Thrombosis Research 141 (2016) 28–34

Contents lists available at ScienceDirect

Thrombosis Research

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

Full Length Article

Association of PEAR1 genetic variants with platelet reactivity in response to dual antiplatelet therapy with aspirin and clopidogrel in the Chinese patient population after percutaneous coronary intervention

Yi Yao, Xiao-fang Tang, Jia-hui Zhang, Chen He, Yuan-liang Ma, Jing-jing Xu, Ying Song, Ru Liu, Xian-min Meng, Lei Song, Jue Chen, Miao Wang, Bo Xu, Run-lin Gao, Jin-qing Yuan *

[a] State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Beilishi Road No. 167, Xicheng District, Beijing 100037, People's Republic of China
[b] Peking Union Medical College, Beijing 100037, People's Republic of China

ARTICLE INFO

Article history:
Received 19 October 2015
Received in revised form 19 February 2016
Accepted 29 February 2016
Available online 2 March 2016

Keywords:
Platelet endothelial aggregation receptor 1
Platelet reactivity
Genetic variants
Aspirin
Clopidogrel

ABSTRACT

Introduction: Platelet Endothelial Aggregation Receptor-1 (PEAR1) is a recently reported platelet transmembrane protein which plays an important role in platelet aggregation. The aim of this study was to investigate whether PEAR1 genetic variations were associated with platelet reactivity as assessed by adenosine diphosphate(ADP)-induced platelet aggregation in Chinese patients treated with aspirin and clopidogrel.

Methods: Patients with coronary heart disease (CHD) who underwent percutaneous coronary intervention (PCI) were enrolled in the study. All patients were on dual antiplatelet therapy with aspirin and clopidogrel. ADP-induced platelet aggregation was measured by thromboelastography and defined as percent inhibition of platelet aggregation (IPA). Patients ($n = 204$) with IPA <30% were identified as high on-treatment platelet reactivity (HPR). Patients ($n = 201$) with IPA >70% were identified as low on-treatment platelet reactivity (LPR). Sixteen single nucleotide polymorphisms (SNPs) of PEAR1 were determined by a method of improved multiple ligase detection reaction.

Results: Among the 16 SNPs examined by univariate analysis, 5 SNPs were significantly associated with ADP-induced platelet aggregation. Minor allele C at rs11264580 ($p = 0.033$), minor allele G at rs2644592 ($p = 0.048$), minor allele T at rs3737224 ($p = 0.033$) and minor allele T at rs41273215 ($p = 0.025$) were strongly associated with HPR, whereas homozygous TT genotype at rs57731889 ($p = 0.009$) was associated with LPR. Multivariate logistic regression analysis further revealed that the minor allele T at rs41273215 ($p = 0.038$) was an independent predictor of HPR and the homozygous TT genotype at rs57731889 ($p = 0.003$) was an independent predictor of LPR.

Conclusions: PEAR1 genetic variations were strongly associated with ADP-induced platelet aggregation in Chinese patients with CHD treated with aspirin and clopidogrel. These genetic variations may contribute to the variability in platelet function. The utility of PEAR1 genetic variants in the assessment and prediction of cardiovascular risk warrants further investigation.

1. Introduction

Dual antiplatelet therapy with aspirin and clopidogrel is the corner stone of treatment for patients with acute coronary syndrome (ACS) or those undergoing percutaneous coronary intervention (PCI). However, platelet aggregation during antiplatelet therapy displays considerable inter-individual variability. Some patients do not acquire adequate platelet inhibition from antiplatelet drugs like aspirin and clopidogrel, resulting in high on-treatment platelet reactivity (HPR) and adverse clinical ischemic events, whereas other patients exhibit excessive responses to antiplatelet drugs, leading to low on-treatment platelet reactivity (LPR) and adverse bleeding events [1,2]. Genetics is known to contribute to such variability in drug response. Thus, genetic

Abbreviations: CHD, coronary heart disease; ACS, acute coronary syndrome; ADP, adenosine diphosphate; PCI, percutaneous coronary intervention; HPR, high platelet reactivity; LPR, low platelet reactivity; PEAR1, platelet endothelial aggregation receptor 1; SNP, single nucleotide polymorphisms; TEG, thromboelastography; LDR, ligase detection reaction; LD, loading dose; BMI, body mass index; PLT, platelet; IPA, inhibition of platelet aggregation; DM, diabetes mellitus; ACEI, angiotensin conversion enzyme inhibitor; ARB, angiotensin receptor blocker; CCB, calcium channel blocker; PPI, proton pump inhibitor; H2RA, H2 receptor antagonist; OR, odds ratio; MAF, minor allele frequency; CI, confidence interval.

* Corresponding author.
E-mail address: dr_jinqingyuan@sina.com (J. Yuan).

http://dx.doi.org/10.1016/j.thromres.2016.02.031
0049-3848/

tests are used to identify patients who carry certain genes (polymorphisms) that modify platelet reactivity and to guide individualized treatment. For example, increasing drug dosage or switching to a new antiplatelet agent like ticagrelor or prasugrel would be a rational approach for those patients with HPR. On the other hand, decreasing dosage or discontinuing drug use or switching to another antithrombotic drug [3–7] would be a better therapeutic strategy for those patients with LPR. However, clinical practice and several studies have shown that even after changing treatment strategy, some patients still display high or low platelet reactivity.

Platelet endothelial aggregation receptor-1 (PEAR1) is a recently reported platelet transmembrane protein which is activated by platelet-to-platelet contact and agonist stimulation. Activated PEAR1 sends signals to enhance and stabilize platelet thrombin in a manner dependent on the functionality of GPIIb/IIIa [8–10]. A few clinical trials have shown that genetic variations in PEAR1 are important determinants of platelet reactivity during aspirin treatment [11,12]. Given the fact that the COX1/thromboxane A2 pathway can be sufficiently inhibited by aspirin, the occurrence of maximal aggregation might be dependent on other signaling pathways, such as the PEAR1 platelet-to-platelet interactive pathway, which has been shown to strongly affect platelet aggregation. However, the influence of PEAR1 genetic variations on ADP-induced platelet aggregation in Chinese patients during dual antiplatelet therapy with aspirin and clopidogrel is largely unknown. The aim of this study was to evaluate the association of PEAR1 genetic variants with platelet reactivity in response to dual antiplatelet therapy in a Chinese patient population undergoing PCI.

2. Methods

2.1. Study population

Patients admitted to Fuwai Hospital of Chinese Academy of Medical Sciences and Peking Union Medical College between November 2011 and March 2013 were considered for enrollment in the study. The inclusion criteria for patient enrollment were: coronary heart disease (CHD) and with percutaneous coronary intervention (PCI). The exclusion criteria were: age <50 years old; lack of platelet function test results; platelet count <100,000/mm^3 or >300,000/mm^3; hemodynamic instability; active bleeding or had bleeding events within 1 year; bleeding diatheses; oral anticoagulation therapy; usage of intensified antiplatelet agents other than standard dual antiplatelet therapy with aspirin and clopidogrel and contraindication to antiplatelet therapy. The study was approved by the Fuwai Hospital Institutional Ethical Review Board. Written informed consent was obtained from all study participants. The study conformed to the principles outlined in the Declaration of Helsinki.

2.2. Study design

All patients received a 300 mg loading dose (LD) of aspirin and a 300 mg LD of clopidogrel before PCI followed by a 100 mg/day maintenance dose of aspirin for life and 75 mg/day of clopidogrel for 1 year. The decision for PCI was based on the coronary angiography results and all interventions were conducted according to the 2010 ESC/EACTS Guideline on Myocardial Revascularization [13], and the 2011 ACCF/AHA/SCAI Guideline for Percutaneous Coronary Intervention [14]. Patients were divided into HPR and LPR groups according to platelet reactivity measured by a thromboelastography platelet-mapping assay. Patients with inhibition of platelet aggregation (IPA) <30% were included in the HPR group. Patients with IPA >70% were included in the LPR group. The cutoff values of IPA used here were based on previous studies which reported that IPA <30% was associated with ischemic events [15,16], whereas IPA >70% was associated with increasing requirement for blood transfusion [17]. The flow diagram for the trial is shown in Fig. 1.

2.3. Platelet function

Blood was collected into two vacutainer tubes 12–36 h after PCI, one tube with 3.2% trisodium citrate anticoagulation and the other with lithium heparin anticoagulation. The vacutainer tubes were filled to capacity and inverted 3–5 times to ensure complete mixing of the anticoagulant. ADP-induced platelet aggregation was measured by a thromboelastograph platelet-mapping assay. A detailed description of this method was outlined previously [18]. The TEG Hemostasis Analyzer (Haemonetics Corp, Braintree, MA) and automated analytical software were used to measure the physical properties. Data were recorded as the percent inhibition of platelet aggregation (IPA): $100–100 \times [(MA_{ADP} - MA_{FIBRIN}) / (MA_{THROMBIN} - MA_{FIBRIN})]$, where MA_{ADP} is the ADP-induced clot strength (measurement of antiplatelet drug effect), MA_{FIBRIN} is the fibrin-induced clot strength (measurement of fibrin contribution), and $MA_{THROMBIN}$ is the thrombin-induced clot strength (maximum clot strength).

2.4. Genetic analysis

Sixteen SNPs of PEAR1 were selected for analysis based on previous reports [11,12,19–27] and the results of HapMap (http://hapmap.ncbi.nlm.nih.gov/) Chinese Han Beijing Databank with the aid of Haploview 4.2 software. HapMap selection criteria include minor allele frequency (MAF) >0.05 and a linkage disequilibrium (LD) measure r^2 threshold of 0.8. SHEsis software (http://analysis.bio-x.cn/myAnalysis.php) was used for haplotype analysis. Blood samples were obtained from each patient and stored in 4 mL ethylene diamine tetraacetic acid (EDTA) anticoagulated vacuum tubes. Genomic DNA was extracted from whole blood samples according to the salting-out protocol. All selected SNPs of PEAR1 were genotyped using a method of improved multiple ligase detection reaction (im LDR) with technical support from the Shanghai Genesky Biotechnology Company. Repeat genotyping was performed on random duplicate samples ($n = 17$) and DNA sequencing techniques were used for quality control.

2.5. Statistical analyses

Sample size was estimated based on a case-control study design (HPR group vs LPR group) with the statistical power $(1\text{-}\beta) = 0.8$, $\alpha = 0.05$, assuming 50% of patients carrying minor allele in LPR group (namely control group) and odds ratio (OR) = 1.8. Thus, $n = 188$ for each group was needed (Statistical software NCSS-PASS 11). Continuous variables were presented as the mean ± standard deviation (SD) and comparisons between groups of means were performed using the Student's *t*-test. Categorical variables were reported as counts (percentages) and Chi-square test (X^2) was used to compare groups. Genotype frequencies were tested for Hardy–Weinberg equilibrium. Any deviation between observed and expected frequencies was tested for significance using the X^2 test. Multivariate logistic regression analysis was used to adjust for potential confounding factors which may affect platelet aggregation including age, sex, platelet count, erythrocyte count, diabetes mellitus, hypertension, hypercholesterolemia, smoking, alcohol use and PPI/H2RA use. All statistical analyses were performed using SPSS ver. 20.0, and a two-tailed probability value <0.05 was considered to be significant.

3. Results

3.1. Clinical data and biochemical characteristics of study subjects

From November 2011 to March 2013, 1483 patients with CHD who underwent PCI were screened for the study. Of those patients, 408 patients were enrolled in the study and divided into two groups according to the inclusion/exclusion criteria and platelet aggregation test results. With successful genotyping, a total of 204 patients with IPA <30%