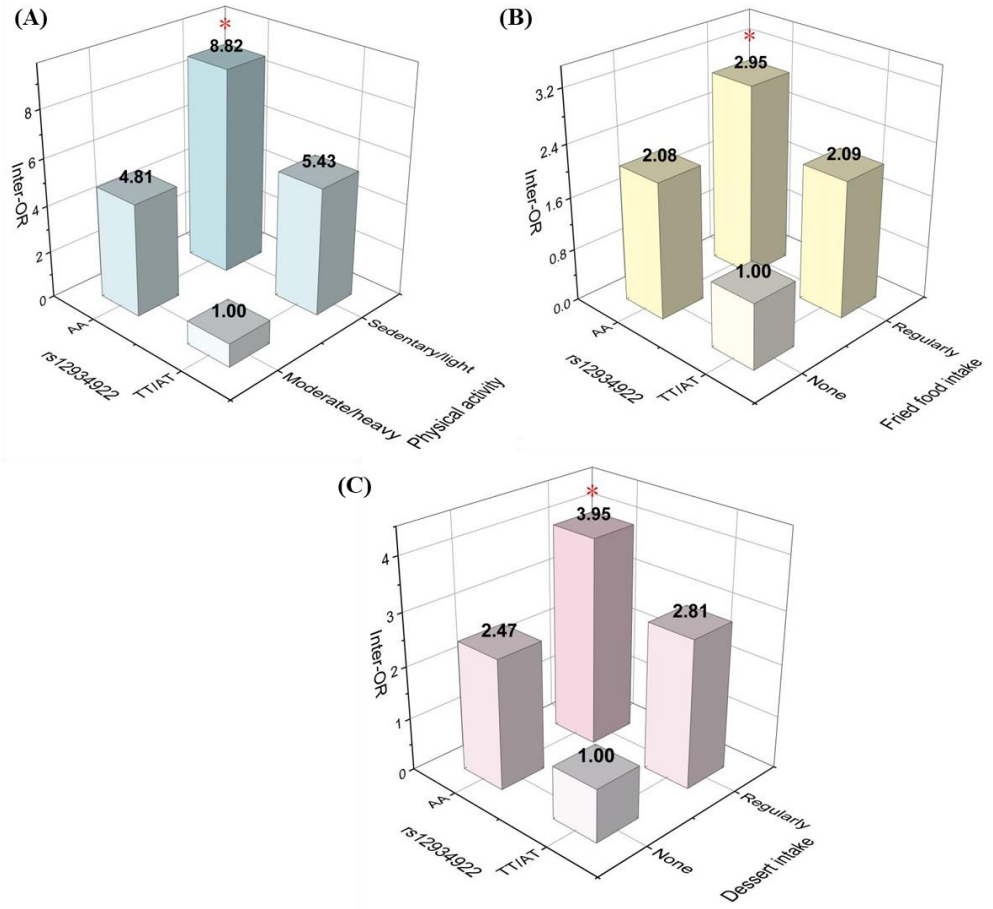

Figure 3. Interactions of the rs12934922 and behaviors (physical activity, fried food intake and dessert intake) on coronary atherosclerosis. *Represents $p<0.05$.

with regular intake of dessert had a 3.95 times higher risk of coronary atherosclerosis (Figure 3-C). There was no significant interaction effects between rs12934922 and overall behavior/dietary score (p=0.183; p=0.186)(Table 5).

For the SNP rs11646692, the distributions of the genotype and physical activity (p=0.013), fried food intake (p=0.005), dessert intake (p=0.015) were different for patient and control groups (Table 6). Compared with the reference group, CC genotype combined with sedentary or light-intensity physical activity had a 3.69 times higher

Table 6. Distributions of rs11646692 variants and selected behaviors in cases and controls

Behaviors	Status	rs11646692	Case n (%)	Control n (%)	*p*
Physical activity	Moderate/heavy	GG/GC	7 (4.3)	59 (11.9)	0.013
		CC	14 (8.7)	49 (9.9)	
	Sedentary/light	GG/GC	74 (46.0)	233 (47.2)	
		CC	66 (41.0)	153 (31.0)	
Fried food intake	None	GG/GC	22 (13.8)	136 (27.5)	0.005
		CC	3 (18.8)	77 (15.6)	
	Regularly	GG/GC	58 (36.3)	156 (31.6)	
		CC	5 (31.3)	125 (25.3)	
Dessert intake	None	GG/GC	2 (12.4)	16 (21.5)	0.015
		CC	18 (11.2)	66 (13.4)	
	Regularly	GG/GC	61 (37.9)	186 (37.7)	
		CC	62 (38.5)	136 (27.5)	
Diet score	0 Score	GG/GC	31 (19.4)	94 (19.0)	0.192
		CC	36 (22.5)	64 (13.0)	
	1 Score	GG/GC	49 (3.6)	198 (4.1)	
		CC	44 (27.5)	138 (27.9)	
Overall score	0 Score	GG/GC	34 (21.3)	156 (31.6)	0.144
		CC	48 (3.0)	13 (2.9)	
	1 Score	GG/GC	46 (28.8)	136 (27.5)	
		CC	32 (2.0)	99 (2.0)	

p, probability for statistic testing

risk (Figure 4-A), CC carriers with regular intake of fried food had a 2.36 times higher risk (Figure 4-B), and CC genotype combined with regular intake of dessert had a 2.39 times higher risk of coronary atherosclerosis (Figure 4-C). There were no effects resulting from the interaction between overall behavioral score (p=0.144) and overall dietary score (p=0.192) with rs11646692 (Table 6).

DISCUSSION

In the present nested case-control study conducted in a Chinese population of Han ethnicity with dyslipidemia, it was found that rs12934922 and rs11646692 on BCO1 were potentially associated with the risk of coronary atherosclerosis; SNP variants on BCO1 and physical activity, fried food intake, as well as dessert intake respectively interacted and affected the development of coronary atherosclerosis.

BCO1 is a key enzyme involved in the conversion of dietary provitamin carotenoids to vitamin A. It catalyzes the centric cleavage at 15, 15' double bond of β-carotene, forming two all-trans retinal molecules which are subsequently oxidized into retinoic acid or reduced into retinol (Vitamin A).[26] Retinoic acid is the transcriptionally active form of vitamin A. By binding to and activating retinoic acid receptor (RAR), all-trans-retinoic acid can inhibit the differentiation of adipocyte. In addition, retinoic acid can block the transcriptional activity of C/EBPβ and decrease the expression of PPARγ. Retinol (Vitamin A) is re-esterified to retinyl ester by lecithin:retinol acyltransfrase (LRAT) and delivered to the liver as the storage of Vitamin A in the body.[27] Previous reports suggested that in addition to the main function as an enzyme for β-carotene metabolism, BCO1 also plays an important role in lipid metabolism. BCO1 may influence the transcription of certain genes such as LRAT, LCAT, ACAT1 and DGAT2, thus ultimately affect the enzymatic activity of the corresponding proteins and the levels of some atherosclerosis-related products, such as cholesteryl esters, triacylglycerols cholesterol and diacylglycerols. In the other hand, BCO1 may be more directly involved in these acyltransferase reactions, by acting as a lipid transporter or an esterification enzyme.[28,29] Study has shown that BCO1 deficient mice were more susceptible to dyslipidemia and hepatic steatosis. For mice with diet-induced obesity, the lack of BCO1 was associated with a significant increase in adiposity, elevated serum free fatty acid levels and fatty liver,[17] which indicates that enzyme activity of BCO1 is substantially related with the processes of lipid metabolism and atherosclerosis in mammals.

Rs12934922 maps to the coding region of BCO1. The allele conversion from A (Arginine) to T (Serine) lowers the enzymatic activity, which contributes to a reduction of vitamin A conversion and higher fasting β-carotene in plasma.[21] Though the availability of vitamin A is limited by the conversion, it may affect health positively by increasing the plasma concentration of powerful antioxidant carotenoids. A considerable amount of studies have shown that, higher level of antioxidant carotenoids concentrations in body is associated with a protection against the development of inflammatory disorder and chronic diseases.[8,30,31]

Rs11646692 and rs11641677 are located around 500bp upstream from the transcription start sites of BCO1 gene, which is a functional clusters region for multiple binding transcription factors. SNP variances in this region may alter transcriptional regulation and gene expression and, consequently, protein level enzyme activity.[32,33] Previously, it was found that GG/GC genotype of rs11646692 was associated with lower risk of dyslipidemia (OR=0.685,

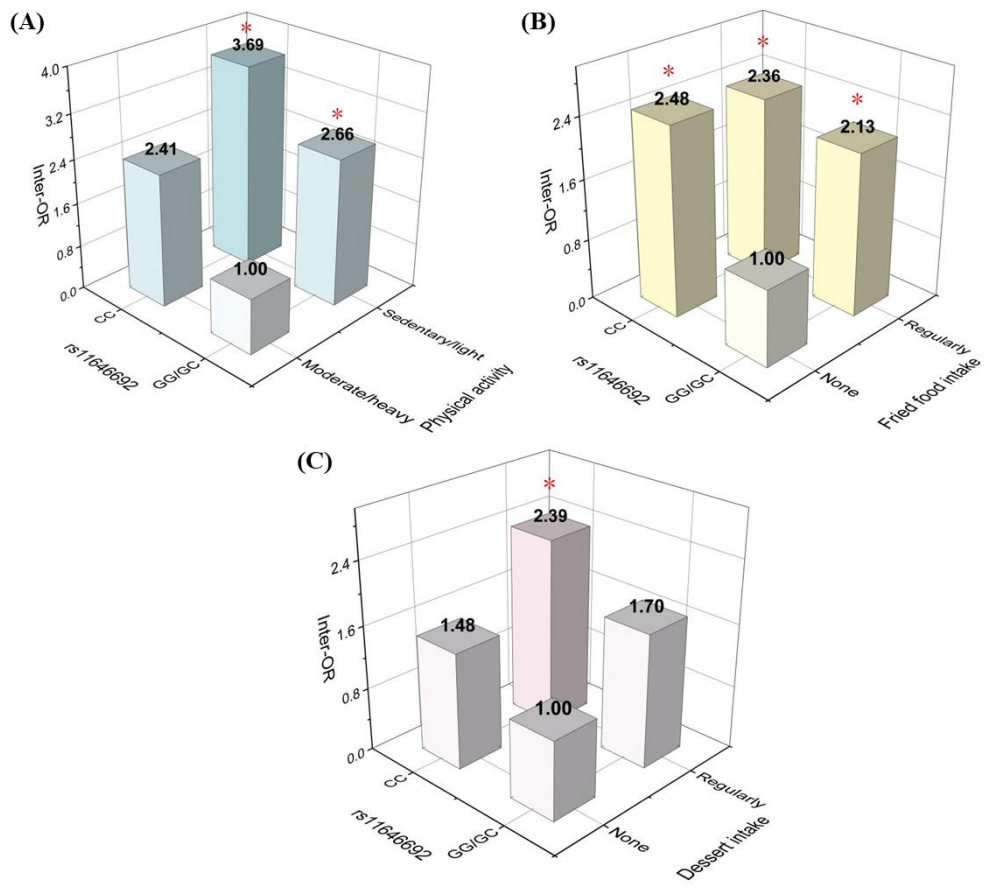

Figure 4. Interactions of the rs11646692 and behaviors (physical activity, fried food intake and dessert intake) on coronary atherosclerosis. *Represents $p<0.05$.

95% CI: 0.529~0.887; $p<0.01$).[19] In the present study, the GG or GC genotype was found to prevent coronary atherosclerosis (OR=0.697, 95%CI: 0.482~1.007; $p=0.054$). However, an association between rs11641677 and coronary atherosclerosis was not observed although the two loci are genetically linked, which could have resulted from the limited statistical power.

Rs6564851 locates 7.7 kb upstream from the BCO1 gene,[16] which is a binding site for transcription factor ISX, which is a transcriptional repressor for BCO1 expression. SNP variances in this region may affect health by influencing the conversion rates of carotenoids and the level of vitamin A in the body.[34] Previous studies identified that G allele of rs6564851 could reduce the catalytic activity of BCO1 and results in higher circulating levels of unconverted β-carotene in plasma,[16,35] suggesting that it may have a substantial impact on oxidation resistance and development of many chronic diseases in the body. However, such explanation has not yet been proved.

Rs7501331 maps the ninth exon coding region of BCO1. Research from Leung WC reported that, the allele conversion from C (Alanine) to T (Valine) leads to a reduced catalytic activity of beta-carotene by about 57%.[36] However, a previous work showed that CT or TT genotype of rs7501331 was associated with a higher risk of high LDL-C in plasma (OR=1.388, 95% CI: 1.038-1.857; $p=0.027$).[19] Due to the limitation of the sample size, a functional relationship between rs7501331 and coronary atherosclerosis was not found in the present associated study.

Coronary atherosclerosisis a complex degenerative trait affected by dozens of risk factors. Among them, personal behaviors such as lack of physical activity, regularly intake of fried food and dessert have long been related to increased risk for coronary atherosclerosis.[37,38] Here, it was confirmed that physical activity, fried food intake and dessert intake were independent risk factors for coronary atherosclerosis. The crossover analysis further demonstrated that, combined physical inactivity, high intake of fried food or dessert by the homozygous risk allele carriers of rs12934922 or rs1646692 could increase susceptibility to coronary atherosclerosis. Since the variations of BCO1 gene were related to circulating levels of β-carotenes or vitamin, which subsequently affected the anti-oxidative capacity and lipid metabolism in the body, there is no doubt that BCO1 gene could synergize with dietary SFA/TFA and have a combined effect on atherogenesis. Reports from prospective studies also found similar results, Casas-Agustench P proved that in MESA cohort, higher intake of saturated fatty acids was associated with higher BMI among individuals with a genetically high risk of premier atherosclerosis traits such as obesity;[39] result from the HUNT cohort study revealed that the interaction between genes and physical activity was associated with WHR and BMI.[40] Those gene-environment interaction exploration indicated that personal hereditary traits, as well as environmental factors should be taken into account for the prediction of complex chronic disease and precision health protection.

Neither the overall behavioral score nor the overall dietary score interacted with BCO1 polymorphisms on the risk of coronary atherosclerosis in the interaction models in this study. The negative results may be due to the unspecific questionnaire that the types of food only is not enough to find the differences in carotenoid levels among diverse foods. The carotenoids are widely found in foods, and the distribution levels of different types of foods vary widely. This research used a general food conception for analysis, such as vegetables, fruits, meat, which may cause adverse interference to the findings. However, the differences in dietary patterns could be primarily responsible for the same. Thus a more specific and detailed study needs to be further implemented based on varied causes and multi-factor synergy, which produce pathological effects in the development of this disease.[41,42] This study had several limitations worth mentioning. Firstly, the sample size of cases in this survey was rather small since the follow-up duration was only three years, individuals selected as controls during that time could become cases later on, which would influence the result of an associated study. However, the limitation was partially overcome by conducting the matched case-control design. The controls were oversampled, for each case such that, at least three controls were selected by matching age and sex, this method strictly controlled any confounding effects and the outcomes from any of the variables could be successfully explained in the results. Secondly, the diet questionnaire used in this study only contained 11 food items, details such as cooking method and specific nutrition intake were not available. Moreover, it was not possible to know the quantitative data of the various food intakes of the study population and the nutrients contained in these foods, and whether the nutrients would interfere with the BCO1 gene polymorphisms on coronary atherosclerosis in the participants. Therefore, it was not possible to calculate the total energy consumed for food intake adjustment, it was also difficult to investigate the relationship between atherosclerosis and single nutrients or food components, which could have provided a better understanding about the observed association and the disease pathogenesis.

In summary, this study identified genetic variants of rs12934922 and rs11646692 in BCO1 genes that were potentially associated with coronary atherosclerosis in the Chinese Han population with dyslipidemia. The study suggests that interaction between BCO1 and personal behaviors may substantially have combined effect on the susceptibility of coronary atherosclerosis. Individuals with the genetic susceptibility for coronary atherosclerosis are advised to enhance their physical activity and lower dietetic intake of fried food and dessert. Further explorations with a validated dietary questionnaire and longer time of follow-up are required to validate the observations made in this study.

ACKNOWLEDGEMENTS

This field work and electronic health records data was supported by Yinzhou Center for Disease Control.

AUTHOR DISCLOSURES

This study does not have any conflicts of interest and funding support.

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