T cell–mediated mechanisms and intracellular signaling pathways.

In the field of hemophilia this study is unprecedented in its size, both in the number of patients and the number of genetic markers that were investigated. Refraining from a genome-wide association study, the authors took a more efficient approach and focused on genes likely to be involved in the immune response. They have strengthened the external validity of their findings by replicating their findings in 3 different cohorts and a subgroup of brother pairs discordant in inhibitor status. This makes the results of this study more persuasive.

Although Astermark and colleagues took great care to validate their results, the scientific and clinical importance of the identified associations needs further clarification. Chance findings among the large number of associations needs further clarification.

The genetic markers that were identified in this study may be regarded as an important departure point for future studies. These novel genetic markers may be used to construct a clinical prediction score for inhibitor development. Ideally, this score would include all important predictors. Before the first treatment with FVIII products it would consist exclusively of genetic predictors. Once treatment with FVIII products is initiated, the risk score would encompass treatment–related factors as well, directing clinicians to take funded treatment decisions. The future will tell whether the predictive value of such a score is sufficient to be clinically relevant. Moreover, it may be challenging to determine genetic markers in young patients before the first hemorrhage occurs.

The next step toward prevention of inhibitors is the characterization of immunologic pathways that are involved in inhibitor development. They may provide potential therapeutic targets for future prevention or treatment of inhibitor development.

Even though the clinical implications of the findings of this study are not yet imminent, they set the stage for further study. Moreover, unraveling the immunologic process of anti-FVIII antibody formation in patients with hemophilia may also provide insight into basic immunologic (patho)physiology and could ultimately not only benefit those with hemophilia, but possibly also a wider group of patients with auto-immune diseases.

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**Comment on Hategan et al, page 1455**

**Counting 1 fibrin molecule at a time**

Robert A. S. Ariëns 1 UNIVERSITY OF LEEDS

In this issue of *Blood*, Hategan et al report on the development of a novel method to study single molecule kinetics of fibrin polymerization.

Each fibrin fiber is composed of thousands of fibrin molecules in width (diameter). For example, a fiber with a thickness of 400 nm consists of around 5000 fibrin molecules in diameter, and a fiber with a diameter of 900 nm consists of 25,000 molecules. Data based on Hategan et al. 1 Schematic representation; not drawn to scale. Fiber is only part filled.

**Using total internal reflection fluorescence microscopy (TIRFM), Hategan and colleagues are able to follow in real time the addition of individual fibrin molecules to growing fibrin fibers during clot formation. In essence, the authors base their calculations on steps of**
fluorescence bleaching during the TIRFM experiments, which they relate back to single fibrin molecules. On the basis of these bleaching experiments the authors developed what they call a molecular calibrator, with which they can count individual fibrin molecules during clot formation using TIRFM. Therefore, accurate quantitative data at the single molecule level is provided, leading for the first time to new insights into the number of fibrin molecules involved in fiber formation, over the entire time course of clot formation. In doing so, the authors calculate that fibrin fibers on average contain several thousand molecules in diameter (see figure). In other words, because fibrin fibers are composed of parallel protofibril strands of half-staggered, overlapping fibrin molecules, this means that fibrin fibers are on average composed of thousands of protofibrils arranged side-by-side.

Armed with TIRFM, Hategan et al first developed a molecular calibration of the method with clots made from well-defined systems using purified proteins. The authors tested 2 different fluorescent probes, which produced similar results, to exclude potential artifacts caused by a particular dye. Several molar ratios of fluorescent probe over fibrinogen in the range of 0.3–4.0 dyes/molecule were investigated to evaluate the system. Clots were produced both by mixing thrombin with the fluorescently labeled fibrinogen beforehand, and by allowing diffusion of thrombin from one side of a microchamber into the opposite side, where fibrinogen was located. Then fibrin formation was studied in plasma, by spiking plasma with fluorescent fibrinogen, and it was found that fibrin fibers grew up to 25,000 molecules and 900 nm in diameter. Fibers showed a range in thickness. Fibers with a thickness of 400 nm for example consisted of around 5000 molecules in diameter. These findings show for the first time the dynamic molecular composition of fibrin fibers in a growing clot.

The study of fibrin polymerization at the molecular level has been the focus of increasing research activity. Alterations in the way fibrin molecules interact influence the structure of the final blood clot. Because fibrin clot structure has been implicated in thrombotic diseases, elucidation of molecular mechanisms in fibrin polymerization is important for our understanding of clot formation and stability. While investigators have previously used light scattering, electron microscopy, and atomic force microscopy to study the qualitative behavior of fibrin molecules during clot formation, none of these previous studies were able to provide an accurate quantitative assessment from single molecules to mature fibers during the process of fibrin clot formation as presented here. Hategan et al for the first time elucidate the fibrin formation process at a single molecule level, and it is truly fascinating to see the live growth of fibrin fibers in terms of the number of molecules that constitute the diameter of a fiber.

There is another reason why the study of fibrin polymerization at the molecular level is so important. Traditional biophysical methods to study fibrin polymerization such as turbidity rely on macroscopic changes and are unable to provide information regarding fibrin structures before the gelpoint. Yet, protofibril formation has already occurred when fibrin reaches the gelpoint. Therefore, new nanoscale methods that analyze fibrin polymerization before gelation are expected to provide new insights into abnormalities of the clotting process, even before a macroscopically visual clot has formed. This study by Hategan et al provides an important new step forward in our understanding of the intriguing process by which individual fibrin molecules produce a 3-dimensional structure and provide the proteaceous backbone with remarkable biophysical and biomechanical properties for the developing blood clot.

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Comment on Switzer et al, page 1469

To give or not to give

Stephanie J. Lee1 1FRED HUTCHINSON CANCER RESEARCH CENTER

In this issue of Blood, Switzer and colleagues report results from a phone survey of 1067 people called on by the National Marrow Donor Program (NMDP) as potential donors because they were preliminary matches with patients in need of a transplant. The study found that people who do not proceed with donation tend to be younger and have lower socioeconomic status, worse self-reported health, and more concerns about donation. The greatest predictor of opting out of donation was high ambivalence. Switzer et al suggest ways that donor ambivalence could be minimized, in hopes of increasing the likelihood of potential donors proceeding with donation.

Hematopoietic cell transplantation from a fully HLA-matched donor is associated with better survival than transplantation from a donor with 1 or more HLA-mismatches. Despite a registry of 10 million potential donors maintained by the NMDP (see figure), the odds of finding a fully matched donor differ considerably based on the race and ethnicity of the recipient (Stephen Spellman, NMDP, written communication, January 3, 2013). If 10 white people search the NMDP registry, 8 will locate a fully matched donor.
Counting 1 fibrin molecule at a time

Robert A. S. Ariëns