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Relationship Between *ABCB1* Polymorphisms, Thromboelastography and Risk of Bleeding Events in Clopidogrel-Treated Patients With ST-Elevation Myocardial Infarction

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ABSTRACT

Introduction: This study sought to investigate the relationship of polymorphisms in *ABCB1* and the predictive value of thromboelastography (TEG) on bleeding risk in clopidogrel-treated patients with ST-elevation myocardial infarction (STEMI).

Methods: 467 consecutive patients with STEMI undergoing percutaneous coronary intervention (PCI) were enrolled. Twenty tag single nucleotide polymorphisms (SNPs) selected from *ABCB1* gene and *CYP2C19**2, *3, *17 were detected by the ligase detection reaction. Platelet reactivity was assessed by TEG. The follow-up period was 12 months.

Results: By receiver operating characteristic curve analysis, the TEG platelet mapping assay value of ADP inhibition had the best predictive value of bleeding academic research consortium definition (BARC) ≥3b bleedings, yielding an area under the curve (AUC) of 0.707 (95% CI 0.662-0.749, p = 0.009; cut-off value >93.4%). ADP inhibition can also predict BARC ≥ 3 bleedings with an AUC of 0.594 (95% CI 0.546-0.640, p = 0.05; cut-off value >92.5%). After adjustment for established risk factors of bleeding including the gain of function *CYP2C19**17 allele, age, female gender, renal function, the multivariable logistic regression model demonstrated that ADP inhibition > 92.5% (OR 2.247, 95%CI 1.082-4.665, P = 0.03), carriage of rs1045642 (OR 2.943, 95%CI 1.195-7.247, P = 0.019) and rs7779562 (OR 0.453, 95%CI 0.219-0.936, P = 0.032) were independent predictors of BARC ≥ 3 bleedings. These associations were validated in a second cohort of 504 STEMI patients.

Conclusions: In STEMI patients treated with clopidogrel after PCI, the *ABCB1* tag SNP rs1045642 is associated with higher risk of bleedings while rs7779562 is associated with lower bleeding risk, and ADP inhibition in TEG has a predictive value of bleedings.

Introduction

The adenosine diphosphate (ADP) receptor blocker clopidogrel is routinely administered for the prevention of cardiovascular events in patients suffering ST-elevation myocardial infarction (STEMI), especially in those undergoing percutaneous coronary intervention (PCI) [1,2]. Although new standard of antiplatelet agents such as prasugrel and ticagrelor are available now, clopidogrel is still one of the most frequently prescribed drugs in many countries. However, interindividual variability in pharmacodynamics response to clopidogrel is widespread in patients treated with this medication [3]. Patients with high platelet reactivity (PR) to ADP are more likely to experience ischemic events, while low PR to ADP may contribute to increased risk of bleeding events [4]. Although the mechanisms have not been fully elucidated, many factors have been reported to involve in the clopidogrel response variability. Gene polymorphisms

Abbreviations: ADP, adenosine diphosphate; STEMI, ST-elevation myocardial infarction; PCI, percutaneous coronary intervention; PR, platelet reactivity; LOF, loss-of-function; GOF, gain-of-function; *ABCB1*, ATP-binding cassette, sub-family B, member 1, also called MDR1; SNP, single nucleotide polymorphisms; TEG, thromboelastography; LDR, ligase detection reaction; BARC, bleeding academic research consortium definition; MI, myocardial infarction; TVR, target vessel revascularization; ST, stent thrombosis; ROC, Receiver operator curve; BMI, body mass index; OR, odds ratio; LVEF, left ventricular ejection fraction; WBC, white blood cell; hs-CRP, high-sensitivity C-reactive protein; DM, diabetes mellitus; CHD, coronary heart disease; CABG, coronary artery bypass grafting; LAD, left anterior descending; LCX, left circumflex; RCA, right coronary artery; LM, left main; ACEI, angiotensin converting enzyme inhibitor; CCB, calcium channel blocker; PPI, proton pump inhibitor.

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play a critical role in clopidogrel metabolism, strongly affecting the prognosis of patients under clopidogrel treatment [5–7].

Clopidogrel is an inactive prodrug that requires intestinal absorption and subsequent biotransformation to active metabolites by cytochrome P450 enzymes. We have recently observed that the *CYP2C19* loss-of-function (LOF) alleles responsible for clopidogrel metabolism had a gene dose effect on the pharmacodynamics and composite ischemic events of clopidogrel in Chinese people after PCI [8]. As for clopidogrel absorption, a key drug transporter involved is the P-glycoprotein at the intestinal barrier, which is encoded by the *ABCB1* (ATP-binding cassette, sub-family B, member 1, also called *MDR1*) gene [9]. The P-glycoprotein is an ATP-dependent efflux pump that transports various molecules across extracellular and intracellular membranes. The increased expression or function of P-glycoprotein on intestinal epithelial cells can affect bioavailability of its substrate drugs, such as clopidogrel. The contribution of the *ABCB1* gene to clopidogrel response continues to be of great interest. More than 50 single nucleotide polymorphisms (SNPs) reside in the coding region of *ABCB1* gene which can possibly cause altered function [10]. Most studies focused on a synonymous SNP C3435T (rs1045642) in the gene. Previous research has shown that the minor T allele causes altered function of P-glycoprotein to affect the absorption of clopidogrel [11].

Antiplatelet effect can be evaluated through clinical outcomes and laboratory platelet function tests. Although accumulating data from large studies underscore the importance of high on-treatment PR to ADP as a prognostic risk factor of ischemic events, the association between on-treatment PR and bleeding events is less clear[4]. Thus, we are interested in exploring a potential link between thromboelastography (TEG) results and bleeding events. Meanwhile, epidemiological evidence on *ABCB1* gene-association with clopidogrel response is largely inconsistent [12–14]. In contrast to the numerous studies linking *ABCB1* polymorphisms to an increased risk of ischemic events, there is less evidence about the relation of *ABCB1* gene and bleeding events. We previously found a significant association between *ABCB1* C3435T and bleeding events. In order to further investigate the relation of other *ABCB1* polymorphisms on the risk of bleeding and ischemic events, we analyzed tag SNPs across the *ABCB1* gene in Chinese STEMI patients treated with clopidogrel, and assessed the association of the *ABCB1* polymorphisms in the context of *CYP2C19* status to reveal an independent relation of *ABCB1* gene variants with clinical outcome. Here, we demonstrated that in STEMI patients treated with clopidogrel after PCI, the *ABCB1* tag SNP rs1045642 is associated with higher risk of bleedings while rs7779562 is associated with lower bleeding risk, and ADP inhibition in TEG has predictive value of bleeding risk.

Methods

Study population

Between January 2011 and July 2012, 467 consecutive patients with STEMI were enrolled in our prospective, randomized, single-center study. The inclusion criteria were: age of >18 years, had an uneventful PCI, and could be followed up for >1 year after PCI. The major exclusion criteria were hemodynamic instability, active bleeding and bleeding diatheses, oral anticoagulation therapy, use of intensified antiplatelet agents other than standard dual antiplatelet therapy, contraindication to antiplatelet therapy, non-cardiac disease with a life expectancy of <1 year, or inability to follow the protocol. The Institutional Review Board approved the study protocol, and the patients were provided written informed consent for participation and agreed to the TEG testing and genotype determination. The study conformed to the principles outlined in the Declaration of Helsinki.

Study design

All patients were pre-treated with aspirin and a loading dose of 300 mg clopidogrel before PCI, followed by a maintenance dose of 100 mg/day aspirin for life and 75 mg/day clopidogrel for 1 year. The decision for PCI was based on the coronary angiography results, and all interventions were conducted according to the current standard guidelines. The stent type was chosen by the operator, and tirofiban was administered if a glycoprotein IIb/IIIa receptor inhibitor (GPI) was required. Anticoagulation with low-molecular-weight heparin (enoxaparin) or unfractionated heparin was initiated before angiography in all patients.

Selection of tag SNPs

Using the pairwise tagging approach, tag SNPs were selected from the HapMap CHB databank (HapMap Data Rel 27 PhaseII + III, Feb09, on NCBI B36 assembly, dbSNP b126) with aid of tag SNPs' online software (http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap27_B36/#search). The selected tag SNPs covered the complete *ABCB1* region, from 5,000 bp upstream to 5,000 bp downstream. Common variants were defined as those with a minor allele frequency (MAF) greater than 0.05, with a linkage disequilibrium (LD) measure r^2 threshold of 0.8. Twenty tag SNPs were identified to capture 86 percent of SNPs over the entire *ABCB1* gene. Before analysis, one tag SNP rs2032582 was excluded because of significant deviation from Hardy-Weinberg equilibrium in the study population ($p < 0.001$). No such deviation was detected in all other enrolled tag SNPs.

Genetic analysis

Genomic DNA was extracted from peripheral whole blood samples according to a salting-out protocol. All 20 selected SNPs were genotyped using the ligase detection reaction (LDR) and a commercially available detection system (ABI3130XL DNA Analyzer System; Applied Biosystems, USA). Repeat genotyping was performed on random duplicate samples ($n = 21$), and sequencing techniques were used to ensure quality control. Based on the known association of *CYP2C19* genetic variation with pharmacological response and adverse outcomes in clopidogrel-treated patients, we assessed the LOF alleles *CYP2C19**2 (rs4244285, c. 681G > A) and *CYP2C19**3 (rs4986893, c. 636G > A), and the gain of function (GOF) allele *CYP2C19**17 (rs12248560, g. -808C > T) to show the relation of *ABCB1* polymorphisms to clinical adverse outcomes.

Thromboelastograph platelet-mapping assay

Blood was collected at least 6 h after the patient had taken the clopidogrel dose in a vacutainer tube containing 3.2% trisodium citrate and lithium heparin. The vacutainer tube was filled to capacity and inverted three to five times to ensure complete mixing of the anticoagulant. Modified TEG® used four channels to detect effects of antiplatelet therapy with arachidonic acid (AA) and adenosine diphosphate (ADP) activators. A detailed description of this method is outlined previously [15]. The TEG Hemostasis Analyzer (Haemonetics Corp, Braintree, MA) and automated analytical software were used to measure the physical properties.

The percentage of platelet inhibition by clopidogrel was computed as the contribution of ADP-stimulated platelets to maximal clot strength (ADP inhibition): $100\text{-}100 \times [(MA_{ADP}\text{-}MA_{FIBRIN})/(MA_{THROMBIN}\text{-}MA_{FIBRIN})]$, where MA_{ADP} is the ADP-induced clot strength (measurement of clopidogrel effect), MA_{FIBRIN} is the activator-induced clot strength (measurement of fibrin contribution), and $MA_{THROMBIN}$ is the thrombin-induced clot strength (maximum clot strength).