not. Platelets on collagen-coated microdots show a similar extraboundary spreading, which required $\alpha_{IIb}\beta_3$ and was inhibited by eptifibatide, an $\alpha_{IIb}\beta_3$ antagonist. Consistent with the need for α-granule secretion, platelets from a patient with gray platelet syndrome failed to extend beyond the microprinted surface. Additional analysis by Sakurai et al demonstrated the importance of actin cytoskeleton, but not microtubules, to extraboundary spreading and showed a role for Rac1 and myosin light-chain kinase in its regulation. Rho kinase had a negative effect on the process. Platelets from a patient with Wiskott-Aldrich syndrome also failed to spread.

These data are exciting because they show that platelets sense substrate borders and respond by "self-depositing" matrix proteins to alter the boundary and extend their ability to move beyond it. This suggests that platelets can, in response to spatial cues, polarize their secretion to peripheral regions at the edges of the cell. Release from the centralized granulomere may also represent polarized secretion, although that is unclear. Clearly, actin and Rac1/RhoA are important, but what else is involved? Platelet secretion is mediated by membrane proteins called soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs), which facilitate granule–plasma membrane fusion for granule content release. There are 2 classes of SNAREs: v-SNAREs from vesicles/granules and t-SNAREs from the target membrane (the plasma membrane). Platelets contain 4 major v-SNAREs (vesicle-associated membrane protein-2, -3, -7, and -8); each contributes to platelet function.[4,5] VAMP-8 is thought to be the dominant form, mediating the fast secretion events. VAMP-7 mediates secretion from a spatially distinct population of granules. Previous work by the Flaumenhaft group, using time-lapse microscopy, showed that VAMP-7$^+$ granules translocate to the platelet periphery during spreading,[6] in contrast to VAMP-8$^+$ granules, which concentrate in the granulomere. Translocation of VAMP-7$^+$ granules was proposed to provide a membrane reservoir for filopodia and lamellipodia formation. More recent studies, using VAMP-7 knockout mice, have confirmed the importance of VAMP-7–mediated secretion to platelet spreading.[7] These studies also showed interaction between VAMP-7 and key actin cytoskeletal regulators, which may be responsive to the platelet's boundary detection system.

What does this work tell us about platelet function? First, Sakurai et al suggest that this mode of boundary detection and response may facilitate platelet adhesion to small, subclinical vascular lesions. Platelet adhesion must be sufficiently stable to withstand the shear forces of blood flow, and the ability to "self-deposit" matrix and to polarize P-selectin exposure would strengthen their contacts with neighboring endothelial cells. Similarly, this process could stabilize attachments of platelets at the periphery of a growing thrombus. These data might also explain phenotypes where secretion in suspension is modestly affected but bleeding is significant.[8] Defects in secretion polarization may not be readily detected in suspension assays but could be critical in vivo. The most exciting implication of this work is the realization that platelets are not only detecting biochemical changes in the vasculature, but are also deciphering spatial and physical cues. Understanding how this detection system transduces signals to the platelet secretory machinery will undoubtedly yield new insights into how individual platelets integrate into a growing thrombus.

*Conflict-of-interest disclosure: The authors declare no competing financial interests.* ■

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# Warfarin pharmacogenomics and African ancestry

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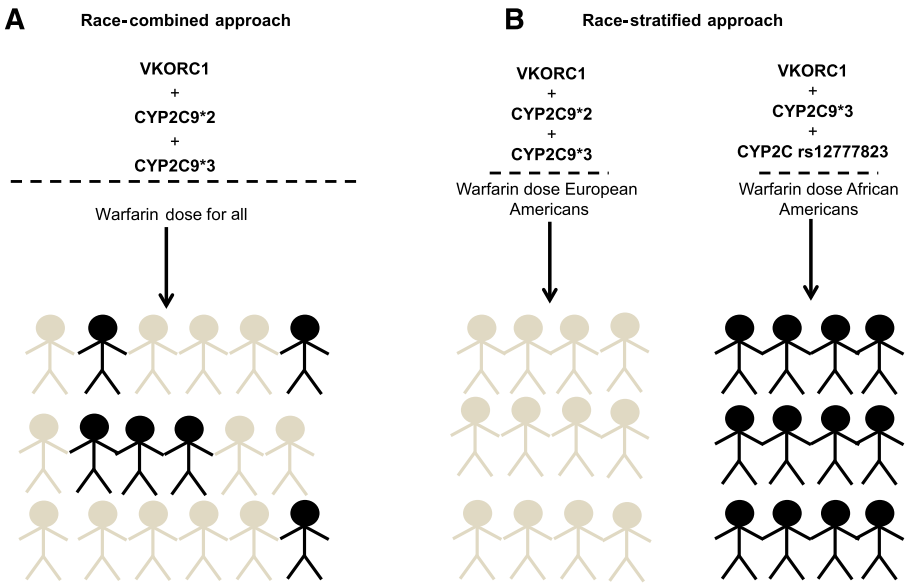
In this issue of *Blood*, Limdi and coauthors demonstrate that racially informed warfarin pharmacogenetic algorithms perform better than traditional algorithms, which previously excluded genetic variants that are unique to patients with African ancestry.[1]

Approximately 34 million warfarin prescriptions are filled annually in the United States to reduce the morbidity and mortality associated with various hypercoagulable states.[2] Warfarin, a vitamin K antagonist, is a potent anticoagulant with proven efficacy when patients reach therapeutic anticoagulation goals. However, warfarin demonstrates significant interpatient variability in dose requirements, and it has a narrow therapeutic index. This makes warfarin difficult to manage despite the use of widespread multidisciplinary anticoagulation clinics dedicated to its management and monitoring. Warfarin's variability in dosing requirements and narrow therapeutic index contribute to this drug being a leading cause of the adverse drug events requiring




The concept of race-combined approaches to warfarin pharmacogenetics compared with a race-stratified approach. (A) The race-combined approach assumes that the genetic variants included in the model will demonstrate equal effects on dosing requirements regardless of genetic ancestry. (B) The race-stratified approach illustrates the concept that genetic ancestry could influence the genetic predictors that are useful for warfarin pharmacogenetics in patient subpopulations.

hospitalizations in the United States.[3] Therefore, clinicians and researchers have been working for years to optimize the efficacy and limit the adverse events associated with warfarin.

Genetic variants that influence the target of warfarin's pharmacodynamic effects (vitamin K epoxide reductase complex 1 [*VKORC1*]) and its primary metabolism pathway (cytochrome P450 2C9 [*CYP2C9*]) have been suggested among the predictors of warfarin efficacy and adverse events. However, 2 recent large genotype-guided trials designed to evaluate their prospective usefulness generated incongruent results.[4,5] The Clarification of Oral Anticoagulation through Genetics (COAG) trial suggested that genotype-guided dosing was not more beneficial than clinical-based dosing.[4] However, the European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) trial demonstrated that genotype-guided therapy was superior to clinical based dosing.[5] The inconsistent reports were puzzling, and the scientific community generated several hypotheses on factors that may explain the observed results. A leading hypothesis was generated from the observation that COAG contained substantially more racial heterogeneity when compared with EU-PACT (~27% black vs ~1% black). This hypothesis is feasible if we consider the fact that the genotype-based dosing algorithm was based upon data largely generated in cohorts of individuals of European ancestry. Furthermore, individuals of African ancestry have differing inheritance patterns of genetic variation when compared with Europeans.[6] This observation has been associated with heterogeneity of genetic predisposition for disease risks and pharmacogenetic responses on many occasions in the literature. Therefore, it was logical to investigate the effects of African ancestry on the effectiveness of the largely European-ancestry–based genotype-based algorithms from COAG and EU-PACT.

Limdi et al investigated the effectiveness of ancestry-informed ("race-stratified") genotype-guided dosing compared with the COAG algorithm that was based primarily on data generated in cohorts of European ancestry ("race-combined" approach) (see figure). The race-stratified genotype-guided dosing algorithm included recently discovered genetic variants that have been associated with warfarin response in African Americans.[7,8] Limdi et al's cohort was composed of 1357 participants, 44% of whom were African American. In this diverse cohort, the COAG algorithm (race-combined approach) demonstrated variability in usefulness based upon race. Only 1 of the 3 included genetic markers was equally predictive of dose requirements for African Americans and European Americans (CYP2C9*3), whereas the other 2 genetic markers demonstrated a significantly larger effect on dosing requirements in European Americans when compared with African Americans (CYP2C9*2 and VKORC1). This could have resulted in a much larger than required warfarin dose reduction in African Americans. This suggests that the COAG algorithm (race-combined approach) may not be optimal for dosing warfarin in African Americans. Using the race-stratified approach, similar results were obtained for CYP2C9*2, which was more influential on dosing requirements for European Americans when compared with African Americans. Interestingly, the CYP2C gene cluster variant that was previously associated with warfarin response in African Americans demonstrated a significantly greater effect on warfarin dosing in African Americans compared with European Americans in the present study.

Limdi et al have generated timely data that should influence the debate on the usefulness of genetics in personalized approaches for warfarin therapy. This study suggests that race-combined genetic-based algorithms are not "one size fits all" tools that can be broadly applied to individuals from varying genetic ancestries. This study is one of the larger studies at the current moment to examine the aforementioned approaches in a large number of participants from heterogeneous ancestries. Overall, the study demonstrates that the inclusion of ancestry-specific genetic markers for African Americans could benefit this subpopulation of patients that have a high risk of being exposed to warfarin. However, the inclusion of the African-ancestry–specific genetic variants in the race-stratified algorithms did not completely explain the variability observed in warfarin dosing requirements for African Americans. Therefore, additional race-stratified genotype-guided trials for warfarin dosing and efficacy are warranted for patients from various genetic ancestries.

Warfarin remains one of the most widely used drugs in the United States. Therefore, approaches to improve its efficacy and limit adverse drug-related events associated with warfarin should remain a public health priority. Ancestry-informed genotype-guided strategies are promising approaches for improving efficacy and limiting warfarin toxicity.

*Conflict-of-interest disclosure: The author declares no competing financial interests.* ■

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