Conclusions from mouse monoclonal antibodies in the pharmacologic inhibition of TAFI-Ab show that activation of TAFI as well as TAFIa can be targeted. T/TM as well as plasmin-mediated TAFI activation seems important. *In vivo* studies are scarce due to lack of cross-reactivity with mouse or rat TAFI.

Pharmacologic inhibition of TAFI-Nb has shown that TAFI and the activation of TAFI and TAFIa can be targeted. Further, due to their size, VHH has better clot penetration. The short half-life and lack of cross-reactivity with mouse and rat TAFI hampers studies in mouse models. TAFI also plays a role in inflammation, with TAFIa inactivating C3a, C5a, thrombin cleaved osteopontin, and bradykinin.

Prof. Gils explained that in time the toolbox of TAFI(a) inhibitors allows to investigate the role of TAFI inhibition using animal models whilst the availability of assays to measure the extent of TAFI activation allows to explore the role of TAFI (activation) in different pathologies.

Phenotyping and Genotyping of Platelet Disorders

Written by Rita Buckley

Investigation of patients with mild bleeding disorders might provide novel information on the regulation and role of platelet proteins. It might even identify new targets for prevention of thrombosis. However, gene mutations require phenotypic support to assign causation, according to findings from the observational study, Genotyping and Platelet Phenotyping [GAPP; ISRCTN77951167; UKCRN ID 9858]. Steve P. Watson, PhD, University of Birmingham, Birmingham, United Kingdom, presented results to date from the study.

Several factors have contributed to the fact that platelet function disorders are heavily underdiagnosed, including the absence of a "gold standard" point-of-care assay of platelet function and the variable penetrance of bleeding in families with inherited disorders of platelet function. The GAPP study is testing the hypothesis that a proportion of patients who present with excessive bleeding have a previously unrecognized impairment in platelet function that may explain their propensity to bleed in conditions that would not normally be associated with severe bleeding.

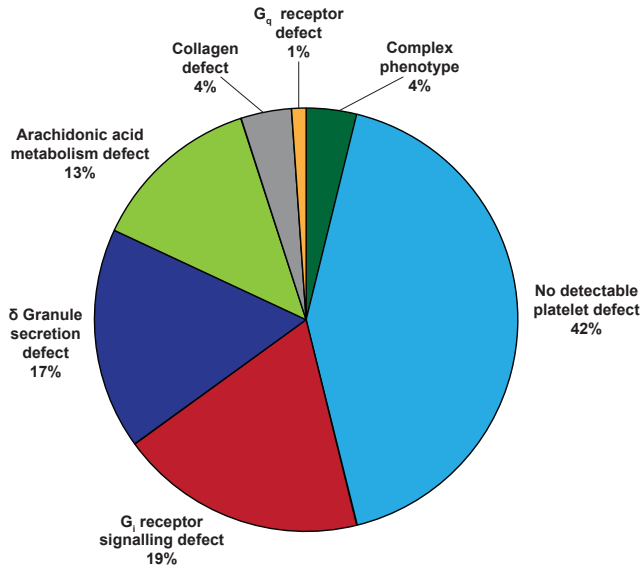
The study uses a combination of platelet phenotyping and a combination of targeted and whole exome gene sequencing to identify candidate mutations underlying platelet dysfunction. The effect of a small number of missense mutations discovered in the study on protein function is being investigated through expression studies in immortalized cell lines

To date more than 520 participants, including patients with excessive bleeding suggestive of inherited platelet dysfunction and healthy volunteers, have been recruited to this multicenter study. The main inclusion criteria for patients are patients of any age with excessive bleeding who are willing to participate and are able to provide informed consent. Exclusions include known platelet disorders, such as Glanzmann's thrombasthenia, Bernard Soulier syndrome, Hermansky Pudlak syndrome, and May Hegglin anomaly.

Today, light transmission aggregometry (LTA) is used worldwide for the study of heritable platelet function disorders (PFDs), but interpretation of results is complicated by the feedback effects of adenosine diphosphate (ADP) and thromboxane A(2) [TxA(2)] and the overlap with the response of healthy volunteers [Dawood BB et al. *Blood* 2012].

The GAPP study performed lumi-aggregometry on 9 platelet agonists in patients with suspected PFD and in healthy volunteers. Abnormal LTA or adenosine triphosphate (ATP) secretion test results were identified in 58% of patients in the GAPP study. In 84% of these, the patterns of response were consistent with defects in Gi receptor signaling, the TxA(2) pathway, and dense granule secretion. Targeted genotyping identified three participants with function-disrupting mutations in the p2Y (12), ADP, and TxA(2) receptors (Figure 1). Prof. Watson noted that the majority of defects were in platelet feedback pathways: ADP, thromboxane, and secretion.

Figure 1. Classification of Defects



The majority of defects are in platelet feedback pathways: ADP, thromboxane and secretion.

Adapted from Dawood BB et al. Evaluation of participants with suspected heritable platelet function disorders including recommendation and validation of a streamlined agonist panel. *Blood* 2012;120:5041–5049.

Research illustrates that detailed phenotypic analysis using a rationalized panel of LTA agonists and simultaneously measuring ATP secretion is a powerful tool for the diagnosis of PFDs and in guiding targeted genetic investigations [Watson SP et al. *J Throm Haemost* 2013] .

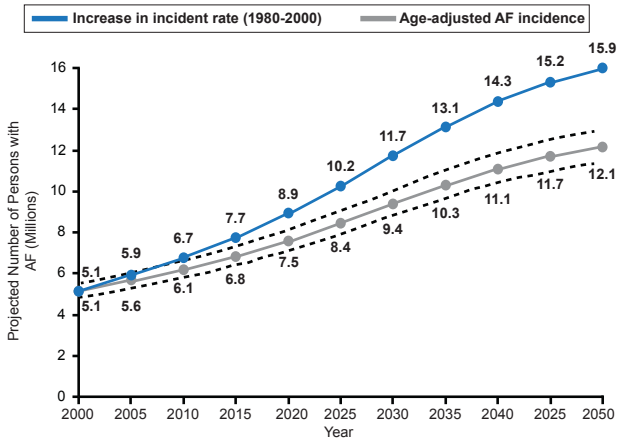
Anticoagulant Treatment Requires Special Care in the Elderly

Written by **Rita Buckley**

Atrial fibrillation (AF) is a leading reason for physicians to prescribe warfarin treatment in the elderly. The prevalence of AF increases with age [Chen LY, Shen WK. *Heart Rhythm* 2007]. By 2050, the projected number of persons with AF in the United States will exceed 10 million (Figure 1) [Miyasaka Y et al. *Circulation* 2006], and 12%, or 1 in 8 Americans, will be aged ≥75 years, said Elaine M. Hylek, MD, MPH, Boston University Medical Center, Boston, Massachusetts, USA, who presented on the need to optimize anticoagulant treatment in the elderly.

Warfarin is effective in the prevention of stroke in AF, but is underused in clinical care [Hylek EM et al. *Circulation* 2007]. They found that of 472 patients, 32% were aged ≥80 years, and 91% had ≥1 stroke risk factor. The cumulative incidence of major hemorrhage for patients aged ≥80 years was 13.1 per 100 person-years versus 4.7 for those aged ≤80 years (p=0.009). Within the first year, 26% of patients aged ≥80 years stopped taking warfarin, with 81% due to perceived safety issues. Rates of major hemorrhage and warfarin termination were highest among patients with $CHADS_2$ scores of ≥3.

Figure 1. Projected Number of Persons With Atrial Fibrillation in the United States Between 2000 and 2050



Reproduced from Miyasaka Y et al. Secular Trends in Incidence of Atrial Fibrillation in Olmsted County, Minnesota, 1980 to 2000, and Implications on the Projections for Future Prevalence. *Circulation* 2006;114(2):119-125. With permission from the American Heart Association.

A review of emergency hospitalizations for adverse drug events in older Americans relating to hematological agents is shown in Table 1 [Budnitz DS et al. *N Engl J Med* 2011].

Nonetheless, the decision to not resume warfarin therapy in the 90 days following gastrointestinal tract bleeding (GIB) is associated with increased risk for thrombosis and death [Witt DM et al. *Arch Intern Med* 2012]. In their analysis of 442 patients with warfarin-associated index GIB, 260 patients (58.8%) resumed warfarin therapy. This strategy was associated with a lower adjusted risk for thrombosis (HR, 0.05; 95% CI, 0.01 to 0.58) and death (HR, 0.31; 95% CI, 0.15 to 0.62), without significantly increasing the risk for recurrent GIB (HR, 1.32; 95% CI, 0.50 to 3.57).

Dr. Hylek reports that AF stroke is associated with a 30-day mortality rate of 24% among individuals not taking antithrombotic therapy. Given the morbidity and mortality related to AF stroke, it is important to remain attentive to the factors that increase the risk of hemorrhage. Blood pressure control reduces the risk of both ischemic stroke and intracranial hemorrhage. Concomitant use of aspirin should be avoided. The risk of falls in the older adult is multifactorial and a major source of serious injury. Interventions to reduce this risk should be sought.

Table 1. Hazards of Warfarin

Therapeutic Category and Adverse-Event Manifestation	Annual National Estimate of Hospitalizations (%; 95% CI)	Proportion of ED Visits Resulting in Hospitalization (%)
Hematologic agents		
Intracranial hemorrhage	5.6 (2.1–9.1)	99.7
Hemoptysis	2.0 (1.1–2.8)	73.6
Gastrointestinal hemorrhage	40.8 (29.9–51.7)	84.7
Genitourinary hemorrhage	4.7 (3.2–6.2)	42.4
Epistaxis	6.1 (4.3–8.0)	10.6
Skin or wound hemorrhage	6.8 (4.5–9.1)	24.5
Other type of hemorrhage	5.3 (2.7–8.0)	27.5
Elevated INR, abnormal laboratory values, or drug toxicity not otherwise described	23.7 (16.8–30.6)	59.5

ED=emergency department.

Multiple strategies can be used to improve the quality of anticoagulant therapy in the elderly. These call on physicians to stay current with physiological changes in their patients that occur with increasing age, identify hazards amenable to intervention, improve management of antithrombotic drugs, and implement strategies to optimize the use of anticoagulants in the elderly.