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Male-specific association of the *APC* rs383830 T allele with the risk of coronary heart disease

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ABSTRACT. *APC* is a tumor suppressor gene that is involved in the processes of cell migration and adhesion, transcriptional activation, and apoptosis. The goal of this study was to evaluate the contribution of the *APC* rs383830 polymorphism to coronary heart disease (CHD) in Han Chinese. A total of 783 patients with CHD and 737 controls were tested in the current association study. Although our study did not identify an association between the *APC* rs383830 polymorphism and CHD, a breakdown analysis by gender indicated there was a significant contribution of the rs383830 T allele to the risk of CHD in males (P = 0.046, odds ratio = 1.267, 95% confidence interval = 1.004-1.598). In conclusion, our study suggested a male-specific association of the *APC* rs383830 polymorphism with CHD.

Key words: Coronary heart disease; *APC*; rs383830; Male

INTRODUCTION

Coronary heart disease (CHD) is a form of heart disease in which the coronary circulation fails to provide enough blood circulation for cardiac muscle and the surrounding tissue. CHD is one of the leading causes of human death in developed countries (Abbott et al., 2004). The incidence of CHD is also rapidly increasing in low- and middle-income countries, including India and China (Celermajer et al., 2012), and CHD is expected continue to dominate mortality trends in the coming decades (Lin et al., 2013).

Twin and family studies have shown a strong genetic component in the development of CHD (Marenberg et al., 1994; Murabito et al., 2005). Estimates have placed genetic predisposition as accounting for 40-60% of the susceptibility to CHD (Roberts and Stewart, 2012). Recent studies have identified a number of genetic markers for susceptibility to CHD (Blankenberg et al., 2010). Although current genome wide association studies (GWAS) have reported a substantial number of genetic variants underlying CHD (Prins et al., 2012), over 95% of the genetic variants influencing disease risk remain undiscovered. Accordingly, CHD has represented a major focus for genetic studies that continue to enrich our understandings of its pathophysiology.

APC, the adenomatous polyposis coli tumor suppressor gene (Rubinfeld et al., 1997), has been shown to be associated with an inherited syndrome of colorectal cancer known as familial APC (Goss and Groden, 2000). The APC protein is involved in various important processes including cell migration and adhesion, transcriptional activation, and apoptosis (Park et al., 2014). A recent GWAS has demonstrated that the *APC* rs383830 polymorphism can also serve as a CHD susceptibility biomarker in Europeans (Angelakopoulou et al., 2012). However, no association was found between this polymorphism and CHD in European populations (Angelakopoulou et al., 2012). These discrepancies suggest that the role of *APC* rs383830 in the risk of CHD might vary in different ethnic groups. Considering the complexity of CHD, further validation is needed in other populations for previously identified markers. CHD has been observed to have different genetic predictors (Qi and Campos, 2011) and various disease prevalences (Hasan et al., 2011; Freund et al., 2012) in different ethnic populations. To validate the contribution of *APC* rs383830 to CHD in a Han Chinese population, we recruited 783 patients with CHD confirmed by angiography and 737 angiography-normal individuals, and performed a case-control association study.

MATERIAL AND METHODS

Sample collection

We recruited a total of 1520 unrelated individuals for the current study of Han Chinese ethnicity originating from Ningbo city in the Eastern China. The study cohorts consisted of 783 patients with CHD confirmed by angiographic evidence that the diameter of stenosis was greater than 50% in any of the main coronary arteries, or by a history of prior angioplasty or coronary artery bypass surgery; 737 controls chosen from hospital patients who had less than 50% stenosis in the major coronary artery, and did not have a history of CAD or electrocardiographic signs of CAD. All samples were collected between May 2012 and April 2013 at Ningbo First Hospital of Zhejiang Province, China. All individuals had been examined through

standardized coronary angiography (Kirisli et al., 2013) according to Seldinger's method (Gagliardi et al., 1990), and the results were judged by at least two independent cardiologists. Blood samples (2 mL) were collected from patients in a fasting state. The blood samples were collected and processed by the same investigators. Demographic information regarding the presence of traditional coronary risk factors including body mass index, hypertension, diabetes, smoking, and serum cholesterol were collected from all participants. Blood samples were stored at -80°C in 3.2% citrate sodium-treated tubes until analyzed. The study protocol was approved by the Ethics Committees of Ningbo First Hospital, and informed consent was obtained from all patients. None of the patients had congenital heart disease, cardiomyopathy, or severe liver or kidney disease.

Single nucleotide polymorphism (SNP) genotyping

Human genomic DNA was isolated from peripheral blood samples using a nucleic acid extraction automatic analyzer (Lab-Aid 820, Zeesan Biotech Co., Ltd., Xiamen City, China). Genotyping was performed on the Sequenom® Mass-ARRAY iPLEX® platform according to the manufacturer instructions **(**Sequenom Inc., San Diego, CA, USA**)**. Polymerase chain reaction (PCR) for genotyping experiments was performed on an ABI Geneamp® PCR System 9700 Dual 384-Well Sample Block Module (Applied Biosystems, Foster City, CA, USA). Complete iPLEX® Gold Genotyping Reagent Set (Sequenom Inc.) was used in the genotyping. Final volume of PCR components were as follows: 1.8 μL ddH_2O, 1 μL 0.05 mM primers, 0.1 μL 25 mM dNTP, 0.4 μL 25 mM $MgCl_2$, 1 μL DNA template, 0.5 μL 10X buffer, and 0.2 μL PCR polymerase. PCR procedures included an initial denaturation stage at 94°C for 15 s, followed by 45 amplification cycles of 94°C for 20 s, 56°C for 30 s and primer extension at 72°C for 1 min, and a final extension stage at 72°C for 3 min. Primer sequences for rs383830 are 5'-ACG TTG GAT GCT GTT ACT CAT GTT GCC TTG-3' for the forward primer, and 5'-ACG TTG GAT GAT TTA CAC CCA CAG GAC TTC-3' for the reverse primer. The extension program consisted of 1) 1 cycle at 94°C for 30 s for an initial denaturation stage; 2) 40 cycles of amplification of 94°C for 5 s, 52°C for 5 s, 80°C for 5 s; 3) 5 cycles of amplification of 52°C for 5 s and 80°C for 5 s; 4) a final extension at 72°C for 3 min. Extension primer is 5'-CCAGGACTTCCAAAAATTAAAATAAAGT-3'. After purifying the products and transfer to a SpectroCHIP, MALDI-time-of-flight mass spectrometry (Sequenom Inc.) was used for SNP genotyping.

Statistical analyses

Comparison of genotype and allele frequencies between patients with CHD and controls were determined by the CLUMP22 software with 10,000 Monte Carlo simulations (Sham and Curtis, 1995). Consistency of the genotype frequencies with Hardy-Weinberg equilibrium (HWE) was performed by the Arlequin program (version 3.5) **(**Excoffier and Lischer, 2010). A two-sided P value < 0.05 was considered to indicate a statistically significant result.

RESULTS

A case-control comparison of both the genotype and allele frequencies for the rs383830 polymorphism is presented in Table 1.

Table 1. Genotype and allele distribution for *APC* rs383830 in cases and controls.

rs383830	Genotype (counts)			χ^2	P (d.f. = 2)	HWE	Allele (counts)		χ^2	P (d.f. = 1)	OR (95%CI)
	TT	AT	AA				T	A			
Cases (N = 783)	31	254	498			0.845	316	1250			
Controls (N = 737)	26	216	495	2.13	0.345	0.685	268	1206	1.95	0.163	1.138 (0.949-1.363)

All SNP data were in HWE. No significant difference for the frequency of the rs383830 T allele between patients and controls was observed [20.2% versus 18.2%, χ^2 = 1.950, d.f. = 1, P = 0.163, odds ratio (OR) = 1.138, 95% confidence interval (CI) = 0.949-1.363]. SNP rs383830 genotype frequencies were shown to exhibit no critical differences between patients with CHD and controls in either dominant or recessive models (data not shown) (**Table S1**).

Since gender is a predictor of CHD risk, we further stratified the data by gender with respect to allele and genotype frequencies (Table 2).

Table 2. Gender-stratified association of *APC* rs383830 with CHD.

Gender	Genotype (counts)			χ^2	P (d.f. = 2)	HWE	Allele (counts)		χ^2	P (d.f. = 1)	OR (95%CI)
rs383830	TT	AT	AA				T	A			
Male cases (N = 537)	23	177	337			0.968	223	851			
Male controls (N = 420)	10	124	286	4.39	0.111	0.421	144	696	3.99	0.046	1.267 (1.004-1.598)
Female cases (N = 246)	8	77	161			0.743	93	399			
Female controls (N = 317)	16	92	209	1.292	0.524	0.167	124	510	0.08	0.777	0.959 (0.711-1.293)

In the comparison between male groups, the frequency of the T allele of rs383830 was significantly higher in patients than in controls (20.8% versus 17.1%; χ^2 = 3.99, d.f. = 1, P = 0.046; OR = 1.267, 95%CI = 1.004-1.698). However, no significant differences were observed between patients and controls in the female subgroup (P > 0.05). In addition, our analysis did not detect any effect of age on the likelihood of having CHD (data not shown) (**Table S2**).

We have identified a sex difference in *APC* gene variation in the present CHD case-control study (males: allele P = 0.046, females: allele P = 0.777). Our results cannot exclude the possibility that premenopausal women have reduced cardiovascular disease compared to men, but we note that the incidence of cardiovascular disease in women increases following menopause **(**Murphy and Steenbergen, 2014). In men, a decrease in mortality from CHD across all age groups over time has been reported, whereas in the youngest women (age <55 years) a notable increase in mortality from CHD has been identified. Estrogen can affect the heart and blood vessels **(**Han et al., 2013), and sexual dimorphism is frequently observed in the prevalence and severity of cardiovascular diseases (Ober et al., 2008; Gulati et al., 2012). The rs974819 polymorphism of *PDGFD* has been shown to be associated with an increased risk of CHD in Han Chinese, with a sex-dependent genetic effect (Zhou et al., 2012). Furthermore, an association has been identified between sex-specific allelic variants within *MYH15*, *VEGFA*, and *NT5E* and an increased risk of coronary microvascular dysfunction in men but not in women **(**Yoshino et al., 2014). A strong association between rs702553 and stroke has also only been found in young men but not in women (Liao et al., 2010).

DISCUSSION

Apoptosis can be controlled by the regulation of a number of genes that can be classified into three categories: effectors of apoptosis, suppressors of apoptosis, and intermediate regulators of apoptosis (Rezvani et al., 2000). TNF belongs to the intermediate regulators of apoptosis (Tong and Coulombe, 2006; McFerrin et al., 2012), and is a tumor suppressor gene that plays a significant role in the pathogenesis of atherosclerosis (Nair et al., 2014). Studies using mouse models have demonstrated that enhanced expression of an apoptotic gene (TNFR1) leads to accelerated atherosclerosis and reduced smooth muscle cell multiplication in the aged wild-type arteries (Zhang et al., 2010). Furthermore, another apoptotic gene polymorphism (*TNFA* -238G>A) was shown to decrease the risk of CHD among nonsmokers in a Han Chinese population (Hou et al., 2009), although there was no significant association of CHD with polymorphism of *TNFSF4*, another apoptotic gene (Cheng et al., 2011).

Our study found an association between the *APC* rs383830 polymorphism and CHD in males. Our sample size is comparatively small; therefore, we cannot exclude the possibility that the overall negative findings might be due to a lack of power. In addition to rs383830, other *APC* polymorphisms have also been examined in previous studies. Plevová et al. (2008) suggested that the c.645+32C>T substitution in the *APC* gene was a non-pathogenic SNP that occurred in approximately 16% of the Czech population. A significant correlation was observed between the *APC* p.I1307K gene variant and colorectal neoplasia in Ashkenazi Jews with otherwise average disease risk (Boursi et al., 2013). Mostowska et al. (2014) revealed significantly increased *APC* rs11954856 and rs351771 frequencies in Polish women with ovarian cancer. In addition, and of more direct relevance to this study, it has also been reported that an association exists between the rs383830 polymorphism of APC and CAD in Europeans (Angelakopoulou et al., 2012). Thus, our study does not exclude the role of other *APC* polymorphisms in the susceptibility of CAD.

In conclusion, our findings support a significant association between the *APC* rs383830 polymorphism and CHD in males, and suggest that there are ethnic differences in the allele frequency of the tested SNP between Han Chinese and other populations.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary material

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