Original Article
Association between *APOE* gene and myocardial bridge in Chinese Han population: a case-control study

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Abstract: Studies found that *APOE* gene play an important role in the development of cardiovascular disease. To investigate the genetic association between myocardial bridge (MB) and *APOE* gene, we conducted this case-control study and genotyped 5 SNPs using ligase detection reaction in 419 patients and 504 controls. The results indicated that, in total group, rs405509, rs769450, rs439401, rs7259004 and rs2075650 showed no significant association with myocardial bridge. The *p* value are all more than 0.05 and the OR [95% CI] value all included "1", both in the allele analysis and in the genotype analysis. In both the female-subgroup and male-subgroup, when subdivided by genders, these 5 SNPs showed no significant association with myocardial bridge. Linkage disequilibrium (expressed in D' and r^2) for *APOE* in the Chinese cases and controls are demonstrated and according to the results of Linkage disequilibrium, the haplotype of the first 3 SNPs (rs405509, rs769450, rs439401) in the first block were analyzed. This is the first case-control association study on *APOE* gene and myocardial bridge. Considering the size of our sample sets (power > 90%), our results suggest that the *APOE* gene may not play a major role in myocardial bridge in the Chinese Han population.

Keywords: Myocardial bridge, *APOE*, association study, SNP

Introduction

The coronary arteries usually run an epicardial course and are surrounded by coronary perivascular adipose tissue. If the original trabecular artery network fails to move outward in the coronary artery development process, any one segment of the coronary artery or its branch is covered by cardiac muscle fiber. This segment of vessel is called a mural coronary artery (MCA), while the bridge-like myocardial fiber bundle covering the artery is called a myocardial bridge (MB) [1]. Myocardial bridge that partially covers the coronary artery is a congenital anatomical variant and almost exclusively present in the left anterior descending coronary artery (LAD) [2]. In 1737, it was first mentioned by Rayman. In the early 1920s, MB was first described by Grainicianu. The current gold standard for diagnosing MB is coronary angiography with the typical systolic compression of the epicardial coronary vessel (milking effect). MB usually locates in the middle segment of

the left anterior descending (LAD) coronary artery [3, 4]. The reported prevalence of MBs varies between 5% and 86% [5-7].

There are reports of pathological observations indicating that the virtual absence of atheromatous changes in the tunneled coronary segments while affecting the pre-bridged segments of the coronary [8, 9]. It is unclear whether the structure of the myocardial bridge, the structural relationship between the overlying myocardium and the tunneled coronary vessel may determine which bridge will compress the coronary artery. For clinical and medical-legal practice the most important issues are to identify significant hemodynamic bridging, its consequences, any associated pathologies, and if this is the case, the identification of a causal link between MB/its consequences and death [10]. MB leads to a decreased blood flow through the coronary artery distal to the tunneled area, although this decrease has minor consequences under normal circumstances

Table 1. Allele and genotype frequency of the 5 loci in total group

	SNP ID	Position[a]	MAF	Alleles		OR (95% CI)	*P*-value	Genotypes			HWe P[b]	*P*-value
						Allele and genotype frequency of the 5 loci						
1	Rs405509	45408836	T = 0.4927	A (freq)	C (freq)			A/A (freq)	A/C (freq)	C/C (freq)		
	Case			585 (0.712)	237 (0.288)	1.031823 [0.840572~1.266589]	0.764544	209 (0.509)	167 (0.406)	35 (0.085)		0.786919
	Control			677 (0.705)	283 (0.295)			244 (0.508)	189 (0.394)	47 (0.098)	0.245706	
2	Rs769450	45410444	A = 0.326	A (freq)	G (freq)			A/A (freq)	A/G (freq)	G/G (freq)		
	Case			168 (0.204)	654 (0.796)	0.988602 [0.784993~1.245022]	0.922363	18 (0.044)	132 (0.321)	261 (0.635)		0.989273
	Control			198 (0.206)	762 (0.794)			22 (0.046)	154 (0.321)	304 (0.633)	0.659334	
3	Rs439401	45414451	T = 0.3942	C (freq)	T (freq)			C/C (freq)	C/T (freq)	T/T (freq)		
	Case			327 (0.398)	495 (0.602)	0.965501 [0.798433~1.167528]	0.717232	63 (0.153)	201 (0.489)	147 (0.358)		0.684531
	Control			390 (0.406)	570 (0.594)			83 (0.173)	224 (0.467)	173 (0.360)	0.474305	
4	Rs7259004	45432557	C = 0.1996	C (freq)	G (freq)			C/C (freq)	C/G (freq)	G/G (freq)		
	Case			186 (0.226)	636 (0.774)	1.031862 [0.825198~1.290282]	0.783265	28 (0.068)	130 (0.316)	253 (0.616)		0.529603
	Control			212 (0.221)	748 (0.779)			25 (0.052)	162 (0.338)	293 (0.610)	0.672885	
5	Rs2075650	45395619	G = 0.1337	A (freq)	G (freq)			A/A (freq)	A/G (freq)	G/G (freq)		
	Case			760 (0.925)	62 (0.075)	0.993897 [0.698202~1.414822]	0.972883	352 (0.856)	56 (0.136)	3 (0.007)		0.980824
	Control			888 (0.925)	72 (0.075)			411 (0.856)	66 (0.138)	3 (0.006)	0.843519	

[a]Based on HapMap database release #21. [b]Deviated from Hardy-Weinberg equilibrium.

Table 2. Allele and genotype frequency of the 5 loci in female group

	SNP ID	Position[a]	MAF	Alleles		OR (95% CI)	*P*-value	Genotypes			HWe P[b]	*P*-value
						Allele and genotype frequency of the 5 loci						
1	Rs405509	45408836	T = 0.4927	A (freq)	C (freq)			A/A (freq)	A/C (freq)	C/C (freq)		
	Case			233 (0.728)	87 (0.272)	1.060333 [0.754350~1.490431]	0.735934	83 (0.519)	67 (0.419)	10 (0.062)		0.938570
	Control			245 (0.716)	97 (0.284)			86 (0.503)	73 (0.427)	12 (0.070)	0.508726	
2	Rs769450	45410444	A = 0.326	A (freq)	G (freq)			A/A (freq)	A/G (freq)	G/G (freq)		
	Case			58 (0.181)	262 (0.819)	0.860196 [0.584052~1.266903]	0.445647	7 (0.044)	44 (0.275)	109 (0.681)		0.696888
	Control			70 (0.205)	272 (0.795)			8 (0.047)	54 (0.316)	109 (0.637)	0.694446	
3	Rs439401	45414451	T = 0.3942	C (freq)	T (freq)			C/C (freq)	C/T (freq)	T/T (freq)		
	Case			125 (0.391)	195 (0.609)	0.936174 [0.685646~1.278243]	0.678093	24 (0.150)	77 (0.481)	59 (0.369)		0.886475
	Control			139 (0.406)	203 (0.594)			29 (0.170)	81 (0.474)	61 (0.357)	0.811357	
4	Rs7259004	45432557	C = 0.1996	C (freq)	G (freq)			C/C (freq)	C/G (freq)	G/G (freq)		
	Case			72 (0.225)	248 (0.775)	1.016129 [0.704804~1.464971]	0.931665	10 (0.062)	52 (0.325)	98 (0.613)		0.908111
	Control			76 (0.222)	266 (0.778)			9 (0.053)	58 (0.339)	104 (0.608)	0.805830	
5	Rs2075650	45395619	G = 0.1337	A (freq)	G (freq)			A/A (freq)	A/G (freq)	G/G (freq)		
	Case			293 (0.916)	27 (0.084)	0.967681 [0.557126~1.680781]	0.907131	134 (0.838)	25 (0.156)	1 (0.006)		0.809636
	Control			314 (0.918)	28 (0.082)			145 (0.848)	24 (0.140)	2 (0.012)	0.385060	

[a]Based on Hap Map database release #21. [b]Deviated from Hardy-Weinberg equilibrium.

[11]. However, it could lead to a series of morphological changes, both within the affected coronary artery and in the vascularized myocardium which, when associated with other cardiovascular pathologies and intrinsic or extrinsic factors, may increase the risk of sudden cardiac death (SCD) [11].

The apolipoprotein E gene (APOE) is located on the long arm of chromosome 19 and consists of four exons and three introns. It encodes a 299 amino acid glycoprotein which plays a key role in the lipid metabolism and participates in the transport of cholesterol [12, 13]. Previous studies have shown that APOE have important roles in the development a number of diseases such as Alzheimer's disease, atherosclerosis and coronary heart disease [14]. In order to evaluate the genetic association between *APOE* gene and MB, we conducted this case-control study. Actually, this is the first time to do such a kind of research, especially in Chinese Han Population.

Materials and methods

Patient and control subjects

The sample set consisted of 419 unrelated myocardial bridge patients and 504 normal controls of Chinese Han population recruited from the Department of Cardiology of Ruijin Hospital appended to Shanghai Jiao Tong University School of Medicine. All patients were diagnosed by senior physicians based on standard clinical, endoscopic, radiologic, and histological criteria. Controls were randomly selected from healthy persons under routine health screening. This study was approved by the Research Ethics Committee of Ruijin Hospital, Shanghai, China. And informed consents were obtained from all subjects before blood sampling.

Genotyping

Genomic DNA was isolated from EDTA peripheral blood using QIAamp blood extraction kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The genotyping of for *APOE* single nucleotide polymorphisms was carried out by the Shanghai Generay Biotech Co., Ltd. (http://www.generay.com.cn/) using allelic specific multiple ligase detection reactions according to the standard protocol.

All primers were designed using the genomic sequences in the GenBank (http://www.ncbi.nlm.nih.gov) and listed in Supplementary Table 1. To test the validity of this procedure, about 10% samples were then confirmed by direct DNA sequencing.

Statistical analysis

Hardy-Weinberg equilibrium testing (HWE), *P*-value computations [P > 0.05], in both of the healthy control and patient groups, the allele and genotype frequency analysis were all performed on SHEsis software (http://analysis.bio-x.cn) [15-17]. All tests were two-tailed and statistical significance was assumed at P < 0.05.

Results

The results of the association study were shown in **Tables 1**-**3**. Linkage disequilibrium (expressed in D' and r^2) for *APOE* in the Chinese cases and controls are demonstrated in **Figure 1**. According to the results of linkage disequilibrium, the haplotype of the first 3 SNPs in the first block was analyzed, and the results were shown in **Table 4**.

In **Table 1**, rs405509, rs769450, rs439401, rs7259004 and rs2075650 showed no significant association with myocardial bridge. The *P* value are all more than 0.05 and the OR [95% CI] value all included "1", both in the allele analysis and in the genotype analysis. In **Table 2**, it showed the female-subgroup results when subdivided by gender. These 5 SNPs showed no significant association with myocardial bridge. In **Table 3**, it showed the male-subgroup results when subdivided. Also as what showed above, these 5 SNPs showed no significant association with myocardial bridge. **Figure 1A-C**) showed that rs405509, rs769450 and rs439401 formed one hot block (LDs in the total group, in the female subgroup and in the male subgroup were all > 0.85). Based on **Figure 1**, the results of this hot block were showed in **Table 4**.

Discussion

Myocardial bridge is a common benign lesion on coronary angiography (CAG). It occurs when a band of cardiac muscle overlies an intramural segment of a coronary artery, the intramural

Table 3. Allele and genotype frequency of the 5 loci in male group

	SNP ID	Position[a]	MAF	Alleles		OR (95% CI)	*P*-value	Genotypes			HWe P[b]	*P*-value
						Allele and genotype frequency of the 5 loci						
1	rs405509	45408836	T = 0.4927	A (freq)	C (freq)			A/A (freq)	A/C (freq)	C/C (freq)		
	Case			352 (0.701)	150 (0.299)	1.021235 [0.790095~1.319993]	0.872477	126 (0.502)	100 (0.398)	25 (0.100)		0.986897
	Control			432 (0.697)	188 (0.303)			154 (0.497)	124 (0.400)	32 (0.103)	0.347208	
2	rs769450	45410444	A = 0.326	A (freq)	G (freq)			A/A (freq)	A/G (freq)	G/G (freq)		
	Case			110 (0.219)	392 (0.781)	1.078603 [0.809257~1.437596]	0.605694	11 (0.044)	88 (0.351)	152 (0.606)		0.872359
	Control			128 (0.206)	492 (0.794)			12 (0.039)	104 (0.335)	194 (0.626)	0.674148	
3	rs439401	45414451	T = 0.3942	C (freq)	T (freq)			C/C (freq)	C/T (freq)	T/T (freq)		
	Case			202 (0.402)	300 (0.598)	0.983280 [0.773626~1.249751]	0.890371	39 (0.155)	124 (0.494)	88 (0.351)		0.897341
	Control			252 (0.406)	368 (0.594)			52 (0.168)	148 (0.477)	110 (0.355)	0.852977	
4	rs7259004	45432557	C = 0.1996	C (freq)	G (freq)			C/C (freq)	C/G (freq)	G/G (freq)		
	Case			113 (0.225)	389 (0.775)	1.033797 [0.779032~1.371878]	0.817885	18 (0.072)	77 (0.307)	156 (0.622)		0.523182
	Control			136 (0.219)	484 (0.781)			16 (0.052)	104 (0.335)	190 (0.613)	0.719235	
5	rs2075650	45395619	G = 0.1337	A (freq)	G (freq)			A/A (freq)	A/G (freq)	G/G (freq)		
	Case			467 (0.930)	35 (0.070)	1.019246 [0.643178~1.615201]	0.935297	218 (0.869)	31 (0.124)	2 (0.008)		0.689138
	Control			576 (0.929)	44 (0.071)			267 (0.861)	42 (0.135)	1 (0.003)	0.628749	

[a]Based on Hap Map database release #21. [b]Deviated from Hardy-Weinberg equilibrium.
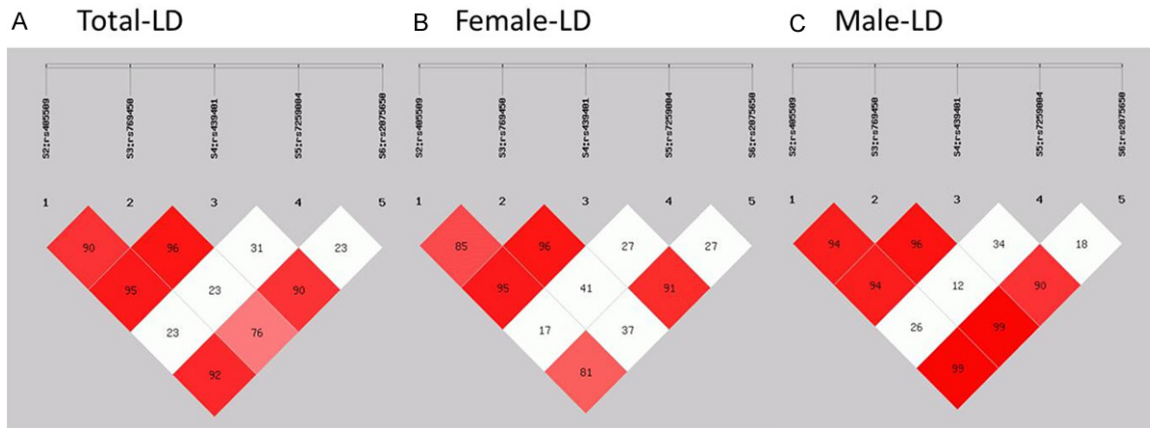
Figure 1. LD analyses in total group, female subgroup and male subgroup.

Table 4. Haplotype analyses in total group, female subgroup and male subgroup

	Case (freq)	Control (freq)	Chi2	Fisher's p	Person's p	Odds Ratio [95% CI]
Haplotype analysis-total						
Loci chosen for hap-analysis: rs405509, rs769450, rs439401						
A A C	12.09 (0.015)	9.64 (0.010)	-	-	-	-
A A T	1.23 (0.001)	1.23 (0.001)	-	-	-	-
A G C	86.98 (0.106)	102.97 (0.107)	0.001	0.981010	0.981000	0.996 [0.736-1.348]
A G T	484.71 (0.590)	563.16 (0.587)	0.156	0.692641	0.692641	1.040 [0.858-1.260]
C A C	151.82 (0.185)	183.18 (0.191)	0.051	0.821554	0.821536	0.973 [0.766-1.236]
C A T	2.86 (0.003)	3.95 (0.004)	-	-	-	-
C G C	76.11 (0.093)	94.22 (0.098)	0.107	0.743229	0.743223	0.948 [0.690-1.303]
C G T	6.21 (0.008)	1.65 (0.002)	-	-	-	-
Haplotype analysis-female						
Loci chosen for hap-analysis: rs405509, rs769450, rs439401						
A A C	7.96 (0.025)	5.70 (0.017)	-	-	-	-
A G C	31.19 (0.097)	39.75 (0.116)	0.597	0.439837	0.439808	0.822 [0.500-1.351]
A G T	193.85 (0.606)	199.56 (0.583)	0.39	0.532456	0.532448	1.106 [0.806-1.520]
C A C	50.04 (0.156)	62.05 (0.181)	0.724	0.394842	0.394801	0.837 [0.556-1.261]
C A T	0.00 (0.000)	2.25 (0.007)	-	-	-	-
C G C	35.81 (0.112)	31.51 (0.092)	0.723	0.395072	0.395031	1.245 [0.751-2.065]
C G T	1.15 (0.004)	1.19 (0.003)	-	-	-	-
Haplotype analysis-male						
Loci chosen for hap-analysis: rs405509, rs769450, rs439401						
A A C	4.11 (0.008)	4.25 (0.007)	-	-	-	-
A G C	55.80 (0.111)	61.98 (0.100)	0.495	0.481832	0.481814	1.147 [0.782-1.683]
A G T	290.73 (0.579)	365.77 (0.590)	0.002	0.968507	0.968491	0.995 [0.781-1.268]
C A C	101.67 (0.203)	121.73 (0.196)	0.157	0.691574	0.691573	1.062 [0.790-1.426]
C A T	2.86 (0.006)	2.02 (0.003)	-	-	-	-
C G C	40.41 (0.081)	64.04 (0.103)	1.489	0.222399	0.222321	0.744 [0.512-1.169]
C G T	5.06 (0.010)	0.20 (0.000)	-	-	-	-
A A T	1.36 (0.003)	0.00 (0.000)	-	-	-	-

segment being referred to as a "tunneled" artery. Studies indicated that MB may cause angina pectoris (AP), myocardial fibrosis, myocardial infarction (MI), life-threatening arrhythmia and even sudden cardiac death [18-22]. It is widely accepted that the proximal portion is prone to an increased happening of atherosclerosis, while the intramural and distal portions of a bridged artery usually remain free from atherosclerotic disease [23].

In the present study, we aimed to investigate the genetic association between *APOE* gene and myocardial bridge. *APOE* gene has been widely known to have an important role in the development of cardiovascular diseases such as coronary atherosclerosis. More recently, CARDIoGRAMplusC4D Consortium et al. have done a GWAS study finding that APOs, for example, rs2075650 in the region of *ApoE-ApoC1,* located in the chromosome 19, have significant relationship with the incidence of coronary artery disease [24]. In their study, the *P* value of this SNP was 5.86×10^{-11}, indicating that *ApoE-ApoC1* may have a significant association with the happening of coronary artery disease.

In this study, we investigate 5 important SNPs of *APOE* gene between myocardial bridge patients and normal controls. Not only the total patient-control group but also the subgroups divided by genders were analyzed. Also, we analyzed the allele frequencies, the genotype frequencies, the LD values, and the haplotype frequencies based on LD. The results showed that there was no significant association between this gene and myocardial bridge.

In conclusion, this is the first time to do such a genetic case-control association study on *APOE* gene and myocardial bridge. Considering the size of our sample sets (power > 90%), our study is an important "the compass" for the researchers to do following study.

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Disclosure of conflict of interest

None.

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References

[1] Niu YJ, Zhang XL, Cao AD, Leng B. Clinical value of the correlations of mural coronary artery compression extent with myocardial bridge length and thickness evaluated by 128-slice CT. Exp Ther Med 2013; 5: 848-852.

[2] Ishii T, Asuwa N, Masuda S, Ishikawa Y. The effects of a myocardial bridge on coronary atherosclerosis and ischaemia. J Pathol 1998; 185: 4-9.

[3] Channer KS, Bukis E, Hartnell G, Rees JR. Myocardial bridging of the coronary arteries. Clin Radiol 1989; 40: 335-359.

[4] Li ZF, Yang SG, Ge JB. Acute myocardial infarction due to myocardial bridge. Chin Med J (Engl) 2012; 125: 3589-3590.

[5] Burnsides C, Edwards JC, Lansing AI, Swarm RL. Arteriosclerosis in the intramural and extramural portions of coronary arteries in the human heart. Circulation 1956; 13: 235-241.

[6] Mohlenkamp S. Update on Myocardial Bridging. Circulation 2002; 106: 2616-2622.

[7] Polacek P, Kralove H. Relation of myocardial bridges and loops on the coronary arteries to coronary occulsions. Am Heart J 1961; 61: 44-52.

[8] Polacek P. Relationship of Myocardial bridging & loop to coronary occlusion. Am Heart J 1961; 61: 44-52.

[9] Ferreira AG Jr, Trotter SE, König B Jr, Décourt LV, Fox K, Olsen EG. Myocardial bridge: morphological and functional aspects. Br Heart J 1991; 66: 364-371.

[10] Iuchi A, Ishikawa Y, Akishima-Fukasawa Y, Fukuzawa R, Akasaka Y, Ishii T. Association of variance in anatomical elements of myocardial bridge with coronary atherosclerosis. Atherosclerosis 2013; 227: 153-158.

[11] Hostiuc S, Curca GC, Dermengiu D, Dermengiu S, Hostiuc M, Rusu MC. Morphological changes associated with hemodynamically significant myocardial bridges in sudden cardiac

death. Thorac Cardiovasc Surg 2011; 59: 393-398.

[12] Yin YW, Li JC, Wang JZ, Li BH, Pi Y, Yang QW, Fang CQ, Gao CY, Zhang LL. Association between apolipoprotein E gene polymorphism and the risk of vascular dementia: a meta-analysis. Neurosci Lett 2012; 514: 6-11.

[13] Kumar NT, Liestol K, Loberg EM, Reims HM, Brorson SH, Maehlen J. The apolipoprotein E polymorphism and cardiovascular diseases--an autopsy study. Cardiovasc Pathol 2012; 21: 461-469.

[14] Mahley RW, Rall SC Jr. Apolipoprotein E: far more than a lipid transport protein. Annu Rev Genomics Hum Genet 2000; 1: 507-537.

[15] Liu J, Li ZQ, Li JY, Li T, Wang T, Li Y, Xu YF, Feng GY, Shi YY, He L. Polymorphisms and haplotypes in the YWHAE gene increase susceptibility to bipolar disorder in Chinese Han population. J Clin Psychiatry 2012; 73: e1276-1282.

[16] Shi YY, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res 2005; 15: 97-98.

[17] Zhang HX, Wang ZT, Lu XX, Wang YG, Zhong J, Liu J. NLRP3 gene is associated with ulcerative colitis (UC), but not Crohn's disease (CD), in Chinese Han population. Inflamm Res 2014; 63: 979-985.

[18] Brodsky SV, Roh L, Ashar K, Braun A, Ramaswamy G. Myocardial bridging of coronary arteries: A risk factor for myocardial fibrosis? Int J Cardiol 2008; 124: 391-392.

[19] Ishikawa Y, Akasaka Y, Suzuki K, Fujiwara M, Ogawa T, Yamazaki K, Niino H, Tanaka M, Ogata K, Morinaga S, Ebihara Y, Kawahara Y, Sugiura H, Takimoto T, Komatsu A, Shinagawa T, Taki K, Satoh H, Yamada K, Yanagida-Iida M, Shimokawa R, Shimada K, Nishimura C, Ito K, Ishii T. Anatomic properties of myocardial bridge predisposing to myocardial infarction. Circulation 2009; 120: 376-383.

[20] Mookadam F, Green J, Holmes D, Moustafa SE, Rihal C. Clinical relevance of myocardial bridging severity: single center experience. Eur J Clin Invest 2009; 39: 110-115.

[21] Yano K, Yoshino H, Taniu*chi M*, Kachi E, Shimizu H, Watanuki A, Ishikawa K. *Myo*cardial bridging of the left anterior descending coronary artery in acute inferior wall myocardial infarction. Clin Cardiol 2001; 24: 202-208.

[22] Schwarz ER, Gupta R, Haager PK, vom Dahl J, Klues HG, Minartz J, Uretsky BF. Myocardial bridging in absence of coronary artery disease: proposal of a new classification based on clinical-angiographic data and long-term follow-up. Cardiology 2009; 112: 13-21.

[23] Loukas M, Von Kriegenbergh K, Gilkes M, Tubbs RS, Walker C, Malaiyandi D, Anderson RH. Myocardial bridges: A review. Clin Anat 2011; 24: 675-683.

[24] CARDIoGRAMplusC4D Consortium, Deloukas P, Kanoni S, Willenborg C, Farrall M, Assimes TL, Thompson JR, Ingelsson E, Saleheen D, Erdmann J, Goldstein BA, Stirrups K, König IR, Cazier JB, Johansson A, Hall AS, Lee JY, Willer CJ, Chambers JC, Esko T, Folkersen L, Goel A, Grundberg E, Havulinna AS, Ho WK, Hopewell JC, Eriksson N, Kleber ME, Kristiansson K, Lundmark P, Lyytikäinen LP, Rafelt S, Shungin D, Strawbridge RJ, Thorleifsson G, Tikkanen E, Van Zuydam N, Voight BF, Waite LL, Zhang W, Ziegler A, Absher D, Altshuler D, Balmforth AJ, Barroso I, Braund PS, Burgdorf C, Claudi-Boehm S, Cox D, Dimitriou M, Do R; DIAGRAM Consortium; CARDIOGENICS Consortium, Doney AS, El Mokhtari N, Eriksson P, Fischer K, Fontanillas P, Franco-Cereceda A, Gigante B, Groop L, Gustafsson S, Hager J, Hallmans G, Han BG, Hunt SE, Kang HM, Illig T, Kessler T, Knowles JW, Kolovou G, Kuusisto J, Langenberg C, Langford C, Leander K, Lokki ML, Lundmark A, McCarthy MI, Meisinger C, Melander O, Mihailov E, Maouche S, Morris AD, Müller-Nurasyid M; MuTHER Consortium, Nikus K, Peden JF, Rayner NW, Rasheed A, Rosinger S, Rubin D, Rumpf MP, Schäfer A, Sivananthan M, Song C, Stewart AF, Tan ST, Thorgeirsson G, van der Schoot CE, Wagner PJ; Wellcome Trust Case Control Consortium, Wells GA, Wild PS, Yang TP, Amouyel P, Arveiler D, Basart H, Boehnke M, Boerwinkle E, Brambilla P, Cambien F, Cupples AL, de Faire U, Dehghan A, Diemert P, Epstein SE, Evans A, Ferrario MM, Ferrières J, Gauguier D, Go AS, Goodall AH, Gudnason V, Hazen SL, Holm H, Iribarren C, Jang Y, Kähönen M, Kee F, Kim HS, Klopp N, Koenig W, Kratzer W, Kuulasmaa K, Laakso M, Laaksonen R, Lee JY, Lind L, Ouwehand WH, Parish S, Park JE, Pedersen NL, Peters A, Quertermous T, Rader DJ, Salomaa V, Schadt E, Shah SH, Sinisalo J, Stark K, Stefansson K, Trégouët DA, Virtamo J, Wallentin L, Wareham N, Zimmermann ME, Nieminen MS, Hengstenberg C, Sandhu MS, Pastinen T, Syvänen AC, Hovingh GK, Dedoussis G, Franks PW, Lehtimäki T, Metspalu A, Zalloua PA, Siegbahn A, Schreiber S, Ripatti S, Blankenberg SS, Perola M, Clarke R, Boehm BO, O'Donnell C, Reilly MP, März W, Collins R, Kathiresan S, Hamsten A, Kooner JS, Thorsteinsdottir U, Danesh J, Palmer CN, Roberts R, Watkins H, Schunkert H, Samani NJ. Large-scale association analysis identifies new risk loci for coronary artery disease. Nat Genet 2013; 45: 25-33.

Supplementary Table 1. List of the primers used in this study

SNP	Forward primer	Reverse primer
rs405509	GAGGAAGGAGGTGGGGCATAG	TGGGCGGCAGCCTCCACATTC
rs769450	TCAGGTGATCTGCCCGTTTC	CCAGCGGAAAAGCATGTATTG
rs439401	CGGCTCTTCTGTCGGAGTCTG	CAAGATGGGCAGAGGGACAAG
rs7259004	TTGTGGGGTAATGAAGGACAC	ACAATGTGCCAGACACCCTAT
rs2075650	TGCCTCTTTACAGGTGGAATC	GTAAGGACACCAGGAAGGCTC