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Effects of genetic variation in protease activated receptor 4 after an acute coronary syndrome: Analysis from the TRACER trial

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ABSTRACT

Variation in platelet response to thrombin may affect the safety and efficacy of PAR antagonism. The Thr120 variant of the common single nucleotide polymorphism (SNP) rs773902 in the protease-activated receptor (PAR) 4 gene is associated with higher platelet aggregation compared to the Ala120 variant. We investigated the relationship between the rs773902 SNP with major bleeding and ischemic events, safety, and efficacy of PAR1 inhibition in 6177 NSTE ACS patients in the TRACER trial. There was a lower rate of GUSTO moderate/severe bleeding in patients with the Thr120 variant. The difference was driven by a lower rate in the smaller homozygous group (recessive model, HR 0.13 [0.02–0.92] $P = 0.042$). No significant differences were observed in the ischemic outcomes. The excess in bleeding observed with PAR1 inhibition was attenuated in patients with the Thr120 variant, but the interactions were not statistically significant. In summary, lower major bleeding rates were observed in the overall TRACER cohort with the hyperreactive PAR4 Thr120 variant. The increase in bleeding with vorapaxar was attenuated with the Thr120 variant, but we could not demonstrate an interaction with PAR1 inhibition. These findings warrant further exploration, including those of African ancestry where the A allele (Thr120) frequency is ~65%.

1. Introduction

Patients with acute coronary syndromes are at risk of subsequent coronary events due to platelet thrombosis on a lipid-rich plaque [1,2]. Antiplatelet therapies are critical to mitigate the risk of atherothrombotic events but at the same time increase the risk of bleeding [2–5]. Bleeding complications are associated with increased mortality, and decision of potency and duration of antiplatelet medications should be based on the assessment of the risk of thrombotic events vs. bleeding complications [6,7]. There are well-established

Abbreviations: PAR, protease activated receptor; NSTE ACS, non-ST-segment elevation acute coronary syndromes; TRACER, Thrombin Receptor Antagonist for Clinical Event Reduction

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inter-individual variations in platelet reactivity and platelet response to anti-platelet agents, and we have shown there is a substantial genetic component to both types of variations [8,9] that may play a role in determining the risk of bleeding and coronary events [10–12]. Many of the challenges with platelet functional assays for assessing bleeding and thrombosis risk can be overcome with nucleic acid-based assays [13]. Thus, identifying genetic biomarkers of variation in platelet reactivity may help with risk stratification of ischemia and bleeding and improve individual tailoring of antiplatelet medications [14–18].

Thrombin is the most potent platelet activator, acting through the protease-activated receptors (PARs) [19]. The PAR1 receptor has classically been identified as the primary mediator of thrombin effects on human platelets since it is activated at a lower thrombin concentration [20]. However, PAR1 signaling is a rapid-on and rapid-off mechanism, whereas thrombin-induced PAR4 signaling has slower activation kinetics, but a sustained response. PAR4 has an overall greater contribution to platelet calcium flux than PAR1 [21,22]. We have previously reported that a single nucleotide dimorphism (G/A), rs773902 of the PAR4 gene (*F2RL3*) determines the Ala120 or Thr120 variants and that the Thr120 variant was associated with higher PAR4 induced platelet aggregation in humans [23]. Notably, the allele frequency of rs773902 G or A varies by race, with the G allele more common in whites than blacks (~80% vs. 37%, respectively) and the A allele less common in whites than blacks (~20% vs. 63%, respectively) [24]. Another rare SNP, rs2227346 (only seen on the rs773902 A allele in blacks), results in a Phe296Val substitution and the Val 296 variant markedly reduces PAR4 signaling [24]. An increased response to thrombin via PAR4 could favor platelet-mediated hemostasis and thrombosis events, but it is unknown whether either PAR4 variant modifies the risk of bleeding or ischemic events *in vivo*. In addition, under conditions where PAR1 is nearly fully inhibited, such as with the use of the potent PAR1 antagonist vorapaxar, the residual response of platelets to thrombin is due to PAR4 activation [25]. Therefore, inter-individual variation in platelet PAR4 reactivity could also influence bleeding and thrombotic events in patients treated with PAR1 antagonists, and especially bleeding when two different platelet signaling pathways are inhibited by aspirin or P2Y12 blockade.

Using the platform of the TRACER trial of vorapaxar vs. placebo in patients with non-ST-segment elevation acute coronary syndromes (NSTE ACS) and the DNA samples collected as part of the trial biorepository, we genotyped participants for the rs773902 and rs2227346 SNPs [26]. The overarching hypothesis of our analysis was that genetically determined variability in PAR4 function would influence the risk of bleeding and thrombotic events among patients who were treated with vorapaxar. In particular, our goals were to test whether rs773902 G or A could mitigate the increased risk of major bleeding observed with vorapaxar and to assess the effect of PAR4 genetic variability in patients following NSTE ACS.

2. Materials and methods

2.1. The TRACER trial

The design and results of the TRACER trial have been reported [26]. In brief, the TRACER trial included 12,944 patients with NSTE ACS and high-risk features within 24 h of hospital presentation. Patients were randomized to vorapaxar 40 mg loading dose and 2.5 daily maintenance dose or matching placebo. Patients had to be treated for a minimum of one year and for the entire duration of the study, which was an event-driven trial. The trial was halted 5 months prior to its planned conclusion after a Data Safety Monitoring Board review, which reported an increased risk of intracranial hemorrhage after the enrollment was completed and the minimum number of endpoint events had been reached (median follow up 502 days). In TRACER vorapaxar was associated with a non-significant reduction of the primary endpoint, a composite of death from cardiovascular causes, myocardial infarction, stroke, recurrent ischemia with rehospitalization, or urgent coronary revascularization and a nominally significant reduction in death from cardiovascular causes, myocardial infarction, stroke. Vorapaxar significantly increased GUSTO moderate or severe bleeding and intracranial hemorrhage.

A TRACER trial biorepository was created which included blood samples for genetic and biomarker analysis. A total of 7927 consented to the supplemental collection of the genetic sample (blood for DNA and RNA analysis).

2.2. Genotyping

Blood samples from patients who consented to voluntary participation in the genetic substudy were collected in DNA PAXgene tubes (PreAnalytiX, Hombrechtikon, Switzerland) for DNA analysis. Because the additional collection of DNA samples was voluntary and subject to individual country regulations, about 60% of the trial cohort had available DNA for genetic analysis. Samples were genotyped for rs773902 and rs222736 using TaqMan SNP Genotyping Assays (Life Technologies Carlsbad, CA) as previously described [24]. Control DNAs for each allele (that had been sequenced) were included on each plate.

2.3. Statistical analysis

Based on the prior platelet function studies, we hypothesized that patients with at least one copy of the hyperreactive rs773902 A allele had a decreased rate of major bleeding. We also hypothesized that bleeding liability with the PAR1 antagonist vorapaxar would be lower in presence of the A allele, as the increased PAR4 function would provide a more effective pathway for thrombin-mediated platelet activation through the PAR4. Finally, we hypothesized that patients with at least one copy of the rs773902 A allele would have increased risk of ischemic events and reduced efficacy from vorapaxar. For this analysis patients were classified in 3 groups: *F2RL3* rs773902 AA homozygous (Thr/Thr120); AG heterozygous (Thr/Ala120), GG homozygous (Ala/Ala120).

Similarly, we hypothesized that patients with at least one copy of the rs2227346 G allele (encodes Val296) are at increased risk of bleeding and relatively higher bleeding risk when treated with vorapaxar, compared with placebo.

The main bleeding outcomes for the study were non-CABG related GUSTO Moderate and Severe and intracranial hemorrhages. The main ischemic outcome was the composite of cardiovascular death, myocardial infarction or stroke. The event accrual period for the analysis was from hospital discharge to 24 months.

To describe the relationship between the rs773902 and clinical events and their timing, Kaplan-Meier rates stratified by genotype were computed for each endpoint and were compared across genotype using the log-rank test. The relationship between the *F2RL3* variant rs773902 genotype and clinical events was assessed by fitting a Cox proportional hazards model for the time-to-first event. The association is characterized by the genotype hazard ratio (HR) and the corresponding 95% confidence interval (CI) and *P*-value. TRACER used self-identified race and ethnicity (called “race”). To minimize population confounders, self-identified non-white patients were excluded in the primary analysis. This analysis was repeated modeling the rs773902 genotype under an additive, dominant, and recessive model.

To describe the relationship between rs773902 and rs227346 variants, vorapaxar and clinical event Kaplan-Meier rates were calculated by genotype overall and by treatment arm for each endpoint of interest. Event counts were compared across genotype using the log-rank test. To determine if the relationship between vorapaxar and clinical events differ by rs773902 genotype, an interaction term between genotype and treatment arm was included in the model and tested. The relationship between treatment and outcome was characterized by the vorapaxar vs. placebo HR and the corresponding 95% CI within each genotype and by