Race Differences in Platelet Reactivity
Is Protease Activated Receptor 4 a Predictor of Response to Therapy?

Michele M. Mumaw, Marvin T. Nieman

Several factors influence the incidence and severity of cardiovascular disease, such as environmental exposures, medical adherence, and genetic polymorphisms. One of the genetic components is a heritable interindividual variation in platelet reactivity, which is greater in black individuals than whites. This correlates strongly with the historical observations that black individuals develop cardiovascular disease at a younger age and have a higher incidence of mortality from the disease. Platelet RNA And eXpression study (PRAX-1) was designed to identify the specific genetic components that correlate with variations in platelet reactivity. This study recently identified an expression profile of mRNAs and microRNAs that are associated with race and protease activated receptor 4 (PAR4) reactivity.

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Thrombin is a potent platelet activator that initiates signaling in platelets and other cells via the G-protein coupled receptors called protease activated receptors (PARs); human platelets express PAR1 and PAR4. PARs are activated by proteolytic cleavage of the N-terminus to expose the tethered ligand, which interacts with the extracellular loops of the receptor. The primary determinant of PAR activation is the nature of the enzyme–substrate interaction, which determines the rate of proteolysis of the N-terminus. PAR1 is more efficiently cleaved by thrombin than PAR4. As a result, PAR4 is often thought of as a low-affinity backup receptor with redundant function because PAR1 and PAR4 initiate overlapping signaling cascades. Like many G-protein coupled receptors, PARs form homo- and hetero-oligomers (Figure). These lateral associations of PAR4 directly influence its activation and signaling. For example, interactions between PAR1 and PAR4 result in a 6- to 10-fold increase in the rate of PAR4 cleavage. PAR4 also forms hetero-oligomers with P2Y12, which regulates arrestin-2 recruitment and AKT signaling in platelets. The interactions with P2Y12 and PAR1 put PAR4 at the center of 2 important pathways for platelet activation. Therefore, any alterations in PAR4 reactivity have the potential to dramatically influence platelet signaling.

The numerous studies on PAR1 have led to the successful development of the antagonist vorapaxar (Zontivity), which was approved for clinical use in 2014. Vorapaxar is a first-in-class antplatelet agent directed to PAR1. Vorapaxar directly competes for the ligand-binding site on PAR1 to prevent activation. Recently, a high-resolution structure of PAR1 bound to vorapaxar was solved and provides exquisite detail regarding its specific contacts with PAR1. In 2012, the results of 2 simultaneous Phase 3 clinical trials with vorapaxar (Thrombin Receptor Antagonist for Clinical Event Reduction in Acute Coronary Syndrome [TRACER] and Thrombin Receptor Antagonist in Secondary Prevention of Atherothrombotic Ischemic Events-Thrombolysis in Myocardial Infarction-50 [TRA 2°P-TIMI 50]) and a subsequent subgroup analysis were reported. The TRACER trial, which was designed to determine the effectiveness of vorapaxar in patients with high-risk acute coronary syndrome, did not reach its primary end point and was terminated early because of a >2-fold increase in the rate of intracranial hemorrhage. In contrast, the TRA 2°P-TIMI 50 trial was designed to determine the effectiveness of vorapaxar at reducing the secondary major cardiovascular events in patients with a history of myocardial infarction, peripheral artery disease, or stroke. The TRA 2°P-TIMI 50 trial showed a 12% reduction in cardiovascular death and ischemic complications in the overall study group.

However, a secondary analysis that excluded individuals with a history of stroke showed that the reduction in primary end points increased to 18%. The combination of these studies ultimately lead to FDA approval with a black box warning about the increased bleeding risk in individuals with a history of stroke, transient ischemic attack, intracranial hemorrhage, or active bleeding. Finally, it is of note that vorapaxar has not been tested as a single agent and is recommended to be used in conjunction with a P2Y12 antagonist. This combination of drugs further drives platelet activation through PAR4 (Figure).

Previously, Edelstein and colleagues examined 154 healthy individuals who self-identified as black or white and determined that differences in platelet reactivity were dependent on PAR4 activation. The degree of aggregation in response to PAR4 agonist peptide (AYPGKF) was higher in platelets from black individuals versus white individuals. The dependence on PAR4 was confirmed by activating platelets with thrombin in the presence of a PAR1 antagonist. From there the authors found that blacks had 4-fold higher expression of phosphatidylycholine transfer protein mRNA, and this correlated with increased PAR4-dependent aggregation and Ca2+ mobilization. In contrast, alterations in the DLK1-DIO3 miRNA cluster were associated with white individuals and decreased PAR4 reactivity. This was the first report to demonstrate that...
PAR4 correlates with differences in platelet activity between black and white populations.

The article by Tourdot and colleagues appearing in this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology* expands on the previous work done by Edelstein et al. This new study examined platelet reactivity in a diverse population of white and black individuals from the Philadelphia area. This report confirmed that platelets from black individuals are more responsive to low concentrations (25–100 μmol/L) of PAR4 agonist peptide and that this effect is caused by an intrinsic property of platelets and not plasma components. To mimic the current standard of care, the authors measured PAR4 activation in the presence of a P2Y12 antagonist and aspirin. These experiments determined that the differences in platelet reactivity were primarily through PAR4 and not thromboxane or ADP signaling. The authors measured PAR4 surface expression on platelets from white and black individuals and found no difference verifying that the observed signaling differences were not simply caused by variable PAR4 expression. Similar to the previous report, Tourdot and colleagues linked the altered PAR4 signaling to the Gq axis. Black individuals had an enhanced Ca2+ response, and this correlated with increased Rap1 activation, pleckstrin phosphorylation, and ultimately α/β activation. The precise mechanism of the heightened Gq signaling through PAR4 is yet to be identified. The authors did not observe obvious differences in expression levels of key platelet proteins, which suggest there are alterations in the regulation of the signaling cascade. Because the differences are specific to PAR4 and not to Gq signaling, in general, future studies will need to examine the initiation of the PAR4 pathway to determine the genetic causes. The first step in this direction was recently published. In this more recent study, Edelstein and colleagues have identified polymorphisms that result in PAR4 sequence variants with altered responses to agonists and are resistant to a PAR4 antagonist. Importantly, the hyper reactive variants are predominantly found in black individuals.

The study by Tourdot and colleagues pinpoints a specific mechanism through enhanced PAR4 activation and Gq signaling that may contribute to the higher risk of stroke and myocardial infarction that is observed in black populations. This study and the recent reports by Edelstein and colleagues are timely and have important clinical implications, given the recent approval of the PAR1 antagonist vorapaxar. For patients on vorapaxar, thrombin signaling will be exclusively funneled through PAR4 (Figure). Therefore, individuals with increased PAR4 reactivity may be less protected from adverse cardiovascular events by vorapaxar. In contrast, heightened PAR4 signaling may protect from the bleeding complications that are observed in specific patient populations on vorapaxar. The answer to these questions will require carefully planned clinical trials. Finally, these studies raise the potential of PAR4 as a therapeutic target. This intriguing possibility will require substantial time and resources to fully develop and validate. The more immediate clinical application of these exciting new findings may lie in the ability to predict patient response to specific therapies. Time will tell how widespread vorapaxar will be used clinically. Monitoring PAR4 activity may aid in these clinical decisions.

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None.

**References**


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