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ORIGINAL ARTICLE

Investigation of associations between ten polymorphisms and the risk of coronary artery disease in Southern Han Chinese

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A large-scale meta-analysis of 14 genome-wide association studies has identified and replicated a series of susceptibility polymorphisms for coronary artery disease (CAD) in European ancestry populations, but evidences for the associations of these loci with CAD in other ethnicities remain lacking. Herein we investigated the associations between ten (rs579459, rs12413409, rs964184, rs4773144, rs2895811, rs3825807, rs216172, rs12936587, rs46522 and rs3798220) of these loci and CAD in Southern Han Chinese (CHS). Genotyping was performed in 1716 CAD patients and 1572 controls using mass spectrography. Both allelic and genotypic associations of rs964184, rs2895811 and rs3798220 with CAD were significant, regardless of adjustment for covariates of gender, age, hypertension, type 2 diabetes, blood lipid profiles and smoking. Significant association of rs12413409 was initially not observed, but after the adjustment for the covariates, both allelic and genotypic associations were identified as significant. Neither allelic nor genotypic association of the other six polymorphisms with CAD was significant regardless of the adjustment. Our results indicated that four loci of the total 10 were associated with CAD in CHS. Therefore, some of the CAD-related loci in European ancestry populations are indeed susceptibility loci for the risk of CAD in Han Chinese.
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INTRODUCTION

It is estimated that genetic factors contribute to about half of coronary artery disease (CAD) cases.[1] As early as in 2003, mutation of *MEF2A* was reported to cause a familial CAD.[2] Afterwards, genome-wide association studies had identified dozens of independent CAD susceptibility loci.[3,4] Some of these loci appeared to confer risk regardless of ethnic differences, such as variants in *CDKN2A/CDKN2B* on 9p21.3,[5–8] while others were revealed to function in an ethnicity-specific manner.[9]

A recent large-scale meta-analysis of 14 genome-wide association studies had identified 13 novel susceptibility loci (rs17114036 on 1p32.2, rs17609940 on 6p21.31, rs12190287 on 6q23.2, rs11556924 on 7q32.2, rs579459 on 9q34.2, rs12413409 on 10q24.32, rs964184 on 11q23.3, rs4773144 on 13q34, rs2895811 on 14q32.2, rs3825807 on 15q25.1, rs216172 on 17p13.3, rs12936587 on 17p11.2 and rs46522 on 17q21.32) for CAD in subjects with European descent.[10] However, evidences on the associations of these single nucleotide polymorphisms (SNPs) with CAD in other ethnicities remain quite inadequate. Moreover, several previously identified CAD loci (rs6725887 on 2q33.1, rs2306374 on 3q22.3, rs12526453 on 6p24.1, rs3798220 on 6q25.3, rs9982601 on 21q22.11, rs3184504 on 12q24.12 and rs11206510 on 1p32.3) were replicated in the report. Likewise, evidences about the associations of these loci with CAD in non-European populations remain insufficient.

Recently, we attempted to perform a case-control study in Southern Han Chinese (CHS). Nine of these loci were excluded from genotyping because there minor allele frequency was $<5\%$ in Han Chinese in Beijing or CHS. Rs12190287 was excluded as well owing to the genotyping conflict in mass spectrometry used in this study. Finally, ten polymorphisms (rs579459, rs12413409, rs964184, rs4773144, rs2895811, rs3825807, rs216172, rs12936587, rs46522 and rs3798220) were genotyped and their associations with CAD in CHS were analyzed.

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MATERIALS AND METHODS

Study population and classification

In this study we selected 1716 CAD patients and 1572 control subjects. All selected participants were screened at the Conduit Room, Department of Cardiology, the First Affiliated Hospital of Sun Yat-Sen University (Guangzhou, China), and were with the ethnic origin Han and the ancestral home from Guangdong. All the subjects were informed to participate in this study and written consent were obtained from them. This study was approved by the ethics committee of Sun Yat-Sen University and complied with the Declaration of Helsinki.

According to the diagnostic guideline declared by American Heart Association, participants with eligible luminal stenosis in main coronary arteries diagnosed by coronary angiography or with myocardial infarction (MI) were classified in CAD group. Subjects with classic pain lasting longer than half an hour, characteristically ischemic electrocardiographic patterns and elevation of cardiac enzymes were diagnosed as MI patients. We excluded those with congenital heart disease, childhood hypertension, type 1 diabetes, myocardial bridge or myocardial spasms. Participants without luminal stenosis in coronary artery diagnosed by coronary angiography and older than 45 (male)/50 (female) years were classified as controls. Information about gender, age, blood lipid profiles, diabetes, hypertension and smoking habits was also collected. Diabetes was diagnosed as a fasting plasma glucose concentration $\geqslant 7.0$ mmol l^{-1}. Hypertension was defined as systolic blood pressure $\geqslant 140$ mm Hg/diastolic blood pressure $\geqslant 90$ mm Hg. Not enough information about medications for lipids, diabetes and hypertension of most subjects were obtained. Therefore, to avoid the influences of medications on the association analysis, only subjects who were confirmed to have never taken medications for lipids, diabetes and hypertension were included for genotyping.

DNA preparation

Peripheral blood was collected, and genomic DNA was extracted from white cells using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to the manufacturer's protocol.

SNP genotyping

SNP genotyping was performed using MassARRAY Genetic Analysis System (Sequenom, San Diego, CA, USA). Primers were designed by software AssayDesigner3.1. The procedure of genotyping was composed of PCR, SAP and extension reaction. The PCR volume contained 0.2 μl of 5 U μl^{-1} HotStar Taq enzyme, 0.4 μl of 25 mM $MgCl_2$, 0.1 μl of 25 mM dNTP Mix, 1 μl of 0.5 μM primer Mix, 1 μl of 10 ng μl^{-1} DNA template, 0.5 μl of 10x PCR buffer and 1.8 μl of water. PCR program was initial denaturing in 94 °C for 15 min, denaturing in 94 °C for 20 s, annealing in 56 °C for 30 s, extension in 72 °C for 1 min, 45 cycles of the denaturing, annealing and extension, final extension in 72 °C for 3 min. The volume of SAP reaction contained 0.17 μl of SAP buffer, 0.3 μl of 1.7 U μl^{-1} SAP enzyme, 1.53 μl of nanopure water. The program of SAP reaction was incubation in 37 °C for 40 min and in 85 °C for 5 min. The volume of extension reaction contained 0.041 μl of iPLEX Enzyme, 0.619 μl of nanopure water, 0.2 μl of iPLEX Buffer Plus, 0.94 μl of iPLEX Extend Primer Mix, 0.2 μl of iPLEX Termination Mix, 7 μl of PCR+SAP product. The program of extension reaction was initial denaturing in 94 °C for 30 s, denaturing in 94 °C for 5 s, annealing in 52 °C for 5 s, extension in 80 °C for 5 s, 40 cycles of the denaturing, annealing and extension, each of the cycle included 5 cycles of the annealing plus extension, then final extension in 72 °C for 3 min.

Table 1 Clinical and biochemical characteristics of the subjects

Characteristics	*Cases* (n = *1716*)	*Controls* (n = *1572*)	P-*value*
Gender (male/female)	1109/607	858/714	0.004
Age[a] (years)	64.18 ± 11.321	60.73 ± 11.628	0.001
Hypertension (n/%)	1098/64.0%	835/53.1%	0.017
T2DM (n/%)	399/23.3%	157/10.0%	0.011
TCH (mmol l^{-1})	4.44 ± 1.311	4.56 ± 1.173	0.054
TG (mmol l^{-1})	1.281 ± 0.426	1.167 ± 0.407	0.022
LDL-C (mmol l^{-1})	2.96 ± 1.040	2.83 ± 1.312	0.018
HDL-C (mmol l^{-1})	1.05 ± 0.237	1.23 ± 0.389	0.002
Smoker (n/%)	321/18.7%	125/7.95%	0.009

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TCH, total cholesterol; TG, triglyceride; T2DM, type 2 diabetes mellitus.
[a]Age at the first diagnosis for CAD and age at the time of enrollment for control.

Statistic analysis

Statistic analysis was performed using Statistics Package for Social Sciences software (SPSS, version 13.0, Chicago, IL, USA). Each SNP was tested for deviation from Hardy–Weinberg equilibrium (HWE) using the χ^2 test. Linkage disequilibrium (LD) was analyzed by HaploView (version 4.2, Cambridge, MA, USA). Allelic and genotypic associations of SNPs with CAD were tested using Pearson's χ^2 test or Fisher exact test. Odds ratio (OR) and 95% confidence interval (CI) were estimated using the χ^2 test. Other risk factors for CAD were incorporated as covariates by multivariable logistic regression analysis. Statistical power was calculated with Quanto system.

RESULTS

Characteristics of the subjects

In all, 1716 cases and 1572 controls were enrolled and successfully genotyped. Their clinical characteristics are shown in Table 1. The rate of males in cases was higher than in controls ($P = 0.004$). The average age of cases was higher than that of controls ($P = 0.001$). Hypertension, type 2 diabetes and smoking were more prevalent among cases than controls ($P = 0.017$, 0.011 and 0.009, respectively). The average levels of triglyceride and low-density lipoprotein cholesterol in cases were higher than in controls ($P = 0.022$ and 0.018), and the average level of high-density lipoprotein cholesterol in cases was lower than in controls ($P = 0.002$). No significant difference was observed in the levels of total cholesterol ($P = 0.054$).

Genotyping results

For each locus, the observed genotype frequencies did not deviate significantly from HWE expectations ($P_{HWE} \geqslant 0.05$, Table 2). No significant LD among these loci was observed ($r^2 \leqslant 0.11$, data not shown).

Allelic associations of four loci with CAD were revealed (Table 2). The risk allele G of rs964184 had an observed OR_{obs} of 3.104 (95% CI = 1.402–7.878, $P_{obs} = 0.001$). The risk allele C of rs2895811 had an OR_{obs} of 1.595 (95% CI = 1.023–2.477, $P_{obs} = 0.037$), and rs3798220 also had a risk allele C with an OR_{obs} of 2.714 (95% CI = 1.266–5.323, $P_{obs} = 0.006$). After adjusting for the covariates of gender, age, hypertension, type 2 diabetes, lipid profiles and smoking status, the allelic associations of the three loci remained ($P_{adj} = 0.020$, $OR_{adj} = 1.848$, 95% CI = 1.127–3.915 for rs964184, $P_{adj} = 0.004$, $OR_{adj} = 1.965$, 95% CI = 1.226–3.000 for rs2895811, and $P_{adj} = 0.015$, $OR_{adj} = 2.571$, 95% CI = 1.147–5.182 for rs3798220). Observed allelic association of rs12413409 was not significant ($P_{obs} = 0.185$); however, after adjusting for the covariates, the association became significant ($P_{adj} = 0.011$, $OR_{adj} = 2.135$, 95% CI = 1.203–5.697). We did not note significant allelic associations between the other six loci and CAD in our population ($P > 0.05$, Table 2). Statistical power estimate indicated that the four replicating loci had strong power, rs216172 and rs46522 had moderate power (0.238 and 0.154, respectively), but rs579459, rs4773144, rs3825807 and rs12936587 had poor power (Table 2).

Genotypic associations of rs12413409, rs964184, rs2895811 and rs3798220 with CAD were also revealed (Table 3). After adjusting for the covariates, rs12413409 was significantly associated with CAD under either a recessive ($P_{adj} = 0.017$, $OR_{adj} = 3.384$, 95%

Table 2 Analysis of allelic association of ten SNPs with CAD

SNP	Band	Gene	Risk allele frequency (case/control)	P_{HWE}	P_{obs}	OR_{obs} (95% CI)	P_{adj}	OR_{adj} (95% CI)	Power	$N_{0.8}$
rs579459	9q34.2	*ABO*	C (0.199/0.174)	0.050	0.436	1.279 (0.713, 2.452)	0.369	1.082 (0.667,1.462)	0.082	326 113
rs12413409	10q24.32	*CYP17A1, CNNM2, NT5C2*	G (0.749/0.710)	0.616	0.185	1.212 (0.841, 1.579)	0.011	2.135 (1.203,5.697)	0.866	2680
rs964184	11q23.3	*ZNF259, APOA5-A4-C3-A1*	G (0.227/0.180)	0.471	0.001	3.104 (1.402, 7.878)	0.020	1.848 (1.127,3.915)	0.642	5045
rs4773144	13q34	*COL4A1, COL4A2*	G (0.406/0.392)	0.325	0.904	1.061 (0.775, 1.623)	0.813	1.046 (0.701,1.348)	0.071	636 062
rs2895811	14q32.2	*HHIPL1*	C (0.330/0.249)	0.077	0.037	1.595 (1.023, 2.477)	0.004	1.965 (1.226,3.000)	0.804	3248
rs3825807	15q25.1	*ADAMTS7*	T (0.873/0.823)	0.146	0.276	1.348 (0.805, 2.325)	0.652	1.157 (0.733,1.921)	0.111	114 052
rs216172	17p13.3	*SMG6, SRR*	G (0.718/0.668)	0.388	0.341	1.195 (0.814, 1.892)	0.277	1.282 (0.860,1.845)	0.238	23 377
rs12936587	17p11.2	*RASD1, SMCR3, PEMT*	A (0.149/0.121)	0.324	0.563	1.207 (0.703, 2.426)	0.702	1.120 (0.643,2.063)	0.092	206 216
rs46522	17q21.32	*UBE2Z, GIP, ATP5G1, SNF8*	T (0.650/0.633)	0.708	0.829	1.030 (0.699, 1.610)	0.436	1.174 (0.787,1.810)	0.154	52 274
rs3798220	6q25.3	*LPA*	C (0.127/0.048)	0.439	0.006	2.714 (1.266, 5.323)	0.015	2.571 (1.147,5.182)	0.680	4552

Abbreviations: CI, confidence interval; MAF, minor allele frequency; $N_{0.8}$, subjects number needed for statistical power = 0.8 under an additive inheritance mode, using the same MAF and case/control ratio in present sample size; OR_{adj}, odds ratio after adjustment for the covariates; OR_{obs}, observed odds ratio; P_{adj}, *P*-value after adjusted for covariates of gender, age, hypertension, diabetes, blood lipid profiles, smoking; P_{HWE}, *P*-values for Hardy–Weinberg equilibrium tests; P_{obs}, observed *P*-value; Power, one-sided statistical power (type I error rate = 0.05) under an additive inheritance mode; SNP, single nucleotide polymorphism.

Table 3 Analysis of genotypic association of ten SNPs with CAD

SNP	Model	P_{obs}	P_{adj}	OR_{adj} (95% CI)
rs579459	Dominant	0.426	0.613	1.337 (0.683, 2.452)
	Recessive	0.721	0.469	0.714 (0.235, 2.067)
	Additive	0.187	0.605	0.969 (0.581, 1.633)
rs12413409	Dominant	0.537	0.208	1.458 (0.880, 1.943)
	Recessive	0.052	0.017	3.384 (1.266, 8.173)
	Additive	0.116	0.030	1.897 (1.197, 3.065)
rs964184	Dominant	0.009	0.019	2.254 (1.638, 7.169)
	Recessive	1.000	0.883	1.324 (0.453, 4.538)
	Additive	0.014	0.032	1.921 (1.182, 10.176)
rs4773144	Dominant	0.331	0.702	0.835 (0.744, 2.535)
	Recessive	0.167	0.356	1.292 (0.809, 2.787)
	Additive	0.690	0.874	1.065 (0.765, 1.520)
rs2895811	Dominant	0.043	0.001	3.007 (1.414, 8.336)
	Recessive	0.225	0.517	1.553 (0.676, 7.281)
	Additive	0.060	0.012	1.978 (1.204, 4.623)
rs3825807	Dominant	0.913	/	/
	Recessive	0.278	0.543	1.185 (0.572, 2.564)
	Additive	0.107	0.486	1.212 (0.810, 1.872)
rs216172	Dominant	0.772	0.434	0.645 (0.389, 2.055)
	Recessive	0.096	0.182	1.400 (0.901, 2.713)
	Additive	0.225	0.608	1.119 (0.763, 1.957)
rs12936587	Dominant	0.786	0.834	0.736 (0.515, 2.284)
	Recessive	0.088	/	/
	Additive	0.134	0.561	1.147 (0.642, 2.008)
rs46522	Dominant	0.625	0.907	0.852 (0.611, 1.735)
	Recessive	0.417	0.166	1.359 (0.887, 2.401)
	Additive	0.803	0.323	1.176 (0.755, 1.734)
rs3798220	Dominant	0.005	0.010	2.924 (1.438, 8.172)
	Recessive	1.000	/	/
	Additive	0.016	0.028	2.689 (1.177, 5.946)

Abbreviations: CI, confidence interval; OR_{adj}, odds ratio after adjustment for the covariates; P_{adj}, *P*-value after adjusted for covariates of gender, age, hypertension, diabetes, blood lipid profiles, smoking; P_{obs}, observed *P*-value; SNP, single nucleotide polymorphism.

CI = 1.266–8.173) or an additive ($P_{adj} = 0.030$, $OR_{adj} = 1.897$, 95% CI = 1.197–3.065) model. Regardless of adjustment for the covariates, rs964184 was significantly associated with CAD under either a dominant ($P_{obs} = 0.009$, $P_{adj} = 0.019$, $OR_{adj} = 2.254$, 95% CI = 1.638–7.169) or an additive ($P_{obs} = 0.014$, $P_{adj} = 0.032$, $OR_{adj} = 1.921$, 95% CI = 1.182–10.176) model. Similar result of rs3798220 was obtained (under a dominant model, $P_{obs} = 0.005$, $P_{adj} = 0.010$, $OR_{adj} = 2.924$, 95% CI = 1.438–8.172; under an additive model, $P_{obs} = 0.016$, $P_{adj} = 0.028$, $OR_{adj} = 2.689$, 95% CI = 1.177–5.946). Genotypic association of rs2895811 was also revealed under a dominant model with or without the adjustment ($P_{obs} = 0.043$, $P_{adj} = 0.001$, $OR_{adj} = 3.007$, 95% CI = 1.414–8.336), or under an additive model with the adjustment ($P_{adj} = 0.012$, $OR_{adj} = 1.978$, 95% CI = 1.204–4.623). No genotypic associations between the other loci and CAD were revealed ($P > 0.05$, Table 3).

DISCUSSION

Ten loci (rs579459, rs12413409, rs964184, rs4773144, rs2895811, rs3825807, rs216172, rs12936587, rs46522 and rs3798220) were previously identified as susceptibility loci for CAD in independent European ancestry populations.[10,11] In this study, the associations of four of these loci with CAD were reproduced in a CHS population (Tables 2 and 3).

After rs2895811 was identified as a susceptibility locus for CAD in European populations,[10] we showed the first positive result about the association between rs2895811 and non-European CAD. Shortly before the submission of this manuscript, a study investigating the association between rs2895811 and Japanese CAD was reported, but negative result was obtained.[9] One possible explanation for the inconsistence is differences between Chinese and Japanese in genetic structures and interactions with environmental factors. For example, reference to the LD structures in 1000GENOMES database (phase 3), stronger LD between rs2895811 and rs7145262 (8.2 kb away from the former) was observed in CHS ($r^2 = 0.9$, $D' = 0.973$) than in JPT ($r^2 = 0.767$, $D' = 0.897$). Possible differences in environmental factors such as latitude, geographical conditions, dietary patterns and so on, may affect fat ingestion, energy metabolism and consequently the progression of CAD.

Before and after the identification of the association of rs964184 with CAD in European populations,[10] the locus was found to be associated with plasma lipid profiles in other ethnicities.[12–16] Recently, it was shown to be associated with type 2 diabetes mellitus in Japanese and metabolic syndrome in Han Chinese.[17,18] But no sufficient evidence, indicating that rs964184 was associated with non-European CAD, is available, except for a small sample size study reporting that this SNP was associated with CAD in Han Chinese.[19] Here our data reveal rs964184 as a susceptibility locus for CAD in Han Chinese, suggesting that the susceptibility may not be European-

exclusive. Rs3798220 had been repeatedly reported to be associated with lipoprotein-A cholesterol levels and CAD in white subjects,[10,11,20–22] but investigations in other descents remain to be conducted. Herein we show that rs3798220 is significantly associated with CAD in CHS. Recently, a study showed that rs3798220 was associated with plasma triglyceride level but not with MI in a Chinese population.[23] This may be owing to the difference in population stratification between the two studies, although both of the two populations were Chinese. Subjects in the previous study were from 15 cities, most of which were in the north of China, and minor allele frequency in case and control groups were both 0.08. Although subjects in our study were all from Guangdong Province (in the south of China), and minor allele frequency in cases and controls were 0.127 and 0.048, respectively. Another explanation with relatively low likelihood is that CAD is not exclusive, albeit the most important, cause of MI. Coronary artery spasm or severe increase of oxygen consumption in myocardium may also lead to MI, which was not excluded in the report. On the other hand, CAD patients without MI symptoms in our study were not excluded from case group.

No replication study about the association between rs12413409 and CAD has been reported. In the present study, we discovered the association in CHS, with adjustment for gender, age, hypertension, type 2 diabetes, smoking and blood lipid profiles (Tables 2 and 3). Recently, rs12413409 was reported to be associated with waist/hip ratio, heart rate and MI in a Chinese population.[23] The association between this SNP and MI was replicated in Japanese population shortly before we submitted this manuscript.[24]

No associations between the other six loci (rs579459, rs4773144, rs3825807, rs216172, rs12936587 and rs46522) and CAD were disclosed in this study. However, statistical power of rs579459, rs4773144, rs3825807 and rs12936587 was poor and they would need a very large sample size to have a power of 0.8 (Table 2), so here we could not exclude the possibility that they have modest effects on CAD.

Rs579459 was recently found to associate with recurrent MI or cardiac death within 5 years after an acute coronary syndrome in Belgians.[25] Besides, it was reported to associate with circulating levels of soluble intercellular adhesion molecule-1, soluble P-selectin and soluble E-selectin (these cytokines function in leukocyte attachment to endothelia, probably the onset step in CAD) in Italians.[26] Although another study stated that this SNP does not associate with cardiovascular diseases in Spanish with rheumatoid arthritis.[27] Moreover, another study showed that it was associated with lipoprotein levels but not with MI in a Chinese population.[23]

A recent study based on Japanese population showed that rs4773144 and rs46522 were not significantly associated with coronary atherosclerosis,[9] consistent with our results.

Here we show that rs3825807 has no association with CAD in CHS. On the basis of the same cohort of Spanish with rheumatoid arthritis, rs3825807, like rs579459 was not associated with cardiovascular diseases.[27] Rs4380028 (uninvestigated in the present study), located together with rs3825807 in the gene *ADAM17TS7*, was not associated with CAD in Japanese.[9]

Except for the abovementioned study of a large-scale meta-analysis,[10] no literature about association of rs216172 and rs12936587 with CAD was retrieved in PubMed database. Their associations were not replicated in this study. Together with the fact that statistical power of rs216172 was not very poor (0.238, Table 2), it was suggested that the association between rs216172 and CAD may be independent of ethnic differences.

In conclusion, our results revealed that four of the ten loci investigated in this study were associated with CAD in CHS but the others were not. Our data suggest that some of the CAD-related loci probably confer risk regardless of ethnic differences, while the others may function in an ethnicity-specific manner. Those non-associated loci with poor statistical power may have modest effects on CAD. Therefore, large-scale population studies are needed to determine the extent of the effects.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Marenberg, M. E., Risch, N., Berkman, L. F., Floderus, B. & de Faire, U. Genetic susceptibility to death from coronary heart disease in a study of twins. *N. Engl. J. Med.* **330**, 1041–1046 (1994).
2 Wang, L., Fan, C., Topol, S. E., Topol, E. J. & Wang, Q. Mutation of MEF2A in an inherited disorder with features of coronary artery disease. *Science* **302**, 1578–1581 (2003).
3 Roberts, R. Genetics of coronary artery disease: an update. *Methodist Debakey Cardiovasc. J.* **10**, 7–12 (2014).
4 Roberts, R. Genetics of coronary artery disease. *Circ. Res.* **114**, 1890–1903 (2014).
5 Willer, C. J., Sanna, S., Jackson, A. U., Scuteri, A., Bonnycastle, L. L., Clarke, R. *et al.* Newly identified loci that influence lipid concentrations and risk of coronary artery disease. *Nat. Genet.* **40**, 161–169 (2008).
6 Myocardial Infarction Genetics Consortium, Kathiresan, S., Voight, B. F., Purcell, S., Musunuru, K., Ardissino, D. *et al.* Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. *Nat. Genet.* **41**, 334–341 (2009).
7 Coronary Artery Disease (C4D) Genetics Consortium. A genome-wide association study in Europeans and South Asians identifies five new loci for coronary artery disease. *Nat. Genet.* **43**, 339–344 (2011).
8 Lu, X., Wang, L., Chen, S., He, L., Yang, X., Shi, Y. *et al.* Genome-wide association study in Han Chinese identifies four new susceptibility loci for coronary artery disease. *Nat. Genet.* **44**, 890–894 (2012).
9 Dechamethakun, S., Ikeda, S., Arai, T., Sato, N., Sawabe, M. & Muramatsu, M. Associations between the CDKN2A/B, ADTRP and PDGFD Polymorphisms and the development of coronary atherosclerosis in Japanese patients. *J. Atheroscler. Thromb.* **21**, 680–690 (2014).
10 Schunkert, H., König, I. R., Kathiresan, S., Reilly, M. P., Assimes, T. L., Holm, H. *et al.* Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. *Nat. Genet.* **43**, 333–338 (2011).
11 Clarke, R., Peden, J. F., Hopewell, J. C., Kyriakou, T., Goel, A., Heath, S. C. *et al.* Genetic variants associated with Lp(a) lipoprotein level and coronary disease. *N. Engl. J. Med.* **361**, 2518–2528 (2009).
12 Weissglas-Volkov, D., Aguilar-Salinas, C. A., Sinsheimer, J. S., Riba, L., Huertas-Vazquez, A., Ordonez-Sanchez, M. L. *et al.* Investigation of variants identified in caucasian genome-wide association studies for plasma high-density lipoprotein cholesterol and triglycerides levels in Mexican dyslipidemic study samples. *Circ. Cardiovasc. Genet.* **3**, 31–38 (2010).
13 Kristiansson, K., Perola, M., Tikkanen, E., Kettunen, J., Surakka, I., Havulinna, A. S. *et al.* Genome-wide screen for metabolic syndrome susceptibility Loci reveals strong lipid gene contribution but no evidence for common genetic basis for clustering of metabolic syndrome traits. *Circ. Cardiovasc. Genet.* **5**, 242–249 (2012).
14 Braun, T. R., Been, L. F., Singhal, A., Worsham, J., Ralhan, S., Wander, G. S. *et al.* A replication study of GWAS-derived lipid genes in Asian Indians: the chromosomal region 11q23.3 harbors loci contributing to triglycerides. *PLoS ONE* **7**, e37056 (2012).
15 Shen, Y., Xi, B., Zhao, X., Cheng, H., Hou, D., Wu, L. *et al.* Common genetic variants associated with lipid profiles in a Chinese pediatric population. *Hum. Genet.* **132**, 1275–1285 (2013).
16 Weissglas-Volkov, D., Aguilar-Salinas, C. A., Nikkola, E., Deere, K. A., Cruz-Bautista, I., Arellano-Campos, O. *et al.* Genomic study in Mexicans identifies a new locus for triglycerides and refines European lipid loci. *J. Med. Genet.* **50**, 298–308 (2013).
17 Tokoro, F., Matsuoka, R., Abe, S., Arai, M., Noda, T., Watanabe, S. *et al.* Association of a genetic variant of the ZPR1 zinc finger gene with type 2 diabetes mellitus. *Biomed. Rep.* **3**, 88–92 (2015).
18 Wu, Y., Yu, S., Wang, S., Shi, J., Xu, Z., Zhang, Q. *et al.* Zinc finger protein 259 (ZNF259) polymorphisms are associated with the risk of metabolic syndrome in a han chinese population. *Clin. Lab.* **61**, 615–621 (2015).

19 Xu, L., Zhou, J., Huang, S., Huang, Y., Le, Y., Jiang, D. *et al.* An association study between genetic polymorphisms related to lipoprotein-associated phospholipase A(2) and coronary heart disease. *Exp. Ther. Med.* **5**, 742–750 (2013).
20 Luke, M. M., Kane, J. P., Liu, D. M., Rowland, C. M., Shiffman, D., Cassano, J. *et al.* A polymorphism in the protease-like domain of apolipoprotein(a) is associated with severe coronary artery disease. *Arterioscler. Thromb. Vasc. Biol.* **27**, 2030–2036 (2007).
21 Shiffman, D., Louie, J. Z., Rowland, C. M., Malloy, M. J., Kane, J. P. & Devlin, J. J. Single variants can explain the association between coronary heart disease and haplotypes in the apolipoprotein(a) locus. *Atherosclerosis* **212**, 193–196 (2010).
22 Koch, W., Mueller, J. C., Schrempf, M., Wolferstetter, H., Kirchhofer, J., Schomig, A. *et al.* Two rare variants explain association with acute myocardial infarction in an extended genomic region including the apolipoprotein (A) gene. *Ann. Hum. Genet.* **77**, 47–55 (2013).
23 Wang, Y., Wang, L., Liu, X., Zhang, Y., Yu, L., Zhang, F. *et al.* Genetic variants associated with myocardial infarction and the risk factors in Chinese population. *PLoS ONE* **9**, e86332 (2014).
24 Matsuoka, R., Abe, S., Tokoro, F., Arai, M., Noda, T., Watanabe, S. *et al.* Association of six genetic variants with myocardial infarction. *Int. J. Mol. Med.* **35**, 1451–1459 (2015).
25 Wauters, E., Carruthers, K. F., Buysschaert, I., Dunbar, D. R., Peuteman, G., Belmans, A. *et al.* Influence of 23 coronary artery disease variants on recurrent myocardial infarction or cardiac death: the GRACE Genetics Study. *Eur. Heart J.* **34**, 993–1001 (2013).
26 Kiechl, S., Paré, G., Barbalic, M., Qi, L., Dupuis, J., Dehghan, A. *et al.* Association of variation at the ABO locus with circulating levels of soluble intercellular adhesion molecule-1, soluble P-selectin, and soluble E-selectin: a meta-analysis. *Circ. Cardiovasc. Genet* **4**, 681–686 (2011).
27 López-Mejías, R., Genre, F., García-Bermúdez, M., Ubilla, B., Castañeda, S., Llorca, J. *et al.* Lack of association between ABO, PPAP2B, ADAMST7, PIK3CG, and EDNRA and carotid intima-media thickness, carotid plaques, and cardiovascular disease in patients with rheumatoid arthritis. *Mediators Inflamm.* **2014**, 756279 (2014).