Int J Clin Exp Med 2016;9(7):14684-14690
www.ijcem.com /ISSN:1940-5901/IJCEM0028274

Original Article

Association of PLCL2 and AP3D1-DOT1L-SF3A2 variants with the risk of coronary artery disease and its clinical phenotypes in a Chinese population

Qi Qian[1], Zhihui Ma[2], Lijuan Chen[3], Chengchun Tang[3], Genshan Ma[3], Zhong Chen[2]

[1]Department of Cardiology, The Second Affiliated Hospital of Nanjing Medical University, No. 121 Jianjiayuan, Nanjing 210011, China; [2]Department of Cardiology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, No. 600 Yishan Road, Shanghai 200233, China; [3]Department of Cardiology, The Affiliated Zhongda Hospital of Southeast University, No. 87 Dingjiaqiao, Nanjing 210009, China

Received March 15, 2016; Accepted June 10, 2016; Epub July 15, 2016; Published July 30, 2016

Abstract: Objective: PLCL2 is expressed in lymphocytes and platelets, and these cells play a pivotal role in the pathogenesis of atherosclerosis. AP3D1-DOT1L-SF3A2, expressed in lymphocytes and endothelial cells, also has an important role in the progression of atherosclerosis. Recently, a genome-wide association study identified PLCL2 and AP3D1-DOT1L-SF3A2 as new susceptibility loci for myocardial infarction in a Japanese population. Therefore, we performed a case-control study to investigate the relationship between the single nucleotide polymorphisms, (SNPs) PLCL2 (rs4618210) and AP3D1-DOT1L-SF3A2 (rs3803915), and the risk of coronary artery disease (CAD) and its clinical phenotypes in a Chinese population. Methods: The subjects (n=447) enrolled included 219 cases with confirmed CAD and 228 controls, and all participants underwent elective coronary angiography. The SNPs rs4618210 and rs3803915 were genotyped by the ligase detection reaction. The distribution of genotypes and alleles between CAD cases and controls and their association with clinical phenotypes and lesion severity were analyzed. Results: There were significant differences in the frequencies of the rs4618210 G allele between CAD patients and controls (OR, 2.32; 95% CI, 1.80~2.98; P<0.01). Analysis of the distribution of genotypes at rs4618210 also revealed a difference between the CAD patients with MI and those with unstable angina (P=0.05). When the 219 CAD patients were divided into three groups (1-, 2-, and 3-vessel disease), the distribution of the two SNPs was not different among the groups, and there was no association between the SNPs and CAD severity (all P>0.05). Furthermore, there was no significant difference in the distribution of SNP rs3803915 between the CAD patients and controls, and there was no association with CAD severity or clinical phenotypes (all P>0.05). Conclusions: In conclusion, in our sample of patients from the east region of China, the PLCL2 gene and not the AP3D1-DOT1L-SF3A2 variant might be associated with risk of CAD and its clinical phenotypes.

Keywords: Single nucleotide polymorphism, coronary artery disease, phospholipase C-like protein 2, AP3D1-DOT1L-SF3A2, gene polymorphism

Introduction

Coronary artery disease (CAD) and its severest form, myocardial infarction (MI), are important causes of worldwide morbidity and mortality [1, 2]. CAD results from atherosclerosis, which is the blockage of major cardiac vessels supplying blood to the heart; progressive narrowing of the arterial lumen causes myocardial ischemia and angina pectoris [3]. Since 1990, there has been an explosion of studies examining genetic markers that may increase the risk of MI/CAD [4]. These include genetic linkage analyses of candidate genes, cardiometabolic risk factors and genome-wide association studies (GWAS) [5-11]. Several reports of well-powered candidate gene studies have been identified by a several large-scale GWAS. In CAD, 51 loci with GWAS significance ($P<5\times10^{-8}$) have collectively been identified by a recent large-scale GWAS mainly in subjects of Caucasian descent [11].

Among these susceptibility loci, some of them belong to pathways involved in immune or inflammatory responses [8, 12-14]. The phospholipase C-like protein 2 (PLCL2) gene is located on chromosome 3p24.3. PLCL2 is expressed in lymphocytes and platelets, and is an impor-

Table 1. Baseline characteristics of the study population

	Controls	CAD
Numbers, n	228	219
Gender, male (%)	101 (44.3)	145 (66.2)†
Age, years	65.2±15.4	66.7±11.2†
Hypertension, n (%)	120 (52.6)	144 (65.8)†
Diabetes mellitus, n (%)	25 (10.8)	65 (29.9)†
Smokers, n (%)	135 (61.6)	203 (89.0)†
Hemoglobin (Hb) (g/l)	130.7±24.1	133.0±18.5
FBS (mmol/l)	5.8±1.6	6.3±2.0*
TC (mmol/l)	4.1±1.1	4.5±1.3*
HDL (mmol/l)	1.3±0.47	1.2±0.3†
LDL (mmol/l)	2.6±0.9	2.7±1.3*

Data are mean ± SD or number (%), as appropriate; *P<0.05 vs controls. †P<0.01 vs controls; CAD, coronary artery disease; FBS, fasting blood sugar; TC; total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein.

tant PLCL that lacks phospholipase catalytic activity [15]. PLC-L2 also plays an important role in the regulation of Ins (1, 4, 5) P (3) in the endoplasmic reticulum [15]. PLCL2 is likely to be involved in the pathogenesis of the chronic inflammatory process of MI through dysregulation of the immune system [16]. AP3D1-DOT1L-SF3A2 is expressed in lymphocytes and endothelial cells, which have an important role in the pathogenesis of atherosclerosis [17]. Recently, two novel susceptibility loci, PLCL2 on chromosome 3p24.3 (rs4618210:A>G) and AP3D1-DOT1L-SF3A2 on chromosome 19p13.3 (rs3803915:A>C), have been identified as new susceptibility loci for MI due to atherosclerosis in a Japanese population [17].

Due to differences in geographical or ancestral origin of samples, it is not clear whether these two loci are associated with risk of CAD, clinical phenotypes and coronary severity in a Chinese Han population. Thus, the present case-control study was performed to examine the association between SNPs rs4618210 of the PLCL2 gene and rs3803915 of the AP3D1-DOT1L-SF3A2 gene with CAD, its clinical phenotypes and the severity of coronary artery disease in a Chinese population.

Methods

Subjects

A total of 447 Chinese subjects, aged 34 to 90 years, were consecutively enrolled in this study. Since all subjects had symptoms of chest discomfort and there was CAD was suspected, all subjects underwent coronary angiography. CAD was present in 219 patients: 51 were diagnosed with MI, 147 with unstable angina pectoris (UAP), and 21 with stable angina pectoris (SAP). Exclusion criteria were: a contraindication to heparin; autoimmune diseases; or severe kidney, liver or malignant disease. Smoking habits were identified by the information obtained in the patients' questionnaire. The study was approved by the local Ethical Committee, and all participants gave informed consent for participation.

Coronary angiography

All patients underwent elective coronary angiography using the Judkins method, and CAD was defined by angiography as stenosis that obstructed at least one main coronary artery by ≥50%. CAD patients were grouped according to the number of stenosed vessels (1-, 2-, and 3-vessel disease). Those subjects without coronary stenosis were included as controls.

DNA extraction and genotyping

Peripheral venous blood was drawn from each participant. Genomic DNA was extracted using a QIAamp DNA Blood kit (Qiagen, Valencia, CA). The SNPs were genotyped by the ligase detection reaction [18, 19] using TaqMan genotyping assays on an ABI Prism 377 Sequence Detection System according to the manufacturer's instructions (Applied Biosystems, Foster City, CA).

Statistical analysis

The statistical software package, SPSS 15.0, was used for all statistical calculations. Results are presented as mean ± SD for continuous variables, and as proportions for categorical variables. Age, hemoglobin (Hb), total cholesterol (TC), low density lipoprotein (LDL) and high density lipoprotein (HDL) levels were considered continuous variables. Hypertension, diabetes mellitus, smoking status and gender were classified as categorical variables. Allele and genotype frequencies among cases and controls were compared with values predicted by the Hardy-Weinberg equilibrium using the χ^2-test. Relative risks of CAD associated with each genotype were calculated separately by binary logistic regression analysis. For each odds ratio (OR), 95% confidence intervals (CIs)

Table 2. Genotype and allele distribution at rs4618210 and rs3803915 in CAD cases and controls

	Control (n/%)		CAD (n/%)	OR (95% CI)
rs4618210				
AA	48/21.1		42/19.2	AA vs AG 0.93 (0.56~1.55)
AG	97/42.5		79/36.1	AG vs GG 1.45 (0.96~2.20)
GG	83/36.4		98/44.7	GG vs AA 1.35 (0.81~2.24)
P		0.19		
Relative frequencies of alleles				
Allele A	362/57.9		163/37.2	
Allele G	263/42.1		275/62.8	2.32 (1.80~2.98)
P		0.00†		
rs3803915				
AA	17/7.5		11/5.0	AA vs CA 1.63 (0.73~3.68)
CA	88/38.6		93/42.5	CA vs CC 0.89 (0.60~1.30)
CC	123/53.9		115/52.5	CC vs AA 1.45 (0.65~3.22)
P		0.47		
Relative frequencies of alleles				
Allele A	122/26.8		115/26.3	
Allele C	334/73.2		323/73.7	1.03 (0.76~1.38)
P		0.88		

P is the significance level of comparison between CAD and controls. The χ^2 test was used for genotypes and likelihood ratio test was used for alleles in the analysis. N, number of individuals with percentage of the total group in parenthesis; OR, odds ratio; 95% CI, 95% confidence interval; †P<0.01.

Table 3. Distribution of genotypes at rs4618210 and rs3803915 among CAD patients according to the severity of CAD

		Number of vessels involved			*p* value
		One	Two	Three	
rs4618210	AA, n (%)	17/23.9	14/18.7	11/15.1	0.58
	AG, n (%)	21/29.6	29/38.7	29/39.7	
	GG, n (%)	33/46.5	32/42.7	33/45.2	
rs3803915	AA, n (%)	3/4.2	5/6.7	3/4.1	0.16
	CA, n (%)	35/49.3	23/30.7	35/47.9	
	CC, n (%)	33/46.5	47/62.7	35/47.9	

For abbreviations, see **Table 1**.

were calculated. Two-tailed *p*-values <0.05 were considered significant.

Results

There were 447 subjects enrolled in the study; there were 219 CAD cases and 228 controls. Baseline characteristics of all subjects are shown in **Table 1**. There was no significant difference in baseline hemoglobin concentration between the CAD cases and controls. A higher prevalence of hypertension, diabetes mellitus, smokers, and higher fasting blood sugar and main lipid parameters was found in patients with CAD, and the CAD patients were also older than the controls (all P<0.05).

There was no significant deviation from the Hardy-Weinberg equilibrium in all the samples studied. Of the 447 subjects participating in the study, analysis of AA, AG and GG genotype frequencies of rs4618210 and AA, CA and CC genotype frequencies of rs3803915 did not reveal any significant differences between CAD patients and controls. The prevalence of individual alleles at the rs4618210 locus differed significantly between the CAD and control groups (P<0.01), whereas the alleles at another locus, rs3803915, were not significantly different (P>0.05) (**Table 2**).

When the 219 patients that had CAD were divided into three groups based on the number of diseased vessels (1-, 2-, and 3-vessel disease), there was no significant differences in the genotypes of the two SNPs among the three groups (P=0.58 and 0.16, respectively) (**Table 3**).

Table 4. Distribution of genotypes at rs4618210 and rs3803915 among different clinical phenotypic subgroups of CAD

	Clinical phenotypic subgroups of CAD		OR (95% CI)
	AP	MI	
rs4618210			
AA	30/17.9	12/23.5	AA vs AG 0.4 (0.2~1.0)
AG	68/40.4	11/21.6	AG vs GG 2.5 (1.1~5.4)
GG	70/41.7	28/54.9	GG vs AA 1.0 (0.4~2.2)
P	0.05		
Relative frequencies of alleles			
Allele A	128/38.1	35/34.3	
Allele G	208/61.9	275/65.7	1.2 (0.7~1.9)
P	0.6		
rs3803915			
AA	9/5.4	2/3.9	AA vs CA 1.7 (0.3~8.2)
CA	68/40.5	25/49.0	CA vs CC 0.6 (0.3~1.2)
CC	91/54.2	24/47.1	CC vs AA 1.2 (0.2~5.9)
P	0.55		
Relative frequencies of alleles			
Allele A	86/25.6	29/28.4	
Allele C	250/74.4	73/71.6	0.9 (0.5~1.4)
P	0.6		

AP, angina pectoris; MI, myocardial infarction.

As shown in **Table 4**, when an analysis of the clinical phenotype of CAD subgroups was conducted, the distribution of genotypes at the rs4618210 variant between the AP and MI groups was of borderline significance (P=0.05); however, the distribution of the three genotypes at rs3803915 between the AP and MI groups was not significantly different (P=0.55). In addition, further analysis was done to determine if there was a relationship between the two clinical phenotypic subgroups of CAD and alleles, but no significant associations were found (data not shown).

Discussion

The present study provided evidence of an association of alleles at rs4618210 with risk of CAD: carriers with the rs4618210 G allele had a 1.3-fold higher risk of CAD than non-risk allele A. There was also a difference in the distribution of genotypes at rs4618210 between the clinical phenotypes of MI and angina. Our study further supported an association of rs4618210 with CAD and its clinical phenotypes, and these results confirm previous findings in a Japanese population [17].

In the study by Hirokawa et al. [17], several mechanisms were proposed to explain the connection between established MI and genes PLCL2 and AP3D1-DOT1L-SF3A2. Since FAS indirectly affects the expression of PLCL2 and mature B cells undergo FAS-mediated apoptosis, it is possible that PCL2 may regulate B-cell maturation, leading to the formation of atherosclerotic lesions. Furthermore, DOT1L is negatively correlated with endothelin-1, which plays an important role in vasospasm and vasoconstriction. In addition, the binding capacity of AP3D1 is regulated by angiotensinogen, suggesting that AP3D1 might be related to atherosclerosis in concert with a potent atherosclerotic substrate, angiotensin II. Thus, PLCL2 and AP3D1-DOT1L-SF3A2 might play an important role in the pathogenesis of MI via the promotion of atherosclerosis (and vasospasm) to different extents [17]. In the case of multifactorial disorders like CAD, it has always been challenging to establish a mechanistic relationship between a particular allele and disease development.

In the present study, although we did not replicate the association between the common

AP3D1-DOT1L-SF3A2 variant and susceptibility to CAD, our findings implied that rs4618210 might contribute to the formation of CAD. Allele G might be a risk allele for CAD, and those carrying the AG genotype may be an increased risk for MI, which is similar to results reported before [17]. Our data and others support the notion that PLCL2 may be used for CAD risk stratification and outcome evaluations. The discrepancies could be explained partly by anthropometric characteristics. The G allele frequency was higher in Chinese subjects than in Caucasians. Potential genetic variants associated with an increased risk of obstructive CAD may ultimately guide clinical decision making and help improve strategies for the management of patients with CAD and MI. However there are still many key issues that must be overcome before the genomic data can be successful applied to individual patient management [21].

In the present study, the distribution of these two SNPs was not related to the severity of CAD. This suggests that SNP rs4618210 is only associated with the susceptibility to CAD and clinical phenotypes, but not the severity of coronary lesions. Since this was an observational study and the sample size was relatively small, the study was most likely underpowered to show associations between allele frequency and the severity of coronary lesions. Even so, all participants in our study underwent coronary angiography, which allowed evaluation of stenosis in each coronary artery and accurate classification of patients as CAD cases or controls.

In conclusion, in our sample of patients from the east region of China, the PLCL2 gene variant might be associated with the risk of CAD and its clinical phenotypes. Our study results needed to be confirmed by in a larger population, and additional functional experiments are necessary to further increase our understanding of the role of PLCL2 in the pathogenesis of CAD and its clinical phenotypes.

Acknowledgements

The study was partially funded by Shanghai Pudong New Area Science and Technology Development Fund (PKJ2014-Y09) (Dr. Zhong Chen) and Shanghai Municipal Commission of Health and Family Planning Research Project (201440309) (Dr. Zhong Chen). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Disclosure of conflict of interest

None.

Authors' contribution

Z C conceived and designed the study, finished the finial manuscript preparation. Q Q, ZH M, participated in the laboratory tests and data collection, finished the data analysis and primary manuscript writing. LJ C, CC T and GS M helped interpret the results. All authors read and approved the final manuscript.

Abbreviations

CAD, coronary artery disease; CI, confidence interval; ECG, electrocardiogram; FBS, fasting blood sugar; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MI, myocardial infarction; OR, odds ratio; PLCL2, phospholipase C-like protein 2; SAP, stable angina pectoris; SNP, single nucleotide polymorphism; TC, total cholesterol; UAP, unstable angina pectoris.

Address correspondence to: Zhong Chen, Department of Cardiology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, No. 600 Yishan Road, Shanghai 200233, China. E-mail: zhongchen-7498@hotmail.com

References

[1] Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Blaha MJ, Dai S, Ford ES, Fox CS, Franco S, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Huffman MD, Judd SE, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Mackey RH, Magid DJ, Marcus GM, Marelli A, Matchar DB, McGuire DK, Mohler ER 3rd, Moy CS, Mussolino ME, Neumar RW, Nichol G, Pandey DK, Paynter NP, Reeves MJ, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Wong ND, Woo D, Turner MB; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics--2014 update: a report from the American Heart Association. Circulation 2014; 129: e28-e292.

[2] Dai X, Wiernek S, Evans JP, Runge MS. Genetics of coronary artery disease and myocardial infarction. World J Cardiol 2016; 8: 1-23.

[3] Lusis AJ, Fogelman AM, Fonarow GC. Genetic basis of atherosclerosis: part I: new genes and pathways. Circulation 2004; 110: 1868-73.

[4] Luo AK, Jefferson BK, Garcia MJ, Ginsburg GS, Topol EJ. Challenges in the phenotypic characterisation of patients in genetic studies of coronary artery disease. J Med Genet 2007; 44: 161-5.

[5] LeBlanc M, Zuber V, Andreassen BK, Witoelar A, Zeng L, Bettella F, Wang Y, McEvoy LK, Thompson WK, Schork AJ, Reppe S, Barrett-Connor E, Ligthart S, Dehghan A, Gautvik KM, Nelson CP, Schunkert H, Samani NJ; CARDIoGRAM Consortium, Ridker PM, Chasman DI, Aukrust P, Djurovic S, Frigessi A, Desikan RS, Dale AM, Andreassen OA. Identifying Novel Gene Variants in Coronary Artery Disease and Shared Genes With Several Cardiovascular Risk Factors. Circ Res 2016; 118: 83-94.

[6] Nikpay M, Goel A, Won HH, Hall LM, Willenborg C, Kanoni S, Saleheen D, Kyriakou T, Nelson CP, Hopewell JC, Webb TR, Zeng L, Dehghan A, Alver M, Armasu SM, Auro K, Bjonnes A, Chasman DI, Chen S, Ford I, Franceschini N, Gieger C, Grace C, Gustafsson S, Huang J, Hwang SJ, Kim YK, Kleber ME, Lau KW, Lu X, Lu Y, Lyytikäinen LP, Mihailov E, Morrison AC, Pervjakova N, Qu L, Rose LM, Salfati E, Saxena R, Scholz M, Smith AV, Tikkanen E, Uitterlinden A, Yang X, Zhang W, Zhao W, de Andrade M, de Vries PS, van Zuydam NR, Anand SS, Bertram L, Beutner F, Dedoussis G, Frossard P, Gauguier D, Goodall AH, Gottesman O, Haber M, Han BG, Huang J, Jalilzadeh S, Kessler T, König IR, Lannfelt L, Lieb W, Lind L, Lindgren CM, Lokki ML, Magnusson PK, Mallick NH, Mehra N, Meitinger T, Memon FU, Morris AP, Nieminen MS, Pedersen NL, Peters A, Rallidis LS, Rasheed A, Samuel M, Shah SH, Sinisalo J, Stirrups KE, Trompet S, Wang L, Zaman KS, Ardissino D, Boerwinkle E, Borecki IB, Bottinger EP, Buring JE, Chambers JC, Collins R, Cupples LA, Danesh J, Demuth I, Elosua R, Epstein SE, Esko T, Feitosa MF, Franco OH, Franzosi MG, Granger CB, Gu D, Gudnason V, Hall AS, Hamsten A, Harris TB, Hazen SL, Hengstenberg C,Hofman A, Ingelsson E, Iribarren C, Jukema JW, Karhunen PJ, Kim BJ, Kooner JS, Kullo IJ, Lehtimäki T, Loos RJ, Melander O, Metspalu A, März W, Palmer CN, Perola M, Quertermous T, Rader DJ, Ridker PM, Ripatti S, Roberts R, Salomaa V, Sanghera DK, Schwartz SM, Seedorf U, Stewart AF, Stott DJ, Thiery J, Zalloua PA, O'Donnell CJ, Reilly MP, Assimes TL, Thompson JR, Erdmann J, Clarke R, Watkins H, Kathiresan S, McPherson R, Deloukas P, Schunkert H, Samani NJ, Farrall M; CARDIoGRAMplusC4D Consortium. A comprehensive 1,000 Genomes-based genome-wide association meta-analysis of coronary artery disease. Nat Genet 2015; 47: 1121-30.

[7] Ghosh S, Vivar J, Nelson CP, Willenborg C, Segrè AV, Mäkinen VP, Nikpay M, Erdmann J, Blankenberg S, O'Donnell C, März W, Laaksonen R, Stewart AF, Epstein SE, Shah SH, Granger CB, Hazen SL, Kathiresan S, Reilly MP, Yang X, Quertermous T, Samani NJ, Schunkert H, Assimes TL, McPherson R. Systems Genetics Analysis of Genome-Wide Association Study Reveals Novel Associations Between Key Biological Processes and Coronary Artery Disease. Arterioscler Thromb Vasc Biol 2015; 35: 1712-22.

[8] Xu C, Yang Q, Xiong H, Wang L, Cai J, Wang F, Li S, Chen J, Wang C, Wang D, Xiong X, Wang P, Zhao Y, Wang X, Huang Y, Chen S, Yin D, Li X, Liu Y, Liu J, Wang J, Li H, Ke T, Ren X, Wu Y, Wu G, Wan J, Zhang R, Wu T, Wang J, Xia Y, Yang Y, Cheng X, Liao Y, Chen Q, Zhou Y, He Q, Tu X, Wang QK. Candidate pathway-based genome-wide association studies identify novel associations of genomic variants in the complement system associated with coronary artery disease. Circ Cardiovasc Genet 2014; 7: 887-94.

[9] Björkegren JL, Kovacic JC, Dudley JT, Schadt EE. Genome-wide significant loci: how important are they? Systems genetics to understand heritability of coronary artery disease and other common complex disorders. J Am Coll Cardiol 2015; 65: 830-45.

[10] 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.

[11] Ozaki K, Tanaka T. Molecular genetics of coronary artery disease. J Hum Genet 2016; 61: 71-7.

[12] Guven M, Ismailoglu Z, Batar B, Unal S, Onaran I, Karadag B, Ongen Z. The effect of genetic polymorphisms of TLR2 and TLR4 in Turkish patients with coronary artery disease. Gene 2015; 558: 99-102.

[13] Qian Q, Chen Z, Ma G, Jiang Y, Feng Y, Shen C, Yao Y, Ding J, Dai Q, Li Y. Complement factor H Y402H polymorphism, plasma concentration and risk of coronary artery disease. Mol Biol Rep 2009; 36: 1257-61.

[14] Chen Z, Qian Q, Tang C, Ding J, Feng Y, Ma G. Association of two variants in the interleukin-6 receptor gene and premature coronary heart disease in a Chinese Han population. Mol Biol Rep 2013; 40: 1021-6.

[15] Otsuki M, Fukami K, Kohno T, Yokota J, Takenawa T. Identification and characterization of a new phospholipase C-like protein, PLC-L(2). Biochem Biophys Res Commun 1999; 266: 97-103.

[16] Perry HM, Bender TP, McNamara CA. B cell subsets in atherosclerosis. Front Immunol 2012; 3: 373.

[17] Hirokawa M, Morita H, Tajima T, Takahashi A, Ashikawa K, Miya F, Shigemizu D, Ozaki K, Sakata Y, Nakatani D, Suna S, Imai Y, Tanaka T, Tsunoda T, Matsuda K, Kadowaki T, Nakamura Y, Nagai R, Komuro I, Kubo M. A genome-wide association study identifies PLCL2 and AP3D1-DOT1L-SF3A2 as new susceptibility loci for myocardial infarction in Japanese. Eur J Hum Genet 2015; 23: 374-80.

[18] Xiao Z, Xiao J, Jiang Y, Zhang S, Yu M, Zhao J, Wei D, Cao H. A novel method based on ligase detection reaction for low abundant YIDD mutants detection in hepatitis B virus. Hepatol Res 2006; 34: 150-5.

[19] Favis R, Day JP, Gerry NP, Phelan C, Narod S, Barany F. Universal DNAarray detection of small insertions and deletions in BRCA1 and BRCA2. Nat Biotechnol 2000; 18: 561-4.

[20] Zhou Q, Liu K, Wu H, Chen L, Pouranan V, Yuan M, Xiao Z, Peng W, Xiang A, Tang R, Zhang W. Spironolactone rescues Dot1a-Af9-mediated repression of endothelin-1 and improves kidney injury in streptozotocin-induced diabetic rats. PLoS One 2012; 7: e47360.

[21] Morita H. Human genomics in cardiovascular medicine: implications and perspectives. Circ J 2013; 77: 876-85.