

Impact of gender and age on the association of the BUD13-ZNF259 rs964184 polymorphism with coronary heart disease

Xiaofeng Xu, Yirun Li*, Yi Huang**, Huadan Ye*, Liyuan Han*, Huihui Ji*, Xiaoyin Chen*, Nan Wu, Danjie Jiang*, Limin Xu*, Dongjun Dai*, Shiwei Duan*, Xiaomin Chen

Key Laboratory of Ningbo First Hospital and Cardiovascular Center of Ningbo First Hospital, *Medical Genetics Center, School of Medicine, **Department of Neurosurgery, Ningbo First Hospital Ningbo University; Ningbo-China

ABSTRACT

Objective: Coronary heart disease (CHD) is the most common cause of death worldwide. This study aimed to validate the association of the rs964184 polymorphism with the CHD risk and included 874 CHD patients and 776 controls.

Methods: rs964184 polymorphism genotyping was performed using Tm-shift polymerase chain reaction.

Results: A strong association of the rs964184 polymorphism with CHD was found (genotype: $X^2=14.365$, $p=0.001$; allele: $X^2=14.191$, $p=1.67 \times 10^{-4}$; power=0.965). Gender analysis revealed a significant association only in males (genotype: $X^2=12.387$, $p=0.002$; allele: $X^2=12.404$, $p=4.32 \times 10^{-4}$; OR=1.467, 95% CI=1.185–1.817, power=0.945). Age and gender analyses revealed significant associations of the rs964184 polymorphism with CHD in males between the ages of 55 and 65 years (genotype: $X^2=10.070$, $p=0.007$; allele: $X^2=10.077$, $p=0.002$; OR=1.706, 95% CI=1.224–2.377, power=0.996) and in females older than 65 years (genotype: $X^2=9.462$, $p=0.009$; allele: $X^2=9.560$, $p=0.002$; OR=2.112, 95% CI=1.308–3.412, power=0.994). Further subgroup analysis suggested that rs964184 genotypes were significantly associated with TG levels in the patients ($r=0.191$, adjusted $p=1.05 \times 10^{-5}$) and controls ($r=0.101$, adjusted $p=0.026$).

Conclusion: Our results indicate that both gender and age have great impacts on the association of the rs964184 polymorphism with CHD among Chinese. (*Anatol J Cardiol* 2018; 19: 42-9)

Keywords: coronary heart disease, SNPs, BUD13-ZNF259, rs964184, age, gender

Introduction

Coronary heart disease (CHD), which is mainly caused by atherosclerosis, has become the leading cause of death worldwide (1). Although the prevalence of CHD is now decreasing in high-income countries, most deaths caused by CHD occur in low- and middle-income countries (2). The development of CHD is a very complex process. Its risk factors include age, gender, family history, dyslipidemia, hypertension, diabetes, cigarette smoking history, physical inactivity, and even psychosocial issues (3-5). A twin study has established that gender factors play an extremely important role in CHD (6). The interaction between environmental and genetic factors contributes to the morbidity of CHD (7, 8).

A recent genome-wide association study (GWAS) found a significant association of the BUD13-ZNF259 rs964184 polymorphism with triglyceride (TG) levels (9). Another GWAS identified that the rs964184 polymorphism was strongly associated with

low-density lipoprotein cholesterol (LDL-C) levels in Chinese school-age children (10). High LDL-C levels are risk factors for CHD (11). BUD13-ZNF259 is located on 11q23.3 and encodes Zinc finger protein (ZPR1), which is a cytoplasmic zinc finger protein that interacts with tyrosine kinase receptors in quiescent mammalian cells (12). ZPR1 transcription is upregulated in the brain tissues of mice fed a high-fat diet (13). The high expression of BUD13-ZNF259 in neuronal cells can lead to increased H2O2-induced cell death, suggesting that a high-fat diet enhances ZPR1 mRNA expression and might increase vulnerability to oxidative stress (13). We hypothesize that the BUD13-ZNF259 rs964184 polymorphism as a quantitative locus of TG increases the vulnerability of coronary artery endothelial cells to both oxidative stress and inflammatory response after damage. Furthermore, elevated serum TG levels are independent risk factors for CHD (5). The BUD13-ZNF259 rs964184 polymorphism has been shown to affect lipid levels in the blood and thus increase the risk of CHD (14).

Our previous association study in 290 patients and 197 con-

Address for correspondence: Dr. Xiaomin Chen, Key Laboratory of Ningbo First Hospital and Cardiovascular Center of Ningbo First Hospital, Ningbo University Ningbo 315010-China
E-mail: chxmin@hotmail.com

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trols found that the rs964184-G allele of BUD13-ZNF259 was a moderate risk factor for CHD in females [$p=0.05$, odd ratio (OR)=1.49, 95% confidential interval (CI)=1.00–2.22] (15). The aim of the present study was to confirm our previous findings in much larger samples: 874 CHD patients and 776 controls.

Methods

Sample collection

The present retrospective case–control study included 874 CHD patients (625 males and 249 females) and 776 controls (424 males and 352 females). All subjects were Han Chinese and were randomly recruited between May 2008 and July 2015 from Ningbo First Hospital in Zhejiang Province. Approximately 1754 CHD patients and controls were examined by standardized coronary angiography on the basis of the Seldinger technique (16); at least two independent cardiologists were involved in adjudication. The controls were chosen from patients whose vascular stenosis rates were less than 50% in every coronary artery. The CHD patients included patients with a history of prior angioplasty or coronary artery bypass surgeries. Of them, 93 were excluded because of cardiomyopathy, congenital heart, liver or kidney disease. A total of 1661 blood samples from the CHD patients and controls were collected by the same investigator and were stored in 3.2% citrate sodium-treated tubes at -80°C . All blood samples were used for genotyping. Approximately 11 blood samples were not analyzed because of technical problems. Finally, a total of 1650 samples were used for genotyping. Our study was approved by the Institutional Review Board. Written information consent was obtained from all individuals.

SNP genotyping

Human genomic DNA was separated from peripheral blood samples using a nucleic acid extraction automatic analyzer (Lab-Aid 820, Xiamen, China), and all DNA samples were stored in TE buffer. Genotyping was done by Tm-shift polymerase chain reaction (PCR) (17). The sequences of the primers were 5'-gcgggcagggcggcTCACCATCTGATGTACTGTTTTCTc-3' (AS1 primer), 5'-gattaccgTCACCATCTGATGTACTGTTTTCTg-3' (AS2 primer), and 5'-TTCATGGAACCTGAAGTCTAGTGGGA-3' (common primer). PCR amplification was performed on ABI GeneAmp® PCR System 9700 Dual 384-Well Sample Block Module (Applied Biosystems, Foster City, CA). The PCR process included an initial denaturation stage at 95°C for 30 s, followed by 40 cycles of 95°C for 30 s, 59°C for 30 s, 72°C for 30 s, and a final extension stage at 72°C for 5 min. Then, melting curve analysis was conducted on a Roche LightCycler 480® fluorescence quantitative PCR instrument (Roche, Basel, Switzerland). The process included using temperatures of 95°C of 15 s and 60°C of 30 s and then increasing the temperature by $0.11^{\circ}\text{C}/\text{s}$ to 95°C . Melting curve data were acquired by Air borne software provided by Roche automatic clustering based on fluorescence intensity analysis (18).

Statistical analysis

Hardy–Weinberg equilibrium (HWE) was analyzed according to Arlequin software Version 3.5 (Bern, Switzerland) and $p>0.05$ was considered to be in HWE. The differences in the genotype and allele frequencies between the CHD patients and controls were calculated by CLUMP22 software (Denmark Hill, London, UK) with 10,000 Monte Carlo simulations. Power analysis was performed by Power and Sample Size Calculation software, Version 3.0.43 (Nashville, TN, USA). The odds ratio (OR) and their 95% confidence intervals (95% CIs) were calculated using PASW Statistics 18.0 software (SPSS Inc., NY, USA). If the expected frequency for 2×2 and 2×3 chi-square and 2×2 tables was over 25%, Fisher's exact test can be used. Correlation analysis was used Pearson test and performed on both PASW and R statistical software (Stanford, California, USA). Two-sided p -value of <0.05 was considered to be statistically significant.

Results

The clinical and demographic details of the patients and controls [including smoking, hypertension, diabetes, and total cholesterol, high-density lipoprotein cholesterol (HDL-C), LDL-C, and TG levels] have been described in our previous studies (19, 20). A case–control comparison of genotype and allele frequencies for the rs964184 polymorphism is presented in Table 1. No departure of HWE was observed in the patients and controls. Our results revealed a strong association of the rs964184 polymorphism with the risk of CHD at the genotype and allele levels (genotype, $X^2=14.365$, $df=2$, $p=0.001$; allele, $X^2=14.191$, $df=1$, $p=1.67\times 10^{-4}$). The frequency of the rs964184-G allele was significantly higher in the patients than in the controls (25.9% vs. 20.4%; $p=1.67\times 10^{-4}$, OR=1.368, 95% CI=1.162–1.611).

Breakdown analysis by gender showed a significant association in males (genotype: $X^2=12.387$, $df=2$, $p=0.002$; allele: $X^2=12.404$, $df=1$, $p=4.32\times 10^{-4}$). In contrast, no significant association between the patients and controls was observed in females

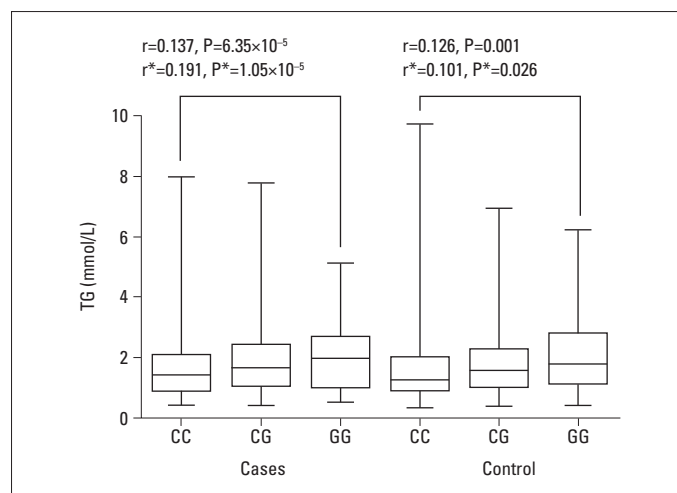


Figure 1. Correlation between TG levels and rs964184 genotypes*
*: indicates that cigarette smokers were excluded. TG - triglyceride

Table 1. Genotype and allele distribution of rs964184*

Gender	Group	Genotype (counts)			X ²	P (df=2)	HWE	Allele (n,%)		X ²	P (df=1)	OR (95% CI)	Power
		GG	GC	CC				G	C				
All	Cases (n=874)	56	341	477			0.724	453 (25.9)	1295 (74.1)				
	Controls (n=776)	32	252	492	14.365	0.001	1.000	316 (20.4)	1236 (79.6)	14.191	1.67*10 ⁻⁴	1.368 (1.162-1.611)	0.965
Males	Cases (n=625)	40	238	347			1.000	318 (25.4)	932 (74.6)				
	Controls (n=424)	15	130	279	12.387	0.002	1.000	160 (18.9)	688 (81.1)	12.404	4.32*10 ⁻⁴	1.467 (1.185-1.817)	0.945
Females	Cases (n=249)	16	103	130			0.523	135 (27.1)	363 (72.9)				
	Controls (n=352)	17	122	213	4.190	0.123	1.000	156 (22.2)	548 (77.8)	3.894	0.049	1.306 (1.001-1.704)	0.504

* P value less than or equal to 0.05 is in bold. 95%CI-95% confidence interval; HWE-hardy Weinberg equilibrium; OR-odds ratio

at the genotype level ($p > 0.05$), although there is a much weaker association at the allele level in females ($X^2 = 3.894$, $df = 1$, $p = 0.049$) than in males. We hypothesized that the inconsistent results in females between the genotype and allele levels results from the inheritance models. A further test showed that there was a strong association between the CHD patients and controls in the dominant model (Table 2, GG+GC vs. CC: $X^2 = 13.209$, $df = 1$, $p = 2.81 \times 10^{-4}$; OR=1.442, 95% CI=1.183–1.757). Moreover, a stratification test by gender showed a significant effect in the dominant model (GG+GC vs. CC) for both males and females (Table 2; males: $X^2 = 11.098$, $df = 1$, $p = 0.001$, OR=1.542, 95% CI=1.194–1.990; females: $X^2 = 4.103$, $df = 1$, $p = 0.043$, OR=1.403, 95% CI=1.011–1.947).

In Table 3, subgroup analysis by the age of the individuals showed a significant difference between the rs964184 polymor-

phism and the risk of CHD in individuals between the ages of 55 and 65 years (genotype: $X^2 = 8.056$, $df = 2$, $p = 0.018$; allele: $X^2 = 7.876$, $df = 1$, $p = 0.005$; OR=1.411, 95% CI=1.109–1.795, power=0.802) and in individuals older than 65 years (genotype: $X^2 = 8.426$, $df = 2$, $p = 0.015$; allele: $X^2 = 8.377$, $df = 1$, $p = 0.004$; OR=1.605, 95% CI=1.163–2.215, power=0.835). As age and gender are two important risk factors for CHD, we further investigate the effect of gender in each age subgroup. As shown in Table 4, a strong association of the rs964184 polymorphism with CHD in males between the ages of 55 and 65 years (genotype: $X^2 = 10.070$, $df = 2$, $p = 0.007$; allele: $X^2 = 10.077$, $df = 1$, $p = 0.002$; OR=1.706, 95% CI=1.224–2.377) and in females older than 65 years (genotype: $X^2 = 9.462$, $df = 2$, $p = 0.009$; allele: $X^2 = 9.560$, $df = 1$, $p = 0.002$; OR=2.112, 95% CI=1.308–3.412). In Table 5, further analysis suggested significant associations

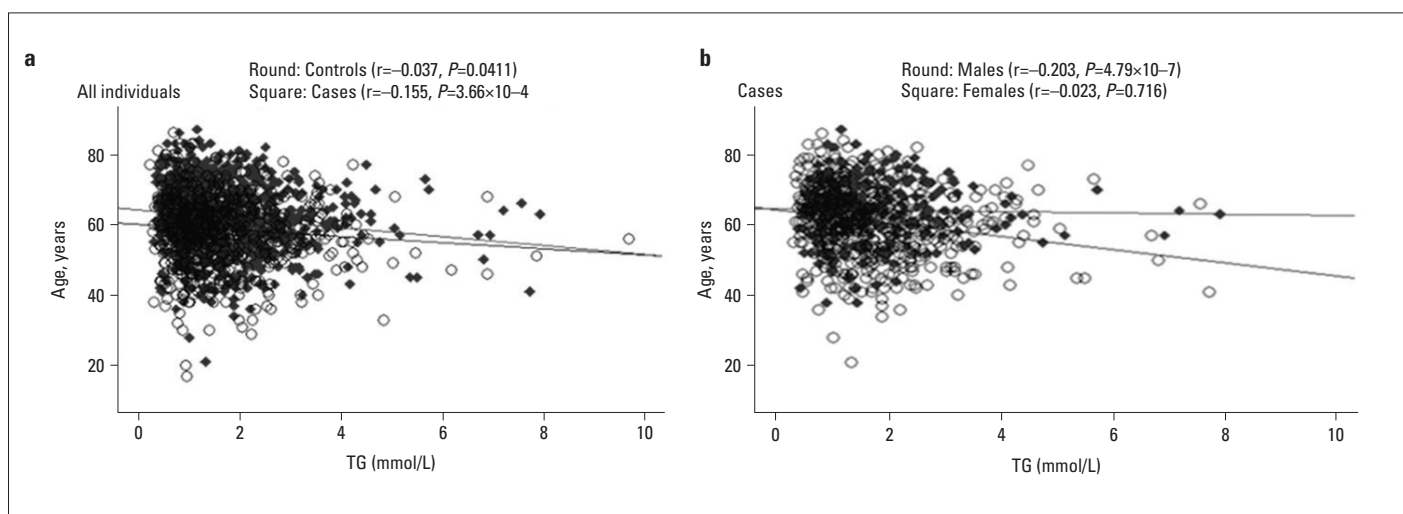


Figure 2. Breakdown correlation between TG levels and age by gender in the patients and controls
A-Correlation between TG levels and age in the subgroups of the patients and controls; B-Correlation between TG levels and age in the subgroup of male and female patients. TG - triglyceride

Table 2. Genetic testing under the dominant and recessive models*

Gender	Group	Dominant	X ²	P (df=1)	OR (95% CI)	Power	Recessive	X ²	P (df=1)	OR (95% CI)	Power
		GG+GC CC					GG GC+CC				
All	Cases										
	(n=874)	397 477					56 818				
	Controls										
	(n=776)	284 492	13.209	2.81*10 ⁻⁴	1.442 (1.183-1.757)	0.999	32 744	4.246	0.039	1.592 (1.019-2.485)	0.787
Males	Cases										
	(n=625)	278 347					40 585				
	Controls										
	(n=424)	145 279	11.098	0.001	1.542 (1.194-1.990)	0.997	15 409	4.166	0.041	1.864 (1.016-3.420)	0.670
Females	Cases										
	(n=249)	119 130					16 233				
	Controls										
	(n=352)	139 213	4.103	0.043	1.403 (1.011-1.947)	0.817	17 335	0.716	0.397	1.353 (0.670-2.733)	0.300

*P value less than or equal to 0.05 is in bold. 95%CI-95% confidence interval; OR - odds ratio

in the dominant model (GG+GC vs. CC) for males between the ages of 55 and 65 years (X²=7.643, df=1, p=0.006, OR=1.748, 95% CI=1.175–2.600) and for females older than 65 years (X²=7.467, df=1, p=0.006, OR=2.218, 95% CI=1.247–3.945).

In Figure 1, TG levels were showed to associated with rs964184 genotypes (patients: r=0.191, adjusted p=1.05×10⁻⁵; controls: r=0.101, adjusted p=0.026). Moreover, a significant reverse correlation was observed between age and TG levels (Fig. 2, Panel A; patients: r=-0.155, p=3.66×10⁻⁴; controls: r=-0.037, p=0.411). Breakdown analysis by gender showed that a signifi-

cant association only existed in male patients (Fig. 2, Panel B; males: r=-0.203, p=4.79×10⁻⁷, females: r=0.023, p=0.716). TG levels reduced with increasing age.

Discussion

In the present study, which contained a higher number of samples than our previous study, we wanted to confirm the association of the rs964184 polymorphism and the CHD risk. Our results revealed a significant association between the BUD13-

Table 3. Comparison of genotype and allele frequencies between cases and controls by age*

Age	Group	Genotype(counts)			X ²	P (df=2)	HWE	Allele (n,%)		X ²	P (df=1)	OR (95% CI)	Power
		GG	GC	CC				G	C				
55<	Cases												
	(n=186)	7	70	109			0.404	84 (22.6)	288 (77.4)				
	Controls (n=236)	10	77	149	1.156	0.561	1.000	97 (20.6)	375 (79.4)	0.509	0.476	1.128 (0.811-1.569)	0.111
55-65	Cases												
	(n=361)	27	149	185			0.796	203 (28.1)	519 (71.9)				
	Controls (n=357)	15	125	217	8.056	0.018	0.641	155 (21.7)	559 (78.3)	7.876	0.005	1.411 (1.109-1.795)	0.803
>65	Cases												
	(n=327)	22	122	183			0.768	166 (25.4)	488 (74.6)				
	Controls (N=183)	7	50	126	8.426	0.015	0.446	64 (17.5)	302 (82.5)	8.377	0.004	1.605 (1.163-2.215)	0.835

*P value less than or equal to 0.05 is in bold. 95%CI-95% confidence interval; HWE-Hardy Weinberg equilibrium; OR-odds ratio

Table 4. Comparison of genotype and allele frequencies between cases and controls by age as well as gender*

Age	Gender	Group	Genotype (counts)	X ²	P(df=2)	HWE	Allele (n,%)	X ²	P(df=1)	OR (95%CI)	Power
			GG GC CC				G C				
55<	Males	Cases									
		(n=149)	7 60 82			0.511	74 (24.8) 224 (75.2)				
		Controls									
		(n=159)	5 53 101	2.418	0.299	0.802	63 (19.8) 255 (80.2)	2.242	0.134	1.337 (0.914-1.957)	0.562
	Females	Cases									
		(n=37)	0 10 27			1.000	10 (13.5) 64 (86.5)				
	Controls										
	(n=77)	5 24 48	2.976	0.226	0.504	34 (22.1) 120 (77.9)	2.354	0.125	0.552 (0.256-1.188)	0.588	
55-65	Males	Cases									
		(n=262)	22 103 137			0.648	147 (28.1) 377 (71.9)				
		Controls									
		(n=172)	5 54 113	10.070	0.007	0.803	64 (18.6) 280 (81.4)	10.077	0.002	1.706 (1.224-2.377)	0.996
	Females	Cases									
		(n=99)	5 46 48			0.215	56 (28.3) 142 (71.7)				
	Controls										
	(n=185)	10 71 104	1.759	0.415	0.842	91 (24.6) 279 (75.4)	0.915	0.339	1.209 (0.819-1.785)	0.275	
>65	Males	Cases									
		(n=214)	11 75 128			1.000	97 (22.7) 331 (77.3)				
		Controls									
		(n=93)	5 23 65	3.216	0.200	0.151	33 (17.7) 153 (82.3)	1.882	0.170	1.359 (0.876-2.108)	0.492
	Females	Cases									
		(n=113)	11 47 55			0.826	69 (30.5) 157 (69.4)				
	Controls										
	(n=90)	2 27 61	9.462	0.009	1.000	31(17.2) 149(82.8)	9.560	0.002	2.112 (1.308-3.412)	0.994	

*P value less than or equal to 0.05 is in bold. 95%CI-95% confidence interval; HWE-Hardy Weinberg equilibrium; OR-odds ratio

ZNF259 rs964184 polymorphism and CHD. In addition, we investigated the effect of age and gender on the association of this polymorphism with CHD.

CHD patients often have abnormal lipid phenotypes, including high LDL-C and low HDL-C as well as TG levels (4, 21). The BUD13-ZNF259 rs964184 polymorphism has been shown to be associated with the risk of dyslipidemia (22, 23). This polymorphism could influence blood lipid levels and increase the risk of cardiovascular (24) and cerebrovascular (17) diseases. Ueyama et al. (25) found that the rs964184-G allele was significantly associated with increases in serum TG levels and decreases in serum HDL-C levels in metabolic syndrome (MetS) patients from Japan (26). Mirhafez et al. (27) suggested that the CG+GG genotypes of the rs964184 polymorphism were associated with increased serum TG and LDL-C levels in MetS patients from northeastern Iran. Zhang et al. (28) showed that patients with the rs964184-CC genotype had the lowest TG level and that those with the rs964184 GG genotype had the highest serum TG level. In the present study, we confirmed a significant association of

the rs964184 polymorphism with TG levels (Fig. 2). In addition, we observed a significant inverse correlation between age and TG levels, suggesting that the older patients have lower TG levels. This result seems to be in contrast with current knowledge that the risk of CHD increases along with increased TG levels and aging (21). We speculate that these phenomena are caused by medicines taken by the patients and controls. Normally, older patients (patients and controls) tend to have a longer history of taking medicines that may reduce TG levels to a lower extent. An epidemiologic study has shown that the incidence of CHD was eight to nine times greater in men and women who aged 55–64 years than in young patients (29). The death rate due to CHD increased quickly in patients aged 55 years and more and was higher in patients aged 65 years or more than in young patients (30). Other studies have suggested that these rates were beginning to level off in younger age groups (31, 32). Scientists have confirmed that genetic polymorphisms may play an important role in the pathogenesis of early onset CHD (33, 34). Here we demonstrated significant differences between the rs964184

Table 5. Genetic testing under the dominant and recessive models by age and gender*

Age	Gender	Group	Dominant	X ²	P(df=1)	Power	OR (95%CI)	Recessive	X ²	P(df=1)	OR (95%CI)	Power
		Rs964184	GG+GC CC					GG GC+CC				
55<	Males	Cases	67 82					7 142				
		(n=149)										
	Females	Cases	10 27					0 37				
		(n=37)										
55-65	Males	Controls	58 101	2.298	0.130	0.573	1.423 (0.901-2.246)	5 154	0.496	0.481	1.518 (0.471-4.892)	0.170
		(n=159)										
	Females	Controls	29 48	1.256	0.262	0.349	0.613 (0.260-1.448)	5 72	2.513	0.113	NA	NA
		(n=77)										
>65	Males	Cases	125 137					22 240				
		(n=262)										
		Controls	59 113	7.643	0.006	0.977	1.748 (1.175-2.600)	5 167	5.364	0.021	3.062 (1.137-8.247)	0.928
		(n=172)										
	Females	Cases	51 48					5 94				
		(n=99)										
		Controls	81 104	1.550	0.213	0.421	1.364 (0.836-2.226)	10 175	0.016	0.899	0.931 (0.309-2.803)	0.051
		(n=185)										
Males	Cases	86 128					11 203					
	(n=214)											
	Controls	28 65	2.821	0.093	0.666	1.560 (0.927-2.625)	5 88	0.007	0.932	0.954 (0.322-2.826)	0.054	
	(n=93)											
Females	Cases	58 55					11 102					
	(n=113)											
		Controls	29 61	7.467	0.006	0.975	2.218 (1.247-3.945)	2 88	4.717	0.030	4.745 (1.024-21.989)	0.886
		(n=90)										

*P value less than or equal to 0.05 is in bold. NA-not applicable. 95%CI-95% confidence interval; OR-odds ratio

polymorphism and CHD in patients aged 55–65 years and those older than 65 years; thus suggested that aging is a robust risk factor for CHD (35).

Epidemiologic evidence has shown that men have higher cardiovascular disease morbidity and mortality rates than women (36). Among conventional risk factors, diabetes mellitus and hyperlipidemia affected women more, while smoking was found to affect men more (37). Along with the decreased secretion of testosterone and the accumulative effect of smoking and weight gain, middle-aged men have higher onset rates of CHD (38). Women with late menopause and women who used postmenopausal estrogens had a significantly lower risk of CHD than those with early menopause and those who never used estrogen; this finding provided evidence that estrogen is a protective factor against CHD (39). In the present study, further analysis by gender showed a significant association of age with TG levels only in males.

CHD is a dynamic inflammatory disease caused by atherosclerosis (40). BUD13-ZNF259 encodes ZPR1 that interacts with

tyrosine kinase receptors (17). A study has shown that the inhibition of spleen tyrosine kinase (Syk) attenuates atherogenesis in mice and reduces the atherogenesis inflammatory process and plaque development (41). The activation of Syk may play an important role in the development of atherosclerosis (42). Using Syk as a target to block the pathway of inflammasomes may be a new anti-atherosclerotic treatment (43). Although there is a lack of evidence supporting the relationship between ZPR1 and Syk, ZPR1 might be associated with atherosclerosis as well as CHD because of its interaction with tyrosine kinase receptors.

Study limitations

Our study had great power to detect a the relative association of the rs964184 polymorphism with CHD risk among Han Chinese. However, the current study has some limitations. First, patients who had no significant vasoconstriction seen during their angiography (<50%) were considered as controls. Their degree of vasoconstriction might be reduced due to a spontaneous re-

channelization. Therefore, healthy controls should be included in future studies. Second, gene–gene and gene–disease interactions should be considered. Third, only one SNP was checked in terms of its association with CHD. Other genetic polymorphisms in selected genes may be functional markers for the risk of CHD.

Conclusions

Our results indicate that both gender and age have a great impact on the association of the rs964184 polymorphism with CHD among Han Chinese. Specifically, the rs964184 polymorphism may increase 70.6% of CHD risk in males between 55 and 65 years, and 111.2% of CHD risk in females older than 65. This gender–age interaction in the association of the BUD13-ZNF259 rs964184 polymorphism with CHD may be worth being validated in other populations.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

Authorship contributions: Concept – X.C.; Design – S.D.; Supervision – H.Y., H.J.; Fundings – S.D., Y.H.; Materials – X.X., N.W.; Data collection &/or processing – X.X., N.W., D.J., H.J., X.C., D.D.; Analysis &/or interpretation – L.H., L.X.; Literature search – Y.H.; Writing – Y.L.; Critical review – X.C.

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