Atherosclerosis 237 (2014) 480–485

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

A multilocus genetic risk score predicts coronary heart disease risk in a Chinese Han population

Lixuan Gui [a], Fangqin Wu [b], Xu Han [a], Xiayun Dai [a], Gaokun Qiu [a], Jun Li [a], Jing Wang [a], Xiaoming Zhang [a], Tangchun Wu [a], Meian He [a, *]

[a] MOE Key Lab of Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science & Technology, Wuhan 430030, Hubei, China
[b] Department of Cardiology, Union Hospital, Huazhong University of Science and Technology, Wuhan 430022, Hubei, China

ARTICLE INFO

Article history:
Received 28 May 2014
Received in revised form
22 September 2014
Accepted 28 September 2014
Available online 18 October 2014

Keywords:
Coronary heart disease
Genetic risk score
Risk prediction

ABSTRACT

Objective: Genome-wide association studies have identified multiple genetic loci associated with coronary heart disease (CHD) risk. However, whether these loci could improve the CHD risk prediction is unclear. **Methods and results**: The present case-control study (1146 CHD cases and 1146 controls) genotyped 19 recently discovered SNPs that associated with CHD risk. As a result, 10 SNPs were successfully replicated with odds ratios (ORs) ranging from 1.16 to 1.78 ($P = 4.6 \times 10^{-2}$ to 5.99×10^{-6}). A genetic risk score was constructed to assess the combined effects of the susceptibility loci on CHD risk. Subject in the second tertile (OR = 1.32, 95% CI, 1.02–1.73, $P = 3.84 \times 10^{-2}$) and the third tertile (OR = 2.62, 95% CI, 2.00–3.43, $P = 3.18 \times 10^{-12}$) had an increased risk of CHD comparing with those in the first genetic risk score tertile after adjustment for traditional risk factors including family history of CHD. Addition of the genetic risk score to the traditional model significantly improved the net reclassification as measured by the net reclassification index (NRI) (4.82%, $P = 0.0001$), however, no significant improvement was observed in discrimination of CHD, the area under the receiver operating characteristic curve (AUC) increased from 0.811 to 0.822 ($P = 0.18$). **Conclusions**: A multilocus genetic risk score was associated with CHD risk in a Chinese Han population. This genetic risk score improved the net reclassification but not improved the CHD discrimination. The potential clinical use of this variations remains to be defined.

1. Introduction

Coronary heart disease (CHD) is one of the leading causes of morbidity, mortality, and disability in the developed and developing countries. The CHD prevalence is increasing rapidly in China in the past few years [1–3]. Discrimination of the population with higher risk of CHD and prevention earlier plays important role to reduce the CHD incidence. Till now, numerous risk predictive models of cardiovascular disease such as the Framingham risk score [4], QRISK [5], and the Systematic COronary Risk Evaluation (SCORE) [6] were established and were used to estimate the CHD risk. However, finding more biomarkers to add to the models and improvement of the risk predictive ability of the models remained to be further investigated.

Recent genome-wide association studies (GWAS) have identified multiple loci associated with CHD risk [7–14]. Most of these GWAS were conducted in Europeans and needed to be validated in other populations. Recently two GWAS were conducted in Han Chinese and found 5 novel susceptibility loci for CHD risk [15,16]. Among these reported susceptibility loci, most variants had modest effects on CHD risk [7–18]. It is indicated that the combined effects of these susceptibility loci on CHD risk, which were measured with the genetic risk score, are stronger than the single variant [19]. Several studies have used the genetic risk score to measure the combined effects of CHD risk alleles and to investigate whether these CHD risk alleles could improve the risk prediction of CHD, however, the results are inconsistent [20–22].

Herein, we genotyped 19 SNPs in 19 recently discovered CHD susceptibility loci, including 5 novel loci identified in Chinese Han population and 14 previously reported variants in European ancestry [7–14] in a case-control study to investigate their

* Corresponding author. MOE Key Lab of Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, 13 Hangkong Rd, Wuhan, Hubei 430030, China.
E-mail address: hemeian@hotmail.com (M. He).

http://dx.doi.org/10.1016/j.atherosclerosis.2014.09.032
0021-9150/

association with CHD risk in a Chinese population. Furthermore, we examined the joint genetic effects of these SNPs on CHD risk and their prediction roles in the discrimination of CHD.

2. Materials and methods

2.1. Ethics statement

The Ethics Committee of Tongji Medical College approved the present study, and written informed consent was obtained from all subjects.

2.2. Study population

We performed a case–control study consisted of 1146 CHD patients and 1146 age- and sex-frequency matched healthy control subjects. The detailed study design has been described elsewhere [18]. Briefly, the patients were consecutively recruited from 3 hospitals (Tongji Hospital, Union Hospital, and Wugang Hospital) in Wuhan city (Hubei province, China) between May 2004 and October 2006. The inclusion criteria for the CHD cases were stenoses ≥50% in at least 1 major coronary artery by coronary angiography and/or a diagnosis of CHD based on the World Health Organization criteria [23]. Among the 1146 CHD cases, 379 subjects were diagnosed with acute myocardial infarction. The control subjects were recruited in a population-based survey and resided in the same communities as the cases. Controls were determined to be free of CHD and peripheral atherosclerotic arterial disease by medical history, clinical examinations, and electrocardiography. The participation rate of the controls is 90.7%. Structured questionnaires were used by trained interviewers to collect information on demographic variables, medical history, medications, and lifestyle habits. Subjects were classified as smokers and nonsmokers. Those who had smoked less than 100 cigarettes in the lifetime were defined as nonsmokers; otherwise, they were defined as smokers.

2.3. SNP selection

We selected 19 recently discovered CHD susceptibility loci from published GWAS papers before July, 2012. The phenotypes studied in these GWAS were myocardial infarction or CHD. Five novel CHD-susceptibility loci identified in GWAS in Chinese Han population were included in the present study [15,16]. We also selected six SNPs that were identified in European population and recently validated in Chinese Han population [7–11,16,17]. Besides, eight SNPs discovered in European population but did not identified in Chinese population were taken forward to replication in the present study [9,12–14]. In total, 19 SNPs in 19 genetic loci, including rs629301 (*CELSR2-PSRC1-SORT1*), rs12617744 (*TTC32-WDR35*), rs1199337 (*MRAS*), rs1842896 (*GUCY1A3*), rs9268402 (*C6orf10-BTNL2*), rs6903956 (*C6orf105*), rs9349379 (*PHACTR1*), rs12190287 (*TCF21*), rs3125055 (*LPA*), rs1333042 (*CDKN2A/B*), rs12413409 (*CYP17A1-NT5C2*), rs2019090 (*PDGFD*), rs7136259 (*ATP2B1*), rs11066280 (*C12orf51*), rs2259816 (*HNF1A*), rs4380028 (*ADAMTS7*), rs9899364 (*RASD1-PEMT*), rs216172 (*SMG6-SRR*), and rs16996148 (*NCAN-CILP2*) (Supplementary Table 1) were selected for further genotyping and analysis. Among the 19 SNPs, 11 SNPs were the index SNPS reported before, and the other 8 SNPs were proxy SNP, which were highly linkage disequilibrium with the reported index SNPs (r^2 ranging from 0.3 to 1; Supplementary Table 1).

2.4. SNPs genotyping

Fasting venous blood was collected in 5-ml EDTA tubes, and genomic DNA was isolated with a Puregene kit (Gentra Systems, Inc., Minneapolis, MN, USA). Genotyping was performed with Sequenom MassARRAY iPLEX platform (Sequenom, Inc. San Diego, CA, USA). A successful genotyping rate of over 95% was achieved for all the 19 SNPs. Among the 19 SNPs, 4 SNPs (rs16996148, rs3125055, rs4148152, and rs7136259) nominally deviated from Hardy–Weinberg equilibrium (HWE) in controls (P value = 0.011, 0.026, 0.016, and 0.0072 respectively). However, they did not deviate from HWE in CHD cases (P = 0.374, 0.219, 0.773, and 0.355 respectively). Because SNP of rs6903956 deviated from HWE in both controls and cases (P value < 0.001), it was excluded from further analysis.

2.5. Statistical analyses

A χ^2 test was used to assess whether the SNPs were in HWE and to determine differences in genotype frequencies between CHD cases and controls. The *t*-test was used to compare mean values of quantitative traits between groups. An unconditional logistic regression was used to calculate odds ratios (ORs), adjusting for traditional risk factors including age, sex, smoking, alcohol consumption, BMI, TC and TG levels, family history of CHD and history of hypertension and diabetes. Genetic risk score was calculated with the 10 replicated significant SNPs. We assumed that each SNP was independent associated with risk of CHD according to an additive genetic model and contributed equally. A genetic risk score was calculated by summing the number of risk alleles at each polymorphic locus [24]. Participants with missing genotype (63 controls and 108 cases) were excluded from genetic risk score calculation. In sensitivity analyses, a weighted genetic risk score was calculated by multiplying the number of risk alleles at each locus (0, 1, or 2) for the corresponding β coefficient from additive multivariate logistic regression model, and then taking the sum of the 10 SNPs. We divided the continuous weighted genetic risk score into tertiles and compared risk between them. We used receiver-operating characteristic curve analysis (plots were made with the ROC Curve function in Analysis, SPSS 12.0, SPSS Inc., Chicago, IL, USA) and calculated the area under the curve (also known as the C statistic) to evaluate discrimination. We tested the null hypothesis of no differences between the AUCs from models incorporating traditional risk factors (age, sex, smoking, alcohol consumption, BMI, TC and TG levels, family history of CHD, history of hypertension, and history of diabetes) with and without the genetic risk

Table 1
General characteristics of CHD cases and controls.

Variables	Cases (n = 1146)	Controls (n = 1146)	P value
Age, years	60.0 ± 10.3	60.5 ± 11.3	0.23
Sex, m/f, (%)	891/255 (77.8/22.2)	901/245 (78.6/21.4)	0.61
Body mass index, kg/m^2	24.4 ± 3.3	23.7 ± 3.1	<0.01
Smoking, no/yes, (%)	401/741 (35.1/64.9)	540/606 (47.1/52.8)	<0.01
Alcohol consumption, no/yes, (%)	822/317 (72.2/27.8)	776/365 (68.0/32.0)	0.03
Total cholesterol, mmol/L	4.35 ± 1.10	4.68 ± 0.92	<0.01
Triglyceride, mmol/L	1.67 ± 1.38	1.61 ± 1.30	0.28
Systolic pressure, mmHg	136.0 ± 25.3	133.6 ± 29.0	0.034
Diastolic pressure, mmHg	82.9 ± 15.2	82.0 ± 11.3	0.11
Past history			
Diabetics, no/yes, (%)	824/314 (72.4/27.6)	1080/65 (94.3/5.7)	<0.01
Hypertension, no/yes, (%)	351/790 (30.8/69.2)	784/361 (68.5/31.5)	<0.01
Family history of CHD, no/yes, (%)	947/158 (85.7/14.3)	1133/10 (99.1/0.9)	<0.01