

## Original Article

# Association analysis of GWAS and candidate gene loci in a Chinese population with coronary heart disease

Min Gao<sup>1</sup>, Haiqin Tang<sup>2</sup>, Xiaodong Zheng<sup>3</sup>, Fusheng Zhou<sup>3</sup>, Wensheng Lu<sup>4</sup>

<sup>1</sup>Department of Electrocardiography, Affiliated Provincial Hospital, Anhui Medical University, Hefei, Anhui, 230001, China; <sup>2</sup>Department of Cardiology, The First Affiliated Hospital, Anhui Medical University, Hefei, 230032, China <sup>3</sup>Department of Dermatology, Anhui Medical University, Hefei, 230032, China; <sup>4</sup>Department of Dermatology, Affiliated Provincial Hospital, Anhui Medical University, Hefei, Anhui, 230001, China

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**Abstract:** Objective: Coronary heart disease (CHD), the most severe form of coronary artery disease (CAD), is a complex disease that involves a variety of genetic and environmental factors. Recently, multiple single nucleotide polymorphisms (SNPs) have been associated with CAD in Caucasians by genome-wide association (GWA) studies. However, the association of these SNPs with CHD in Asian populations has not yet been established. Here, we aim to investigate the genetic etiology of CHD in a Chinese population by genotyping SNPs previously been associated with CHD in other ethnic origin in GWAS or candidate gene studies. Methods: Five SNPs, rs17114036, rs9369640, rs515135, rs579459 and rs8055236, from 5 different loci were genotyped using a sequenom Mass array system in 545CHD patients and 1008 unrelated controls from a Chinese population. Results: Our study showed that SNP rs515135 is strongly associated with CHD in a Chinese Han population ( $P$ -value=0.00333, OR=1.48). We also detected significant difference of SNP rs579459 in APOB gene in patients with severe CAD compared to patients with mild CAD. Conclusion: SNP rs515135 is associated with the susceptibility of CHD in Chinese Han population. The location of rs515135 in the APOB gene supports its potential involvement in the pathogenesis of CAD. Our study data also support that SNP rs579459 may be associated with the severity of CHD.

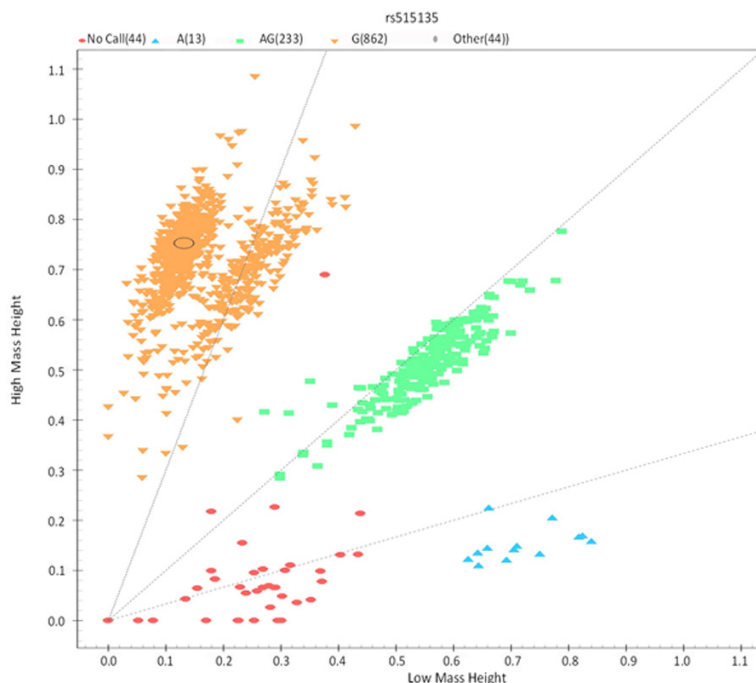
**Keywords:** Coronary heart disease, genetic variants, case-controlled study, single nucleotide polymorphism

## Introduction

Coronary artery disease (CAD) is one of the leading causes of death in the populations both in developed and developing countries. According to the World Health Organization, it was estimated that more than 700,000 people die from CAD each year in China. Coronary heart disease (CHD) is the most severe clinical manifestation of CAD and is the leading cause of death worldwide [1, 2]. CHD is a complex disease characterized by the inheritance of multiple genetic variants in addition to environmental factors which worsen the disease state. Epidemiological studies have identified many risk factors for coronary heart disease, including plasma lipid concentrations, blood pressure, smoking, diabetes and markers of inflammation. Among them, a causal role has been proven only for some factors (for example, low-

density lipoprotein (LDL) cholesterol and blood pressure) primarily through randomized clinical trials of drug therapy. Twin and family studies have demonstrated that a significant proportion (40-50%) of susceptibility to CHD is inherited [3]. A previous linkage study and candidate gene study have identified genetic factors predisposing to CHD. However, the results of CHD genetic studies have not been very satisfactory. Recently, GWAS have proven to be a powerful tool to identify susceptibility genes for common diseases. To date, GWAS studies in Caucasians identified multiple single nucleotide polymorphisms (SNPs) that associated with coronary artery disease (CAD) [4-12]. However, no independent studies of these SNPs have been reported in a Chinese population with CHD. Here, we intend to identify the associations between genetic variants of CHD, specifically, the associations of the SNPs rs17114036, rs9369640,

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**Figure 1.** The genotyping cluster of rs515135.

rs515135, rs579459 and rs8055236 with CHD in a Chinese Han population.

### Materials and methods

#### Patients and controls

A total of 545 blood-unrelated Chinese patients with coronary heart disease and 1008 healthy controls were recruited consecutively from out-patients at the Department of Cardiovascular Internal Medicine at Anhui Provincial Hospital. All subjects were of self-reported Chinese Han ancestry. Coronary heart disease was defined as the following: ① clinical symptoms and an electrocardiogram consistent with the diagnosis of coronary heart disease criteria formulated by the WHO in 1979. ② Coronary angiography revealed that at least 1 coronary artery stenosis was more than or equal to 50%. ③ There was no relationship between cases. Exclusion criteria: ① Associated with other cardiac diseases, such as dilated cardiomyopathy, rheumatic heart disease ② severe disease in the liver and kidney. ③ Previously received intravenous thrombolysis, coronary artery stenting and coronary artery bypass grafting patients. Control subjects were recruited from healthy adult visitors to the hospitals without a history of cardiovascular disease and family

history of coronary artery disease (including first-, second- and third-degree relatives), matched for age (up to 6 years older or younger) and sex with the cases. Participants who consented to the study completed a structured questionnaire, attended a health examination, and had a venous blood sample taken. Information about demographic factors, socioeconomic status, lifestyle (smoking, leisure time, physical activity, and dietary patterns), personal and family history of cardiovascular disease, hypertension and diabetes mellitus was obtained. After written informed consent was obtained, peripheral blood samples were collected from all patients and matched healthy controls. All participants provided their written informed consent. At the same time, we promised reporting of the research results to the participants. The study was approved by the Ethical Committee of the Anhui Provincial Hospital and was performed according to the Declaration of Helsinki Principles.

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#### Definitions

The patients were categorized as the following: the severity of coronary disease was determined to be mild ( $\leq 20$  points) or severe ( $> 20$  points) according to the Gensini scoring system [13]. The family history was considered to be positive if the patient's first-, second-, and/or third-degree relatives had coronary heart disease; the family history was considered to be negative otherwise.

#### Genomic DNA extracting and SNP genotyping

After informed consent, genomic DNA was isolated from peripheral blood of the patients using a Qiagen kit (Hilden, Germany). In addition, genomic DNA of 1008 unrelated healthy individuals was extracted as a control. We selected only SNPs in CAD-related genes with minor allele frequencies of 0.05 in Chinese patients in the HapMap database. We used the

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**Table 1.** Primer sequences of amplification and extension

SNP	Amplification primer sequences	Primer sequences of extension
rs17114036	1) ACGTTGGATGCCTATCAGCAAGAGCTGAAG 2) ACGTTGGATGCGAGAACCTGGTTTCTTGAC	aTAAGTTTTTCAGGAAAAAGTAGTC
rs9369640	1) ACGTTGGATGTGGGCTACTGGAGAATAAGG 2) ACGTTGGATGCGGTGTGTAGATACTGTTGC	gACTGTTGCAAAATCACTGTTAG
rs515135	1) ACGTTGGATGGAACCATCTTGTACTGCAC 2) ACGTTGGATGAGGGCTTACAGCCAAGTAAC	tAAAAACAGCCAAAATGGAACCAAAGA
rs579459	1) ACGTTGGATGTTCTGGTTTGCATGTGTTGG 2) ACGTTGGATGGAAGTAAAGTGACTTGCTG	cGGTTTCTTTTCGCTACACCA
rs8055236	1) ACGTTGGATGGCCAGGCTATTTGTGCATCT 2) ACGTTGGATGCCATACAGTCATTGAGGC	gtCTTTGTTTTTTTCATCTTCCACTCG

**Table 2.** Characters of the study cohorts

Characteristic	CHD patient	Control
	(n=545)	(n=1008)
Age (SD)*	61.5	57.5
Sex (Male/Female)	354/191	605/403
BMI	24.5	23.8
History of HT (%)	40.18%	21.53%
History of T2D (%)	12.29%	3.08%
Current Smoking (%)	57.25%	40.18%
Alcohol intake (%)	38.35%	37.10%
HDL-C mmol/L*	1.02	1.13
LDL-C mmol/L*	2.53	2.27
TG mmol/L*	1.63	1.65
TC mmol/L*	4.55	4.59

CHD, Coronary heart disease; BMI, body mass index (kg/m<sup>2</sup>); HT, hypertension; T2D, type 2 diabetes; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; TC, total cholesterol;. \*Data are means (SD).

SNPs effect size in the references, minor allele frequencies of Chinese (based on HapMap database) and the sample size of this study to calculate the power for detecting a positive association. Excluding SNPs with power less than 0.20, we selected five SNPs (rs17114036, rs9369640, rs515135, rs579459 and rs8055236), which have been shown to significantly associate with CAD, but have not yet been tested in a Chinese population.

All SNPs were genotyped using the Sequenom iPLEX platform (Sequenom, Inc., San Diego, CA, USA) at the State Key Laboratory Incubation of Dermatology, Ministry of Science and Technology, Hefei, Anhui, China. Each genotyping cluster of five selected SNPs is good, especially the genotyping cluster of rs515135 as shown

in **Figure 1**. Besides, the primers designed for sequencing of this study are shown below (**Table 1**).

### Statistical analysis

Hardy Weinberg equilibrium tests of the genotyping data were performed using PLINK (version 1.07). The power of the study population was estimated using the power calculator for genome wide association studies program (<http://www.sph.umich.edu/csg/abecasis/CaTS/>). For allelic association between a SNP and CHD, the *p*-value and corresponding odds ratio (OR) with a 95% confidence interval were computed by Chi-square tests using Pearson's 2X2 contingency tables as implemented in PLINK version 1.07.

For the stratified analysis, differences between the cases and controls in terms of the genotype frequency were tested using the chi squared test and by calculating the odds ratios (ORs) and 95% confidence intervals (CIs).

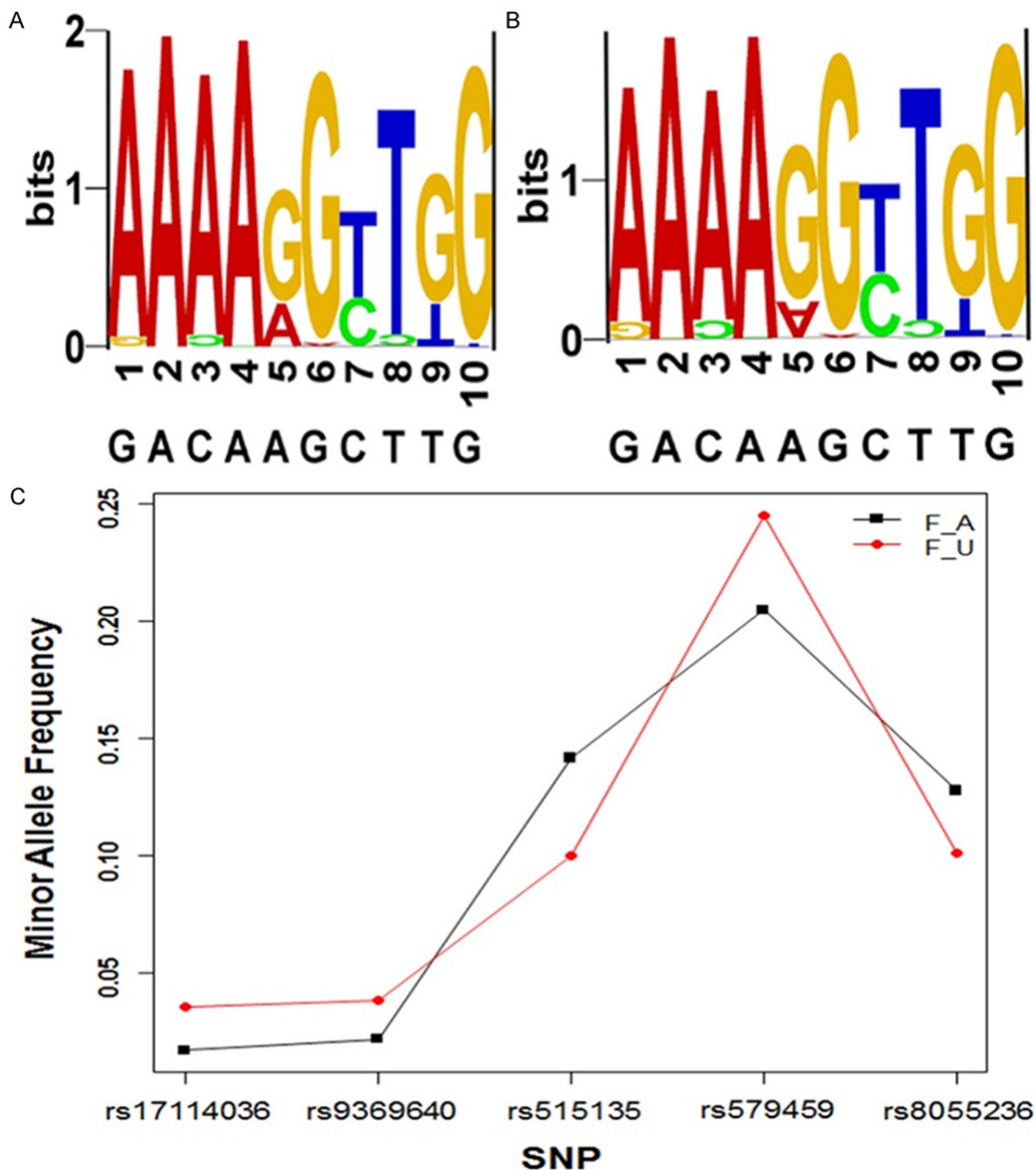
## Results

### Characters of the study cohorts

The demographic details for the patients and control subjects are shown in **Table 2**. Except the means of sex and the frequency of presence of alcohol intake, other characteristics in **Table 2**, CHD patient were significantly different from those in controls.

### Results of the case-controlled study

To demonstrate the genetic susceptibility of SNPs with CHD in a Chinese population, 5 SNPs



**Figure 2.** Allele distribution and minor allele frequency of 5 SNPs in the study patients and controls. A and B are SNP LOGO of 545 blood-unrelated Chinese patients and 1008 healthy controls respectively. (1, 3, 5, 7, 9 shows the minor allele proportion of five SNPs, and 2, 4, 6, 8, 10 shows the major one). C. Minor allele frequency of five SNPs in patients (F\_A) and controls (F\_U).

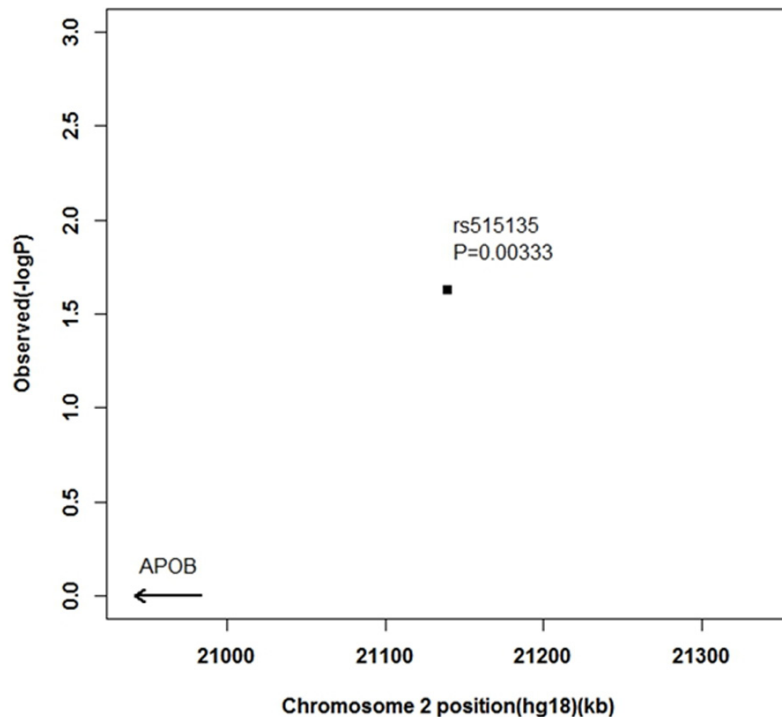
were genotyped in 545 CHD patients and 1008 controls. Allele distribution and minor allele frequency in these patients and controls are shown in **Figure 2**. All of the SNPs were in Hardy-Weinberg equilibrium. Association between the SNPs and CHD is shown in **Table 3**. In five SNPs, rs515135 was significantly associated with CHD in a Chinese population. The

scatter plots of the suggestive association evidence within 2p24-p23 (APOB) for CHD are shown in **Figure 3**. There is still significant association after adjustment of age, sex and BMI. There was no statistically significant association of rs17114036, rs9369640, rs579459 and rs8055236 with CHD in the Chinese population.

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**Table 3.** Association between single-nucleotide polymorphisms and coronary artery diseasesusceptibility in Chinese population coronary artery disease patients (all) versus controls

SNP	Allele(risk)	Mode	Odds ratio (95% CI)	P-value	power
rs17114036	G/A (A)	Dominant	1.003 (0.999-1.007)	0.523	5
		Recessive	2.040 (1.124-3.701)	0.017	67
		Allele	2.093 (1.165-3.761)	0.01167	41.7
rs9369640	C/A (A)	Dominant	1.284 (0.116-14.208)	1	4.7
		Recessive	1.846 (1.062-3.209)	0.028	49.6
		Allele	1.787 (1.049-3.044)	0.03043	32
rs515135	G/A (A)	Dominant	1.638 (1.227-2.187)	0.001	91.4
		Recessive	0.698 (0.214-2.281)	0.776	8.2
		Allele	1.48 (1.138-1.925)	0.00333	54.3
rs579459	C/T (T)	Dominant	1.477 (0.861-2.533)	0.155	28.9
		Recessive	1.285 (0.999-1.652)	0.05	49.9
		Allele	1.261 (1.024-1.554)	0.02917	33.6
rs8055236	G/T (T)	Dominant	1.575 (0.453-5.472)	0.471	87.1
		Recessive	1.337 (0.998-1.791)	0.051	8.1
		Allele	1.305 (0.9978-1.707)	0.05139	28.4



**Figure 3.** The scatter plots of the suggestive association evidences within 2p24-p23 (APOB) for CHD.

### Stratified analysis

Stratified analysis revealed that rs579459 is strongly associated with severe coronary heart disease. Associations between coronary heart

disease severity relative to the controls were not observed for the other four SNPs (**Table 4**).

### Discussion

CHD is the result of a combination of genetic and environmental factors. Until recently, the underlying mechanisms of coronary heart disease formation are only partially understood. More than 200 risk factors have been associated with CHD and, among these, randomized controlled trials have shown that low-density lipoprotein cholesterol (LDL-c) and blood pressure (BP) is causally related to CHD [14-16]. A key factor in reducing the global burden of CAD is early prediction of the disease to target preventive interventions.

Several studies have investigated a number of genes for polymorphisms or mutations that may be associated with coronary heart disease [17]. However, the results genetic studies of coronary heart disease have not been very satisfactory. Recent GWAS studies have identified several genetic variants that are associated with CAD [18-21]. The present study was performed in a Chinese population to determine the positive association signals identified in the GWAS studies with CAD in Caucasians.

We have shown that, there was a significant association of the SNP rs515135 with CHD after age, sex and BMI adjustment in a Chinese population. No association was found between-SNPs rs17114036, rs9369640, rs579459 and

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**Table 4.** Associations between single-nucleotide polymorphisms and coronary artery disease susceptibility in the Chinese population relative to the controls

SNP	Allele (risk)	Mode	Odds ratio (95% CI)	P-value	Power
1) Mild- moderate coronary artery disease vs control					
rs17114036	G/A (A)	Dominant	1.00 (0.999-1.007)	1	NA
		Recessive	3.49 (1.070-11.379)	0.032	70
		Allele	3.55 (1.099-11.490)	0.02386	49
rs9369640	C/A (A)	Dominant	1.00 (0.999-1.007)	1	NA
		Recessive	1.59 (0.706-3.582)	0.259	39
		Allele	1.63 (0.734-3.631)	0.2253	27
rs515135	G/A (A)	Dominant	1.64 (1.082-2.49)	1.90E-02	98
		Recessive	0.52 (0.065-4.126)	1	12
		Allele	1.47 (1.005-2.145)	0.04601	67
rs579459	C/T (T)	Dominant	1.25 (0.575-2.700)	0.577	16
		Recessive	0.95 (0.661-1.371)	0.791	7
		Allele	1.00 (0.745-1.349)	0.989	5
rs8055236	G/T (T)	Dominant	1.70 (1.134-2.561)	0.01	99
		Recessive	0.93 (0.108-7.997)	1	5
		Allele	1.56 (1.079-2.260)	0.01742	83
2) Severe coronary artery disease vs control					
rs17114036	G/A (A)	Dominant	1.00 (0.999-1.007)	1	NA
		Recessive	1.38 (0.574-3.298)	0.472	22
		Allele	1.42 (0.602-3.366)	0.4181	17
rs9369640	C/A (A)	Dominant	0.35 (0.032-3.894)	0.386	34
		Recessive	2.30 (0.814-6.489)	0.117	58
		Allele	1.87 (0.737-4.719)	0.1813	32
rs515135	G/A (A)	Dominant	1.51 (0.956-2.393)	0.076	84
		Recessive	1.01 (1.005-1.023)	0.37	NA
		Allele	1.33 (0.871-2.030)	0.1852	32
rs579459	C/T (T)	Dominant	2.03 (0.717-5.754)	0.216	50
		Recessive	1.79 (1.160-2.757)	0.008	97
		Allele	1.68 (1.154-2.436)	0.00623	90
rs8055236	G/T (T)	Dominant	1.07 (0.656-1.740)	0.792	7
		Recessive	1.14 (0.132-9.873)	1	4
		Allele	1.06 (0.678-1.671)	0.7871	6

Abbreviations: CI, confidence interval; SNP, single-nucleotide polymorphism.

rs8055236 and susceptibility of coronary heart disease.

The SNP rs515135 is located on chromosome 2p23-24 where locates the APOB gene. APOB is the main apolipoprotein component of LDL-C and it plays a central role in lipid metabolism as the major protein component of very-low-density lipoprotein (VLDL) and LDL, and serves as the ligand for the removal of LDL from circulation [22]. The gene encoding for APOB has been cloned and sequenced. It is 43 kb in length

with 29 exons [23]. The APOB gene is known to have several sequences that serve as recognition sites for various restriction enzymes. Several polymorphisms have been identified in the APOB gene, which can result in the deletion of amino acids from the signal peptide and have been postulated to affect APOB secretion and assembly with lipids. In our study, polymorphisms of rs515-135 in the APOB gene in CHD patients were significantly higher compared to healthy controls, suggesting that these variants increase the risk of CHD.

Stratified analysis of rs-579459 revealed that in the recessive mode of inheritance (where C/T+ C/C is compared with T/T), the severe coronary artery disease group had significant ORs compared with both the controls and the group with mild coronary artery disease. The ORs were maintained after Bonferroni's correction (OR: 1.79, 95% CI: 1.16-2.76, *p*-value 0.008, respectively). The ratio of the severe to mild OR for rs579459 (recessive model) is presented

in **Table 4** (1.79/0.95=1.88). The result indicates that rs579459 is strongly associated with severe coronary artery disease. No association was detected for the other four SNPs regarding the severity of coronary artery disease.

The ABO SNP rs579459 had strong association with severe coronary artery disease in our data. Other GWAS studies have also identified ABO as a locus for low density lipoprotein (LDL-C) [24], type-2 diabetes [25], inflammatory risk

biomarkers E-selectin, P-selectin, and sICAM1 [25-27]. These findings suggested that ABO might modulate multiple pathways relating to cardiovascular risk factors, atherosclerosis and thrombosis. The genetic variation(s) in ABO might affect low density lipoprotein and coronary artery disease formation, although further studies are needed to explore its precise role in the development of CHD. There is limitation in the present study, as the cohort is of a limited size, and may not have sufficient power to detect SNPs with a small effect with a true association. Further studies in other ethnic groups and functional studies of the selected SNPs are needed in order to provide more insights into the biological relevance of CHD.

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### Declaration of conflict of interest

None.

**Address correspondence to:** Haiqin Tang, Department of Cardiology, The First Affiliated Hospital, Anhui Medical University, Hefei, 230032, China. E-mail: tanghq898@sina.com; Dr. Wen-Sheng Lu, Department of Dermatology, Affiliated Provincial Hospital, Anhui Medical University, Hefei, Anhui, 230001, China. E-mail: luws2004@sina.com

### References

- [1] Lopez AD, Mathers CD, Ezzati M, Jamison DT and Murray CJ. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. *Lancet* 2006; 367: 1747-1757.
- [2] Mathers CD and Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med* 2006; 3: e442.
- [3] Marenberg ME, Risch N, Berkman LF, Floderus B and de Faire U. Genetic susceptibility to death from coronary heart disease in a study of twins. *New Engl J Med* 1994; 330: 1041-1046.
- [4] Schunkert H, König IR, Kathiresan S, Reilly MP, Assimes TL, Holm H, Preuss M, Stewart AF, Barbalic M, Gieger C, Absher D, Aherrahrou Z, Allayee H, Altshuler D, Anand SS, Andersen K, Anderson JL, Ardissino D, Ball SG, Balmforth AJ, Barnes TA, Becker DM, Becker LC, Berger K, Bis JC, Boekholdt SM, Boerwinkle E, Braund PS, Brown MJ, Burnett MS, Buyschaert I, Carlquist JF, Chen L, Cichon S, Codd V, Davies RW, Dedoussis G, Dehghan A, Demissie S, Devaney JM, Diemert P, Do R, Doering A, Eifert S, Mokhtari NE, Ellis SG, Elosua R, Engert JC, Epstein SE, de Faire U, Fischer M, Folsom AR, Freyer J, Gigante B, Girelli D, Gretarsdottir S, Gudnason V, Gulcher JR, Halperin E, Hammond N, Hazen SL, Hofman A, Horne BD, Illig T, Iribarren C, Jones GT, Jukema JW, Kaiser MA, Kaplan LM, Kastelein JJ, Khaw KT, Knowles JW, Kolovou G, Kong A, Laaksonen R, Lambrechts D, Leander K, Lettre G, Li M, Lieb W, Loley C, Lotery AJ, Mannucci PM, Maouche S, Martinelli N, McKeown PP, Meisinger C, Meitinger T, Melander O, Merlini PA, Mooser V, Morgan T, Muhleisen TW, Muhlestein JB, Munzel T, Musunuru K, Nahrstaedt J, Nelson CP, Nothen MM, Olivieri O, Patel RS, Patterson CC, Peters A, Peyvandi F, Qu L, Quyyumi AA, Rader DJ, Rallidis LS, Rice C, Rosendaal FR, Rubin D, Salomaa V, Sampietro ML, Sandhu MS, Schadt E, Schafer A, Schillert A, Schreiber S, Schrezenmeir J, Schwartz SM, Siscovick DS, Sivananthan M, Sivapalaratnam S, Smith A, Smith TB, Snoop JD, Soranzo N, Spertus JA, Stark K, Stirrups K, Stoll M, Tang WH, Tennstedt S, Thorgeirsson G, Thorleifsson G, Tomaszewski M, Uitterlinden AG, van Rij AM, Voight BF, Wareham NJ, Wells GA, Wichmann HE, Wild PS, Willenborg C, Witteman JC, Wright BJ, Ye S, Zeller T, Ziegler A, Cambien F, Goodall AH, Cupples LA, Quertermous T, Marz W, Hengstenberg C, Blankenberg S, Ouwehand WH, Hall AS, Deloukas P, Thompson JR, Stefansson K, Roberts R, Thorsteinsdottir U, O'Donnell CJ, McPherson R, Erdmann J and Samani NJ. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. *Nat Genet* 2011; 43: 333-338.
- [5] Peden J, Hopewell J, Saleheen D, Chambers J, Hager J, Soranzo N, Collins R, Danesh J, Elliott P, Farrall M, Stirrups K, Zhang W, Hamsten A, Parish S, Lathrop M, Watkins H, Clarke R, Deloukas P, Kooner J, Goel A, Ongen H, Strawbridge R, Heath S, Mälarstig A, Helgadottir A, Öhrvik J, Murtaza M, Potter S, Hunt S, Delepine M, Jalilzadeh S, Axelsson T, Syvanen A, Gwilliam R, Bumpstead S, Gray E, Edkins S, Folkersen L, Kyriakou T, Franco-Cereceda A, Gabrielsen A, Seedorf U, MuTHER C, Eriksson P, Offer A, Bowman L, Sleight P, Armitage J, Peto R, Abecasis G, Ahmed N, Caulfield M, Donnelly P, Froguel P, Kooner A, McCarthy M, Samani N, Scott J, Sehmi J, Silveira A, Hellénus M, van't Hooft F, Olsson G, Rust S, Assman G, Barlera S, Tognoni G,

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- Franzosi M, Linksted P, Green F, Rasheed A, Zaidi M, Shah N, Samuel M, Mallick N, Azhar M, Zaman K, Samad A, Ishaq M, Gardezi A, Fazal-ur-Rehman M, Frossard P, Spector T, Peltonen L, Nieminen M, Sinisalo J, Salomaa V, Ripatti S, Bennett D, Leander K, Gigante B, de Faire U, Pietri S, Gori F, Marchioli R, Sivapalaratnam S, Kastelein J, Trip M, Theodoraki E, Dedoussis G, Engert J, Yusuf S and Anand S. A genome-wide association study in Europeans and South Asians identifies five new loci for coronary artery disease. *Nat Genet* 2011; 43: 339-344.
- [6] Clarke R, Peden JF, Hopewell JC, Kyriakou T, Goel A, Heath SC, Parish S, Barlera S, Franzosi MG, Rust S, Bennett D, Silveira A, Malarstig A, Green FR, Lathrop M, Gigante B, Leander K, de Faire U, Seedorf U, Hamsten A, Collins R, Watkins H and Farrall M. Genetic variants associated with Lp(a) lipoprotein level and coronary disease. *New Engl J Med* 2009; 361: 2518-2528.
- [7] Samani NJ, Erdmann J, Hall AS, Hengstenberg C, Mangino M, Mayer B, Dixon RJ, Meitinger T, Braund P, Wichmann HE, Barrett JH, König IR, Stevens SE, Szymczak S, Tregouet DA, Iles MM, Pahlke F, Pollard H, Lieb W, Cambien F, Fischer M, Ouwehand W, Blankenberg S, Balmforth AJ, Baessler A, Ball SG, Strom TM, Braenne I, Gieger C, Deloukas P, Tobin MD, Ziegler A, Thompson JR and Schunkert H. Genomewide association analysis of coronary artery disease. *New Engl J Med* 2007; 357: 443-453.
- [8] Erdmann J, Grosshennig A, Braund PS, König IR, Hengstenberg C, Hall AS, Linsel-Nitschke P, Kathiresan S, Wright B, Tregouet DA, Cambien F, Bruse P, Aherrahrou Z, Wagner AK, Stark K, Schwartz SM, Salomaa V, Elosua R, Melander O, Voight BF, O'Donnell CJ, Peltonen L, Siscovick DS, Altshuler D, Merlini PA, Peyvandi F, Bernardinelli L, Ardissino D, Schillert A, Blankenberg S, Zeller T, Wild P, Schwarz DF, Tiret L, Perret C, Schreiber S, El Mokhtari NE, Schafer A, Marz W, Renner W, Bugert P, Kluter H, Schrezenmeier J, Rubin D, Ball SG, Balmforth AJ, Wichmann HE, Meitinger T, Fischer M, Meisinger C, Baumert J, Peters A, Ouwehand WH, Deloukas P, Thompson JR, Ziegler A, Samani NJ and Schunkert H. New susceptibility locus for coronary artery disease on chromosome 3q22.3. *Nat Genet* 2009; 41: 280-282.
- [9] Kathiresan S, Voight BF, Purcell S, Musunuru K, Ardissino D, Mannucci PM, Anand S, Engert JC, Samani NJ, Schunkert H, Erdmann J, Reilly MP, Rader DJ, Morgan T, Spertus JA, Stoll M, Girelli D, McKeown PP, Patterson CC, Siscovick DS, O'Donnell CJ, Elosua R, Peltonen L, Salomaa V, Schwartz SM, Melander O, Altshuler D, Merlini PA, Berzuini C, Bernardinelli L, Peyvandi F, Tubaro M, Celli P, Ferrario M, Fétiqueau R, Marziliano N, Casari G, Galli M, Ribichini F, Rossi M, Bernardi F, Zonzin P, Piazza A, Yee J, Friedlander Y, Marrugat J, Lucas G, Subirana I, Sala J, Ramos R, Meigs JB, Williams G, Nathan DM, MacRae CA, Havulinna AS, Berglund G, Hirschhorn JN, Asselta R, Duga S, Sreafico M, Daly MJ, Nemesh J, Korn JM, McCarroll SA, Surti A, Guiducci C, Gianniny L, Mirel D, Parkin M, Burt N, Gabriel SB, Thompson JR, Braund PS, Wright BJ, Balmforth AJ, Ball SG, Hall A, Linsel-Nitschke P, Lieb W, Ziegler A, König I, Hengstenberg C, Fischer M, Stark K, Grosshennig A, Preuss M, Wichmann HE, Schreiber S, Ouwehand W, Deloukas P, Scholz M, Cambien F, Li M, Chen Z, Wilensky R, Matthai W, Qasim A, Hakonarson HH, Devaney J, Burnett MS, Pichard AD, Kent KM, Satler L, Lindsay JM, Waksman R, Knouff CW, Waterworth DM, Walker MC, Mooser V, Epstein SE, Scheffold T, Berger K, Hogue A, Martinelli N, Olivieri O, Corrocher R, McKeown P, Erdmann E, König IR, Holm H, Thorleifsson G, Thorsteinsdottir U, Stefansson K, Do R, Xie C and Siscovick D. Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. *Nat Genet* 2009; 41: 334-341.
- [10] Soranzo N, Spector TD, Mangino M, Kuhnel B, Rendon A, Teumer A, Willenborg C, Wright B, Chen L, Li M, Salo P, Voight BF, Burns P, Laskowski RA, Xue Y, Menzel S, Altshuler D, Bradley JR, Bumpstead S, Burnett MS, Devaney J, Doring A, Elosua R, Epstein SE, Erber W, Falchi M, Garner SF, Ghori MJ, Goodall AH, Gwilliam R, Hakonarson HH, Hall AS, Hammond N, Hengstenberg C, Illig T, König IR, Knouff CW, McPherson R, Melander O, Mooser V, Nauck M, Nieminen MS, O'Donnell CJ, Peltonen L, Potter SC, Prokisch H, Rader DJ, Rice CM, Roberts R, Salomaa V, Sambrook J, Schreiber S, Schunkert H, Schwartz SM, Serbanovic-Canic J, Sinisalo J, Siscovick DS, Stark K, Surakka I, Stephens J, Thompson JR, Volker U, Volzke H, Watkins NA, Wells GA, Wichmann HE, Van Heel DA, Tyler-Smith C, Thein SL, Kathiresan S, Perola M, Reilly MP, Stewart AF, Erdmann J, Samani NJ, Meisinger C, Greinacher A, Deloukas P, Ouwehand WH and Gieger C. A genome-wide meta-analysis identifies 22 loci associated with eight hematological parameters in the HaemGen consortium. *Nat Genet* 2009; 41: 1182-1190.
- [11] Wang F, Xu CQ, He Q, Cai JP, Li XC, Wang D, Xiong X, Liao YH, Zeng QT, Yang YZ, Cheng X, Li C, Yang R, Wang CC, Wu G, Lu QL, Bai Y, Huang YF, Yin D, Yang Q, Wang XJ, Dai DP, Zhang RF,



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- Wan J, Ren JH, Li SS, Zhao YY, Fu FF, Huang Y, Li QX, Shi SW, Lin N, Pan ZW, Li Y, Yu B, Wu YX, Ke YH, Lei J, Wang N, Luo CY, Ji LY, Gao LJ, Li L, Liu H, Huang EW, Cui J, Jia N, Ren X, Li H, Ke T, Zhang XQ, Liu JY, Liu MG, Xia H, Yang B, Shi LS, Xia YL, Tu X and Wang QK. Genome-wide association identifies a susceptibility locus for coronary artery disease in the Chinese Han population. *Nat Genet* 2011; 43: 345-349.
- [12] Butterworth A, Braund P, Farrall M, Hardwick R, Saleheen D, Peden J, Soranzo N, Chambers J, Sivapalaratnam S, Kleber M, Keating B, Qasim A, Klopp N, Erdmann J, Assimes T, Ball S, Balmforth A, Barnes T, Basart H, Baumert J, Bezzina C, Boerwinkle E, Boehm B, Brocheton J, Bugert P, Cambien F, Clarke R, Codd V, Collins R, Couper D, Cupples L, de Jong J, Diemert P, Ejebe K, Elbers C, Elliott P, Fornage M, Franzosi M, Frossard P, Garner S, Goel A, Goodall A, Hengstenberg C, Hunt S, Kastelein J, Klungel O, Klüter H, Koch K, König I, Kooner A, Laaksonen R, Lathrop M, Li M, Liu K, McPherson R, Musameh M, Musani S, Nelson C, O'Donnell C, Ongen H, Papanicolaou G, Peters A, Peters B, Potter S, Psaty B, Qu L, Rader D, Rasheed A, Rice C, Scott J, Seedorf U, Sehmi J, Sotoodehnia N, Stark K, Stephens J, van der Schoot C, van der Schouw Y, Thorsteinsdottir U, Tomaszewski M, van der Harst P, Vasani R, Wilde A, Willenborg C, Winkelmann B, Zaidi M, Zhang W, Ziegler A, de Bakker P, Koenig W, Mätz W, Trip M, Reilly M, Kathiresan S, Schunkert H, Hamsten A, Hall A, Kooner J, Thompson S, Thompson J, Deloukas P, Ouwehand W, Watkins H, Danesh J and Samani N. Large-scale gene-centric analysis identifies novel variants for coronary artery disease. *PLoS Genet* 2011; 7: e1002260.
- [13] Gensini GG. A more meaningful scoring system for determining the severity of coronary heart disease. *Am J Cardiol* 1983; 51: 606.
- [14] Kuulasmaa K, Tunstall-Pedoe H, Dobson A, Fortmann S, Sans S, Tolonen H, Evans A, Ferrario M and Tuomilehto J. Estimation of contribution of changes in classic risk factors to trends in coronary-event rates across the WHO MONICA Project populations. *Lancet* 2000; 355: 675-687.
- [15] Kathiresan S, Manning AK, Demissie S, D'Agostino RB, Surati A, Guiducci C, Gianniny L, Burt NP, Melander O, Orho-Melander M, Arnett DK, Peloso GM, Ordovas JM and Cupples LA. A genome-wide association study for blood lipid phenotypes in the Framingham Heart Study. *BMC Med Genet* 2007; 8 Suppl 1: S17.
- [16] Lanktree MB and Hegele RA. Gene-gene and gene-environment interactions: new insights into the prevention, detection and management of coronary artery disease. *Genome Med* 2009; 1: 28.
- [17] Ozaki K, Ohnishi Y, Iida A, Sekine A, Yamada R, Tsunoda T, Sato H, Hori M, Nakamura Y and Tanaka T. Functional SNPs in the lymphotoxin-alpha gene that are associated with susceptibility to myocardial infarction. *Nat Genet* 2002; 32: 650-654.
- [18] McPherson R, Pertsemlidis A, Kavaslar N, Stewart A, Roberts R, Cox DR, Hinds DA, Pennacchio LA, Tybjaerg-Hansen A, Folsom AR, Boerwinkle E, Hobbs HH and Cohen JC. A common allele on chromosome 9 associated with coronary heart disease. *Science* 2007; 316: 1488-1491.
- [19] Burton P, Clayton D, Cardon L, Craddock N, Deloukas P, Duncanson A, Kwiatkowski D, McCarthy M, Ouwehand W, Samani N, Todd J, Donnelly P, Barrett J, Burton P, Davison D, Donnelly P, Easton D, Evans D, Leung H, Marchini J, Morris A, Spencer C, Tobin M, Cardon L, Clayton D, Attwood A, Boorman J, Cant B, Everson U, Hussey J, Jolley J, Knight A, Koch K, Meech E, Nutland S, Prowse C, Stevens H, Taylor N, Walters G, Walker N, Watkins N, Winzer T, Todd J, Ouwehand W, Jones R, McArdle W, Ring S, Strachan D, Pembrey M, Breen G, St C, Caesar S, Gordon-Smith K, Jones L, Fraser C, Green E, Grozeva D, Hamshere M, Holmans P, Jones I, Kirov G, Moskvina V, Nikolov I, O'Donovan M, Owen M, Craddock N, Collier D, Elkin A, Farmer A, Williamson R, McGuffin P, Young A, Ferrier I, Ball S, Balmforth A, Barrett J, Bishop D, Iles M, Maqbool A, Yuldasheva N, Hall A, Braund P, Burton P, Dixon R, Mangino M, Suzanne S, Tobin M, Thompson J, Samani N, Bredin F, Tremelling M, Parkes M, Drummond H, Lees C, Nimmo E, Satsangi J, Fisher S, Forbes A, Lewis C, Onnie C, Prescott N, Sanderson J, Mathew C, Barbour J, Mohiuddin M, Todhunter C, Mansfield J, Ahmad T, Cummings F, Jewell D, Webster J, Brown M, Clayton D, Lathrop G, Connell J, Dominczak A, Samani N, Marcano C, Burke B, Dobson R, Gungadoo J, Lee K, Munroe P, Newhouse S, Onipinla A, Wallace C, Xue M, Caulfield M, Farrall M, Barton A, Bruce I, Donovan H, Eyre S, Gilbert P, Hider S, Hinks A, John S, Potter C, Silman A, Symmons D, Thomson W, Worthington J, Clayton D, Dunger D, Nutland S, Stevens H, Walker N, Widmer B, Todd J, Frayling T, Freathy R, Lango H, Perry J, Shields B, Weedon M, Hattersley A, Hitman G, Walker M, Elliott K, Groves C, Lindgren C, Rayner N, Timpson N, Zeggini E, McCarthy M, Newport M, Sirugo G, Lyons E, Vannberg F, Hill A, Bradbury L, Farrar C, Pointon J, Wordsworth P, Brown M, Franklyn J, Heward J, Simmonds M, Gough S, Seal S, Stratton M, Rahman N,

## Association of gene loci with coronary heart disease

- Ban M, Goris A, Sawcer S, Compston A, Conway D, Jallow M, Newport M, Sirugo G, Rockett K, Kwiatowski D, Bumpstead S, Chaney A, Downes K, Ghorri M, Gwilliam R, Hunt S, Inouye M, Keniry A, King E, McGinnis R, Potter S, Ravindrarajah R, Whittaker P, Widdon C, Withers D, Deloukas P, Leung H, Nutland S, Stevens H, Walker N, Todd J, Easton D, Clayton D, Burton P, Tobin M, Barrett J, Evans D, Morris A, Cardon L, Cardin N, Davison D, Ferreira T, Pereira-Gale J, Hallgrimsdottir I, Howie B, Marchini J, Spencer C, Su Z, Teo Y, Vukcevic D, Donnelly P, Bentley D, Brown M, Gordon L, Caulfield M, Clayton D, Compston A, Craddock N, Deloukas P, Donnelly P, Farrall M, Gough S, Hall A, Hattersley A, Hill A, Kwiatkowski D, Mathew C, McCarthy M, Ouwehand W, Parkes M, Pembrey M, Rahman N, Samani N, Stratton M, Todd J and Worthington J. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007; 447: 661-678.
- [20] Ding H, Xu Y, Wang X, Wang Q, Zhang L, Tu Y, Yan J, Wang W, Hui R, Wang CY and Wang DW. 9p21 is a shared susceptibility locus strongly for coronary artery disease and weakly for ischemic stroke in Chinese Han population. *Circ-Cardiovasc Gene* 2009; 2: 338-346.
- [21] Lv X, Zhang Y, Rao S, Qiu J, Wang M, Luo X, Zuo X, Su D, Feng X, Yang Y, Ouyang P, Chen Y, Li X, Xiao Y and Ling W. Joint effects of genetic variants in multiple loci on the risk of coronary artery disease in Chinese Han subjects. *Circ J* 2012; 76: 1987-1992.
- [22] Machado MO, Hirata MH, Bertolami MC and Hirata RD. Apo B gene haplotype is associated with lipid profile of higher risk for coronary heart disease in Caucasian Brazilian men. *J Clin Lab Anal* 2001; 15: 19-24.
- [23] Ludwig EH, Blackhart BD, Pierotti VR, Caiati L, Fortier C, Knott T, Scott J, Mahley RW, Levy-Wilson B and McCarthy BJ. DNA sequence of the human apolipoprotein B gene. *DNA* 1987; 6: 363-372.
- [24] Chasman DI, Pare G, Mora S, Hopewell JC, Peloso G, Clarke R, Cupples LA, Hamsten A, Kathiresan S, Malarstig A, Ordovas JM, Ripatti S, Parker AN, Miletich JP and Ridker PM. Forty-three loci associated with plasma lipoprotein size, concentration, and cholesterol content in genome-wide analysis. *PLoS Genet* 2009; 5: e1000730.
- [25] Qi L, Cornelis MC, Kraft P, Jensen M, van Dam RM, Sun Q, Girman CJ, Laurie CC, Mirel DB, Hunter DJ, Rimm E and Hu FB. Genetic variants in ABO blood group region, plasma soluble E-selectin levels and risk of type 2 diabetes. *Hum Mol Genet* 2010; 19: 1856-1862.
- [26] Paterson AD, Lopes-Virella MF, Waggott D, Boright AP, Hosseini SM, Carter RE, Shen E, Mirea L, Bharaj B, Sun L and Bull SB. Genome-wide association identifies the ABO blood group as a major locus associated with serum levels of soluble E-selectin. *Arterioscl Thromb Vas* 2009; 29: 1958-1967.
- [27] Barbalic M, Dupuis J, Dehghan A, Bis JC, Hoogeveen RC, Schnabel RB, Nambi V, Bretler M, Smith NL, Peters A, Lu C, Tracy RP, Aleksic N, Heeriga J, Keaney JF Jr, Rice K, Lip GY, Vasan RS, Glazer NL, Larson MG, Uitterlinden AG, Yamamoto J, Durda P, Haritunians T, Psaty BM, Boerwinkle E, Hofman A, Koenig W, Jenny NS, Witteman JC, Ballantyne C and Benjamin EJ. Large-scale genomic studies reveal central role of ABO in sP-selectin and sICAM-1 levels. *Hum Mol Genet* 2010; 19: 1863-1872.