Atherosclerosis 240 (2015) 40–45

Contents lists available at ScienceDirect

Atherosclerosis

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

Interaction effects between *Paraoxonase 1* variants and cigarette smoking on risk of coronary heart disease in a Singaporean Chinese population

Yi Han [a, b], Rajkumar Dorajoo [c], Tingjing Ke [a, b], Burger Ayala [a, b, h], Xuling Chang [a, b], Chiea-Chuen Khor [c], Rob M. van Dam [d], Jian-Min Yuan [e, f], Woon-Puay Koh [d, g], Jianjun Liu [c, d], Daniel Y.T. Goh [a, b], Yechiel Friedlander [h, **], Chew-Kiat Heng [a, b, *]

[a] Department of Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
[b] Khoo Teck Puat – National University Children's Medical Institute, National University Health System, Singapore
[c] Genome Institute of Singapore, Agency for Science, Technology and Research, Singapore, Singapore
[d] Saw Swee Hock School of Public Health, National University of Singapore and National University Health System, Singapore
[e] Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA
[f] University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA
[g] Duke-NUS Graduate Medical School Singapore, Singapore
[h] School of Public Health and Community Medicine, Hebrew University of Jerusalem, Israel

ARTICLE INFO

Article history:
Received 11 September 2014
Received in revised form
17 January 2015
Accepted 19 January 2015
Available online 25 February 2015

Keywords:
Coronary heart disease
Paraoxonase 1
Cigarette smoking
Interaction
Single nucleotide polymorphism (SNP)
miR-SNP
Singaporean Chinese

ABSTRACT

Objective: Paraoxonase 1 (PON1) plays an important role in reducing the risk of coronary heart disease (CHD). Smoking is known to reduce PON1 activity. We aimed to investigate the effects of interactions between *PON1* variants and smoking on CHD in the Singaporean Chinese population.
Methods: In a case-control study nested within Singapore Chinese Health Study (N = 1914), subjects with and without CHD were classified into never-smokers and ever-smokers (ever smoked at least one cigarette a day for 1 year or longer). Associations at four independent SNPs at the PON1 locus (rs3735590, rs3917550, rs662, rs3917481) with CHD were evaluated using logistic regression, before/after stratification on smoking status. Interactions between smoking and *PON1* variants were analyzed with likelihood ratio tests, by including the SNP*smoking interaction term in regression analyses.
Results: The T allele at the coding SNP, rs662, was associated with higher risk of CHD in ever-smokers only (OR = 1.35, 95% CI 1.08–1.68; adjusted P = 0.036). At the miR-SNP, rs3735590, carrying at least one copy of minor allele T was associated with increased risk of CHD in a dominant manner in never-smokers only (OR = 1.53, 95% CI 1.11–2.11; adjusted P = 0.036). Significant interactions between two *PON1* SNPs and smoking in relation to CHD risk were identified (adjusted P = 0.012 for rs662; adjusted P = 0.044 for rs3735590). These associations remained significant after adjustment for known CHD risk factors and upon correction for multiple tests.
Conclusions: Two *PON1* SNPs, rs662 and rs3735590, were found to significantly interact with cigarette smoking to modulate the risk of CHD in the Singaporean Chinese population.

1. Introduction

Coronary heart disease (CHD) is a complex and multifactorial disorder. Along with its most severe complication, myocardial infarction (MI), it has become a leading cause of death in United States [1] and the number two killer in Singapore [https://www.moh.gov.sg]. Human serum paraoxonase 1 (PON1) is a 43-kDa calcium dependent esterase located on high-density lipoprotein cholesterol (HDL-C) that circulates in plasma [2]. It has been

* Corresponding author. Department of Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore, NUHS Tower Block, Level 12, 1E Kent Ridge Road, Singapore 119228, Singapore.
** Corresponding author. Unit of Epidemiology, Hebrew University-Hadassah Braun School of Public Health, POB 12272, Jerusalem 91120, Israel.
E-mail addresses: yechielf@ekmd.huji.ac.il (Y. Friedlander), paehck@nus.edu.sg (C.-K. Heng).

http://dx.doi.org/10.1016/j.atherosclerosis.2015.01.042
0021-9150/

reported to reduce oxidative stress and the risk of cardiovascular risk by hydrolyzing the pro-inflammatory lipid-peroxides formed by the oxidized low-density lipoprotein (LDL) [3–5]. A *PON1* polymorphism (rs662) results in a nonsynonymous amino acid substitution (Glutamine to Arginine substitution at position 192), which leads to the difference in the hydrolytic activity towards paraoxon [6,7]. This 192Gln variant hydrolyzes paraoxon at a lower rate than 192Arg [8] and is more effective at impeding the oxidation of LDL than 192Arg [9,10]. The reduced anti-oxidative ability of 192Arg may contribute to its association with a higher risk coronary heart disease (CHD) [11,12].

Cigarette smoking is known to lower PON1 activity in patients with CHD [13], and cigarette smoke extract inhibits PON1 activity in a dose- and time-dependent manner [14]. The study of *PON1*-smoking interaction may be able to identify individuals with a high risk of CHD due to the combination of certain *PON1* polymorphisms and smoking status. For this purpose, previous studies have assessed the interactions between a limited number of coding variants in *PON1* and cigarette smoking status on the risk of CHD. However results have been inconclusive [15,16]. For example, a study in the Costa Rican population showed a significant interaction between the rs662 *v*ariant and smoking status to modify CHD risk [15]. In this study, the C allele at rs662 was reported to increase the risk of CHD in non-smokers only. However, another study conducted in a different ethnic group (Asian-Indians) indicated contrasting results for the same *PON1* SNP (rs662), in which the risk allele (C allele) was associated with increased CHD risk among smokers [16].

In this study we aimed to evaluate whether the association of genetic variants in the *PON1* gene region with CHD risk is modified by cigarette smoking status in the Singaporean Chinese population.

2. Material and method

2.1. Study subjects

This case-control study (N = 1914; 688 cases and 1226 controls) was nested within the Singapore Chinese Health Study (SCHS), a prospective cohort of 63,257 Chinese who were recruited between April 1993 and December 1998 [17]. The study population comprises two major dialect groups of Chinese in Singapore, the Hokkiens and the Cantonese. At recruitment, subjects were interviewed in-person using a structured questionnaire which included socio-demographic information, medical history and life style characteristics [17]. Blood was collected from 28,439 participants mostly between 2000 and 2005. During an average follow-up of 15 years, less than 1% of subjects were lost to follow-up.

Cases who had fatal coronary heart disease (CHD) or suffered from non-fatal myocardial infarction (MI) were identified through the Singapore Registry of Births and Deaths and the Hospital Discharge Database respectively. For all non-fatal cases, medical records were retrieved and reviewed by cardiologists and only those who had confirmed MI using the Multi-Ethnic Study of Atherosclerosis criteria (available at: http://www.mesa-nhlbi.org/manuals.aspx), were included. Cases of fatal CHD were only included if there was prior evidence of CHD based on the questionnaire data or the Hospital Discharge Database. For each case, two controls were selected from SCHS participants who were alive and free of CHD at the time of diagnosis or death of the index case and were matched for sex, dialect group, year of birth, year of recruitment and date of blood collection. The Institutional Review Board of the National University of Singapore has approved this study.

2.2. Study variables

Study subjects were classified into never-smokers (N = 1101) and ever-smokers (N = 813) based on their responses (No, Yes) to the following question in the questionnaire, 'Have you ever smoked at least one cigarette a day for 1 year or longer'. An additional variable, pack-year of cigarette smoking, was created and defined as the number of cigarettes smoked per day divided by 20 and multiplied by the number of smoking years to capture the duration and quantity of cigarette smoking, as previously reported [18]. Ever-smokers were classified into light smokers (N = 500) and heavy smokers (N = 313) based on the median of pack-year (median = 30.6). Blood pressure was measured using Omron Automatic Digital Blood Pressure Monitors (HEM 705CP) after the subjects were seated at rest for at least 5 min. Average levels based on three blood pressure measurements were used for the analysis. Hypertension was defined based on subjects' self-report, and/or having a systolic blood pressure (SBP) $\geq$ 140 mmHg and/or a diastolic blood pressure (DBP) $\geq$ 90 mmHg [19]. HbA1c was measured by Biorad Variant II using an ion-exchange HPLC method and diabetes was defined based on subjects' self-report, and/or hemoglobin A1c (HbA1c) $\geq$ 6.5% [20]. Body mass index (BMI) was determined as body weight (kg) divided by the squared height (m^2). Non-fasting blood lipoproteins (high density lipoprotein-cholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL-C) were measured directly with the Siemens Advia 2400 instrument using an elimination/catalase method.

2.3. Genotyping

The current study population was genotyped on Illumina HumanOmniZhongHua-8 BeadChip. Based on quality control procedures (Supplementary Table 1), we excluded 42 duplicate samples, 35 samples with a call rate < 98%, 20 samples with extreme heterozygosities (>mean ± 3standard deviation, SD), 25 samples with 1st degree relatedness, identified from identity-by-state analyses, such as monozygotic twins, full-sibling pairs and parent-offspring (only 1 sample from each pair was retained, prioritizing the cases and samples with higher call-rates) and 36 samples with discordant ethnic membership from Singaporean Chinese ethnicity as identified by principal components analysis. Quality control procedures for SNPs excluded 24,461 non-autosomal SNPs, 467 SNPs with poor call-rates (<95%), 435 SNPs with significant deviations of Hardy–Weinberg Equilibrium (HWE) in controls ($<1 \times 10^{-6}$) and 67,585 SNPs with minor allele frequencies (MAF) < 0.01 (Supplementary Table 2). Eighty nine samples with missing measurements on HDL-C, LDL-C, hypertension and diabetes were excluded and for final statistical analysis, 1914 samples (688 cases and 1226 controls) and 802,635 SNPs that passed quality control procedures were included.

2.4. Selection of PON1 variants

A total of 63 SNPs with minor allele frequencies (MAF) > 1% within the *PON1* gene were selected. From these 63 SNPs, we removed non-informative SNPs by filtering them down to 4 tag SNPs (rs3735590, rs3917550, rs662, rs3917481) that are at low linkage disequilibrium (LD) with each other ($r^2 < 0.25$, Supplementary Table 2).

2.5. Statistical analysis

Statistical analysis of data was carried out with STATA (version 8.2). Differences in means of quantitative traits between cases and controls were determined by the *t*-test. Differences in frequencies