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Original Article

B-cell lymphoma 2 rs17757541 C>G polymorphism was associated with an increased risk of coronary artery disease in a Chinese population

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Abstract: The goal of our study was to evaluate the genetic effects of sixteen single nucleotide polymorphisms (SNPs) in apoptosis-related genes on the development of coronary artery disease (CAD) through a case-control study. A total of 1979 individuals, including 826 CAD cases (aged 67.27±10.26 years) and 1153 non-CAD controls (aged 59.13±10.51 years), were enrolled into the study. The genotypes were determined using a custom-by-design 48-Plex SNPscanTM Kit. The results showed that the *BCL2* rs17757541 C>G polymorphism was associated with increased risk of CAD in homozygote comparison and recessive genetic model. However, no association between the other fifteen SNPs and CAD risk was observed. Stratified analyses indicated a significantly increased risk of CAD associated with the *BCL2* rs17757541 C>G polymorphism among males and younger patients. Therefore, the results indicated that there is a close correlation between the *BCL2* rs17757541 C>G polymorphism and CAD, which suggests that this SNP site should be further studied as a potential biomarker for CAD.

Keywords: BCL2, polymorphism, coronary artery disease, molecular epidemiology, apoptosis

Introduction

Coronary artery disease (CAD) continues to be a leading cause of morbidity and mortality among adults globally and represents a public health challenge in both industrialized and developing countries [1, 2]. CAD is a common complex disease which results from the interplay of genetic and environmental factors. Previous epidemiological studies and clinical trials provided evidence that modification of traditional risk factors for CAD, including diabetes mellitus, smoking and arterial hypertension, would lead to 30% to 40% reduction in clinical events such as myocardial infarction, ischemic heart failure and death [2]. Moreover, apart from common risk factor, population-based studies have repeatedly reported that genetic susceptibility account for around 50% of the risk for CAD, suggesting that the host genetic variants play an important role in the occurrence and development of CAD as well [2, 3].

Apoptosis, a genetically encoded cell death program, is essential for embryonic development, tissue homeostasis, and immune system maintenance in all metazoans [4]. Previous studies have demonstrated that apoptotic cell death involves in the development of atherosclerosis and subsequent plaque rupture, indicating an important role for apoptosis in the pathophysiology of atherosclerosis [5-7]. This programmed cell death is executed by multiple genes, such as *Caspase8* (*CASP8*), *Caspase7* (*CASP7*), *FAS, FAS ligand* (*FASL*)*/FASLG,* tumor protein 53-binding protein 1 (*TP53BP1*), B-cell lymphoma 2 (*BCL2*), erythroblastic leukemia viral oneogene homolog 2 (*ERBB2*), vascular endothelial growth factor receptor 2 (*VEGFR2*)*/KDR* and *C1orf10/Cornulin* (*CRNN*). Caspase-7 is a member of the cysteine-aspartic acid protease family that promulgate diverse biological responses, including programmed cell death and inflammation [8]. Caspase-8 was recognized as an initiator CASP in extrinsic cell-death pathway [9]. CASP8 triggers apoptosis is caused mainly

by death receptor-induced apoptotic signaling and mediated by FAS and FASL [10, 11]. The FASL-FAS system mainly forms the death-inducing signaling complex, plays a role in T-cell-mediated cytotoxicity, apoptosis induction in activated lymphocytes [12, 13]. Serum soluble Fas ligand (sFasL) level is associated with angiographically more severe CAD and considered as a biochemical surrogate of severe coronary atherosclerosis [14]. Apoptosis associated with TP53BP1 exerts a critical effect on both DNA repair and cell cycle control. The TP53BP1 could cooperate with damage sensors and signal transducers to mediate the DNA damage checkpoint [15]. The *BCL2* gene is implicated in prolonged cell survival and it plays a protective effect against apoptosis of macrophage and SMCs in advanced atherosclerotic lesions [16, 17]. ERBB2, a member of the ErbB family of membrane-bound receptor tyrosine kinases, is able to promote cell proliferation and suppress apoptosis which is associated with the atherosclerosis [18]. VEGFR2/KDR can bind to VEGF, stimulating endothelial cell proliferation, maturation of vascular structures, migration and apoptosis inhibition [19]. *C1orf10/CRNN* gene, located on 1q21, was identified as one of the down-regulated genes in esophageal squamous cell carcinoma [20].

Considering the biological and pathological significance of these apoptosis-related genes, we conduct a case-control study to investigate genetic associations of the functional polymorphisms *CASP8* rs1035142 G>T, *CASP7* rs3127075 G>C, *CASP7* rs7907519 C>A, *FAS* rs2234767 G>A, *FASL/FASLG* rs763110 C>T, *TP53BP1* rs560191 G>C, *BCL2* rs17757541 C>G, *BCL2* rs12454712 T>C, *ERBB2* rs1136201 A>G, *VEGFR2/KDR* rs11941492 C>T and six *C1orf10/CRNN* variants to coronary atherosclerosis, and further evaluate the potential value of *Caspases, FAS, FASL/FASLG, TP53BP1, BCL2, ERBB2, VEGFR2/KDR* and *C1orf10/CRNN* genotypes in refined CAD risk assessment.

Materials and methods

Study subjects

This case-control study protocol was reviewed and approved by Ethical Committee of ZhongDa Hospital Affiliated to Southeast University and the clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki. After given the written informed consent to participate, all the subjects were interviewed to collect information on demographic data.

A total of 826 subjects with CAD [males: 592 (71.67%); age: 67.27±10.26 years] were consecutively recruited from Zhongda Hospital Affiliated to Southeast University (Nanjing, China) from July 2005 to December 2008. 1153 controls [males: 510 (44.23%); age: 59.13±10.51 years] were non-CAD subjects recruited from Zhenjiang who participated the physical examination, China. All patients underwent coronary angiography (CAG) and the results were judged by two or three experienced cardiologists. CAD cases were patients having the presence of at least one significant coronary artery stenosis of ≥ 50% luminal diameter on coronary angiography. Non-CAD controls had no symptoms of angina or possible myocardial infarction, and had no history of CAD. Those with congenital heart disease, cardiomyopathy, severe liver or kidney disease, and malignant tumors were excluded from this study.

Single-nucleotide polymorphism genotyping

Blood samples were collected from each subject using vacutainers with ethylenediamine tetra-acetic acid (EDTA). Genomic DNA was extracted from whole blood using the Qiagen DNA Blood Mini Kit (Qiagen, Berlin, Germany). SNPs genotyping were performed utilizing a custom-by-design 48-Plex SNPscanTM Kit (Genesky Biotechnologies Inc., Shanghai, China) as previously described [21]. Repeated analyses were conducted for 4% of randomly selected samples with high DNA quality.

Statistical analyses

All quantitative variables are reported as mean ± standard deviation (SD), and the categorical variables are presented as absolute frequencies or percentages. The differences of the demographic characteristics between the cases and controls were evaluated using the χ^2 test (for categorical variables) and Student's t test (for quantitative variables). The associations of sixteen SNPs genotypes with CAD risk were assessed by calculating odds ratios (ORs) and their 95% confidence intervals (CIs) utiliz-

Table 1. Primary information for sixteen Genotyped SNPs

Genotyped SNPs	Chr[a]	Regulome DB Score[b]	Location	TFBS[c]	Functional Consequence	MAF[d] for Chinese in database	MAF in our controls (n = 826)	*P*-value for HWE[e] test in our controls	Genotyping value (%)
CASP8: rs1035142 G>T	2	No data	3'-Flanking	-	downstream variant 500 B	0.279	0.283	0.612	97.3
CASP7: rs3127075 G>C	10	No data	Intron2	-	intron variant	0.140	0.180	0.946	98.9
CASP7: rs7907519 C>A	10	4	5'-UTR_intron1	-	intron variant	0.289	0.278	0.977	99.5
FAS: rs2234767 G>A	10	4	5'-Flanking	Y	upstream variant 2 KB	0.358	0.335	0.977	98.7
FASL/FASLG: rs763110 C>T	1	2a	5'-Flanking	Y	upstream variant 2 KB	0.298	0.255	0.245	99.3
TP53BP1: rs560191 G>C	15	1f	nonsynon_exon9	-	missense	0.444	0.431	0.003	97.7
BCL2: rs17757541 C>G	18	6	Intron3	-	intron variant	0.134	0.149	0.583	99.3
BCL2: rs12454712 T>C	18	2b	Intron3	-	intron variant	0.477	0.460	0.378	99.4
ERBB2: rs1136201 A>G	17	4	nonsynon_exon17	-	missense	0.221	0.121	0.678	97.4
VEGFR2/KDR: rs11941492 C>T	4	No data	Intron8	-	intron variant	0.314	0.327	0.615	99.4
C1orf10/CRNN: rs3753443 C>T	1	No data	5'-Flanking	Y	upstream variant 2 KB	0.456	0.435	0.853	98.0
C1orf10/CRNN: rs3753444 C>G	1	6	5'-Flanking	Y	upstream variant 2 KB	0.456	0.436	0.944	99.5
C1orf10/CRNN: rs3753446 C>A	1	No data	5'-Flanking	Y	upstream variant 2 KB	0.467	0.458	0.700	99.2
C1orf10/CRNN: rs3829868 C>T	1	No data	nonsynon_exon3	-	missense	0.456	0.437	0.886	99.1
C1orf10/CRNN: rs4285700 C>A	1	6	5'-Flanking	Y	upstream variant 2 KB	0.453	0.436	0.960	99.5
C1orf10/CRNN: rs10888486 C>T	1	No data	3'-UTR_exon3	-	utr variant 3 prime	0.453	0.437	0.951	98.8

[a]Chr, chromosome; [b]http://www.regulomedb.org/; [c]TFBS, transcription factor binding site (http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm); [d]MAF, minor allele frequency, *FAS* rs2234767 G>A and *C1orf10/CRNN* rs3753446 C>A MAF is in CHB+JPT population; [e]HWE, Hardy-Weinberg equilibrium.

Table 2. Main effects of SNPs on CAD risk

Genotyped SNPs	Genotyping		*P*	AB vs. AA OR (95% CI); *P*	BB vs. AA OR (95% CI); *P*
	Case (n = 826) (AA/AB/BB)	Control (n = 1153) (AA/AB/BB)			
CASP8: rs1035142 G>T	438/312/71	585/452/94	0.680	0.92 (0.76-1.11); 0.402	1.01 (0.72-1.41); 0.959
CASP7: rs3127075 G>C	548/250/24	764/334/37	0.834	1.04 (0.86-1.27); 0.672	0.90 (0.53-1.53); 0.707
CASP7: rs7907519 C>A	423/341/62	596/459/88	0.881	1.05 (0.87-1.26); 0.633	0.99 (0.70-1.41); 0.967
FAS: rs2234767 G>A	367/363/88	502/506/128	0.922	0.98 (0.81-1.19); 0.846	0.94 (0.69-1.27); 0.691
FASL/FASLG: rs763110 C>T	456/317/51	625/449/67	0.903	0.97 (0.80-1.17); 0.732	1.04 (0.71-1.53); 0.829
TP53BP1: rs560191 G>C	265/392/151	389/503/233	0.237	1.14 (0.93-1.40); 0.197	0.95 (0.74-1.23); 0.704
BCL2: rs17757541 C>G	575/220/31	823/294/23	0.054	1.07 (0.87-1.31); 0.511	**1.93 (1.11-3.34); 0.019**
BCL2: rs12454712 T>C	255/421/149	341/553/249	0.126	1.02 (0.83-1.25); 0.865	0.80 (0.62-1.04); 0.093
ERBB2: rs1136201 A>G	632/154/13	873/237/18	0.647	0.90 (0.72-1.13); 0.351	1.00 (0.49-2.05); 0.995
VEGFR2/KDR: rs11941492 C>T	393/344/89	520/495/126	0.675	0.92 (0.76-1.11); 0.387	0.93 (0.69-1.26); 0.660
C1orf10/CRNN: rs3753443 C>T	262/403/147	361/551/215	0.861	1.01 (0.82-1.24); 0.941	0.94 (0.72-1.23); 0.657
C1orf10/CRNN: rs3753444 C>G	265/412/149	364/561/218	0.842	1.01 (0.82-1.24); 0.933	0.94 (0.72-1.22); 0.636
C1orf10/CRNN: rs3753446 C>A	232/423/169	338/559/242	0.609	1.10 (0.89-1.36); 0.361	1.02 (0.79-1.32); 0.896
C1orf10/CRNN: rs3829868 C>T	262/413/149	362/557/218	0.807	1.02 (0.84-1.26); 0.816	0.94 (0.73-1.23); 0.669
C1orf10/CRNN: rs4285700 C>A	265/412/149	363/563/217	0.867	1.00 (0.82-1.23); 0.981	0.94 (0.72-1.22); 0.646
C1orf10/CRNN: rs10888486 C>T	261/409/147	361/559/218	0.804	1.01 (0.83-1.24); 0.909	0.93 (0.72-1.21); 0.603

AA/AB/BB means homozygote, heterozygote and mutated homozygote; Bold values are statistically significant (P < 0.05).

ing logistic regression analyses for crude ORs and adjusted ORs when adjusting for age and sex. All the sixteen SNPs were tested for conformity to Hardy-Weinberg expectations by a goodness-of-fit χ^2 test among controls subjects. All statistical tests were considered significant at *P* value < 0.05, and data were analyzed using STATA 12.0 software (StataCorp, College Station, TX, USA).

Results

Characteristics of the study population and sixteen SNPs

Mean age of CAD patients was higher than that of control, and the proportion of female in the CAD group was lower than in the control group (all P < 0.05). Primary information for sixteen Genotyped SNPs is listed in **Table 1**. The genotyping success rate of overall SNP was high, ranging from 97.3% to 99.5%. Among 1979 DNA samples (826 CAD cases and 1153 controls), *BCL2* rs17757541 C>G was successful in 826 (100%) CAD cases and 1140 (98.9%) controls. The concordance rates of repeated analyses were 100%. Minor allele frequency (MAF) of all sixteen SNPs in our controls was similar to MAF for Chinese in database. Genotype distributions of the sixteen SNPs in our control subjects were in accordance with the Hardy-Weinberg equilibrium except *TP53BP1* rs560191 G>C polymorphism.

Associations between sixteen polymorphisms and risk of CAD

The genotype distributions of *CASP8* rs1035142 G>T, *CASP7* rs3127075 G>C, *CASP7* rs7907519 C>A, *FAS* rs2234767 G>A, *FASL/FASLG* rs763110 C>T, *TP53BP1* rs560191 G>C, *BCL2* rs17757541 C>G, *BCL2* rs12454712 T>C, *ERBB2* rs1136201 A>G, *VEGFR2/KDR* rs11941492 C>T and six *C1orf10/CRNN* variants in the cases and the controls were presented in **Table 2**. No significant differences were found in genotype frequencies of sixteen SNPs between cases and controls (all P>0.05). Compared with the rs17757541 CC homozygote genotype, the GG genotype was associated with a significantly increased risk of CAD (OR = 1.93, 95% CI = 1.11-3.34, P = 0.019, **Table 2**), and the association was weakened but still statistically significant when adjusting for age and sex (OR = 1.87, 95% CI: 1.03-3.41, P = 0.041, **Table 3**). In the recessive model, when the *BCL2* rs17757541 CC/CG genotypes were used as the reference group, the GG homozygote genotype exhibited an increased risk for CAD (adjusted OR = 1.85, 95% CI = 1.02-3.36, P = 0.044, **Table 3**). However, logistic regression analyses demonstrated no significant association of the *CASP8* rs1035142 G>T, *CASP7* rs3127075 G>C, *CASP7* rs7907519 C>A, *FAS* rs2234767 G>A, *FASL/FASLG* rs763110 C>T, *TP53BP1* rs560191 G>C, *BCL2* rs12454712 T>C, *ERBB2* rs1136201 A>G,

Table 3. Logistic regression analyses of associations between *BCL2* rs17757541 C>G polymorphism and risk of CAD

Genotype	Cases (n = 826)[a]		Controls (n = 1153)		Crude OR (95% CI)	*P*	Adjusted OR[b] (95% CI)	*P*
	N	%	n	%				
BCL2: rs17757541 C>G								
CC	575	69.61	823	72.19	1.00		1.00	
CG	220	26.63	294	25.79	1.07 (0.87-1.31)	0.511	1.05 (0.83-1.31)	0.705
GG	31	3.75	23	2.02	**1.93 (1.11-3.34)**	**0.019**	**1.87 (1.03-3.41)**	**0.041**
CG+GG	251	30.39	317	27.81	1.13 (0.93-1.38)	0.213	1.11 (0.89-1.38)	0.362
CC+CG	795	96.25	1,117	97.98	1.00		1.00	
GG	31	3.75	23	2.02	**1.89 (1.10-3.27)**	**0.022**	**1.85 (1.02-3.36)**	**0.044**
G allele	282	0.17	340	0.15				

[a]The genotyping was successful in 826 (100%) CAD cases, and 1140 (98.9%) controls for *BCL2* rs17757541 C>G; [b]Adjusted for age and sex. Bold values are statistically significant ($P < 0.05$).

Table 4. Stratified analyses between *BCL2* rs17757541 C>G polymorphism and CAD risk by sex and age

Variable	*BCL2 rs17757541 C>G* (case/control)[a]				Adjusted OR[b] (95% CI); *P*				
	CC	CG	GG	CG+GG	CC	CG	GG	CG+GG	GG vs. (CG+CC)
Sex									
Male	410/363	156/132	26/10	182/142	1.00	1.05 (0.80-1.37); *P*: 0.744	**2.30 (1.10-4.84); *P*: 0.028**	1.13 (0.87-1.47); *P*: 0.342	**2.27 (1.09-4.76); *P*: 0.029**
Female	163/460	64/162	5/13	69/175	1.00	1.11 (0.79-1.57); *P*: 0.531	1.09 (0.38-3.09); *P*: 0.878	1.11 (0.80-1.55); *P*: 0.527	1.05 (0.37-2.99); *P*: 0.921
Age									
< 60	121/410	48/145	8/10	56/155	1.00	1.12 (0.76-1.65); *P*: 0.558	**2.71 (1.05-7.02); *p*: 0.040**	1.22 (0.85-1.77); *P*: 0.28	**2.63 (1.02-6.76); *P*: 0.045**
≥ 60	454/413	172/149	23/13	195/162	1.00	1.05 (0.81-1.36); *P*: 0.709	1.61 (0.80-3.22); *P*: 0.178	1.09 (0.86-1.40); *P*: 0.472	1.59 (0.80-3.17); *P*: 0.188

[a]The genotyping was successful in 826 (100%) CAD cases, and 1140 (98.9%) controls for *BCL2* rs17757541 C>G; [b]Adjusted for age and sex (besides stratified factors accordingly) in a logistic regression model. Bold values are statistically significant ($P < 0.05$).

V*EGFR2/KDR* rs11941492 C>T and six *C1-orf10/CRNN* polymorphisms with CAD risk.

Stratification analyses of BCL2 rs17757541 C>G polymorphism and risk of CAD

We further evaluated the influence of *BCL2* rs17757541 C>G genotypes on CAD risk after stratifying the participants by age and sex. The analysis stratified by sex showed that the increased risk of CAD was evident among male subjects carrying GG genotype (OR = 2.30, 95% CI = 1.10-4.84, P = 0.028), and the similar effects were also shown in the recessive model (OR = 2.27, 95% CI = 1.09-4.76, P = 0.029). When stratified by age (≤ 60 or > 60 years old), younger subjects (≤ 60 years old) carrying GG genotype had a higher susceptibility to CAD than those with the CC genotype (OR = 2.71, 95% CI = 1.05-7.02, P = 0.040) or the combined CG/CC genotypes (OR = 2.63, 95% CI = 1.02-6.76, P = 0.045). Whereas no significant association of *BCL2* rs17757541 C>G polymorphism with CAD risk was observed in female subgroup or the group older than 60 years old (**Table 4**).

Discussion

A large number of studies on genetic risk factors predisposing to CAD have been published recently, such as *MTHFR, endothelial nitric oxide synthase (NOS3) gene*, but apoptosis-related genes are much less well studied [22, 23]. In this study, we determined the association between functional polymorphisms *CASP8* rs1035142 G>T, *CASP7* rs3127075 G>C, *CASP7* rs7907519 C>A, *FAS* rs2234767 G>A, *FASL/FASLG* rs763110 C>T, *TP53BP1* rs560191 G>C, *BCL2* rs17757541 C>G, *BCL2*

rs12454712 T>C, *ERBB2* rs1136201 A>G, *VEGFR2/KDR* rs11941492 C>T and six *C1orf10/CRNN* variants and the risk of CAD in a Chinese population. Our data revealed important genetic contribution of the *BCL2* rs17757541 C>G polymorphism to the development of CAD, particularly among men and young subjects, whereas no significant association between the other fifteen SNPs and CAD risk was found.

Apoptosis has been accepted as an important mechanism in the pathophysiology of atherosclerosis, in addition to other human pathologies including cancer and diabetes [24]. Previous investigations have shown that endothelial cell apoptosis may contribute to the initiation of atherogenesis, whereas apoptosis of macrophages and VSMCs is significantly increased at sites of plaque rupture, and *BCL2* was demonstrated to be involved in these processes [5, 6, 16]. The *BCL2* gene, originally discovered in a follicular B cell lymphoma, is located on chromosome 18q21.3 and consists of 3 exons and 2 promoters [25]. *BCL2* localized in the mitochondrial membrane and prevented the apoptosis-associated release of cytochrome *c*, blocking the cytochrome *c*-mediated caspase-9-dependent apoptosis events [6, 26, 27]. To date, a large number of studies have investigated the contributions of *BCL2* SNPs to the susceptibility of different cancer types [16, 28, 29]. Recently, the rs17757541 C>G polymorphism was identified to be associated with an increased risk of gastric cardiac adenocarcinoma [25]. In this study, we found subjects with the *BCL2* rs17757541 GG variant genotype had a 0.93-fold increased risk of CAD compared to those with CC genotype. The phenomenon may be related to the SNP rs17757541-G allele which modulates the activity of *BCL2*. However, further investigations are warranted to clarify the possible mechanisms of *BCL2* rs17757541 C>G polymorphism.

The frequencies of genetic polymorphisms often vary greatly depending on ethnic background. HapMap International Project has shown large racial differences in rs17757541-G allele frequency between the Sub-Saharan African population (0.000) and the rest populations, including Asians (0.093-0.167) and European (0.158-0.174). In the present Chinese study, the frequency of rs17757541-G allele was 0.149 among 1153 controls, which was similar to that reported in Chinese Han population (0.134) in PubMed SNP Database (http://www.ncbi.nlm.nih.gov/snp).

Although this is the first study to our knowledge to describe the *BCL2* rs17757541 C>G polymorphism and the risk for CAD with a relatively large sample size and a high statistical power (considering *BCL2* rs17757541 C>G mutant alleles in the control group, OR, CAD samples and control samples, the power of our analysis ($\alpha = 0.05$) was 0.9997 in 826 CAD cases and 1153 controls with adjusted OR 1.87 for *BCL2* rs17757541 C>G), however, several limitations should be taken into consideration. Firstly, the controls enrolled from hospitals were inpatients that received QCA and did not get CAD. Thus, selection bias was inevitable because the participants were not fully representative of the underlying source populations. Secondly, the adjusted ORs were calculated based on only adjusting for age and sex due to the lack of original data. Thus, if biochemical indicators (such as HDL or triglyceride) were available to allow for adjustment, a more precise analysis could be performed. Thirdly, the polymorphisms of genes investigated in this study were chosen as candidates based on their functional considerations, and may not give a broad, comprehensive view of genetic variability of *BCL2*. Further fine-mapping studies in the susceptible region of the variants are needed.

In conclusion, our study firstly revealed that the *BCL2* rs17757541 GG variant genotype was significantly associated with an increased risk of CAD in a Chinese population, suggesting potential implications for genotyping the *BCL2* rs17757541 C>G polymorphism in CAD risk appraisal. However, the exact functional relevance of the *BCL2* rs17757541 SNP remains elusive. Due to the limitations mentioned above, replication in other populations and further well-design studies are needed to confirm the general validity of our findings and to clarify the molecular pathways of this association.

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Disclosure of conflict of interest

None.

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