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THROMBOSIS AND HEMOSTASIS

Comment on Sadler et al, page 3277

Unraveling von Willebrand factor deficiency

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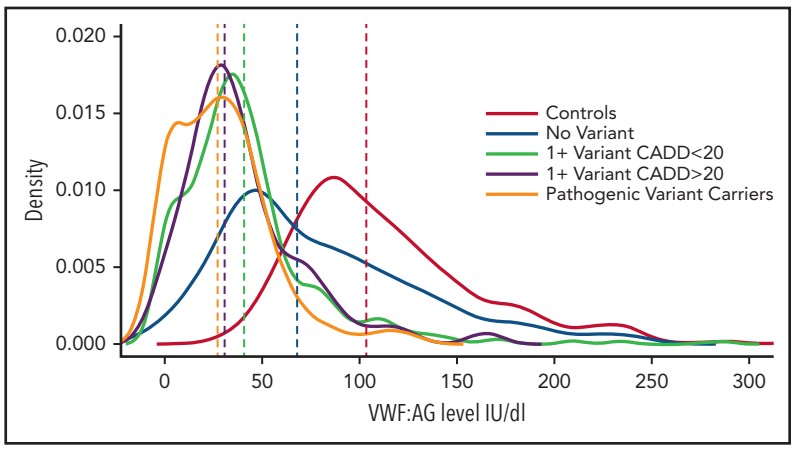
von Willebrand factor (VWF) deficiency has important hemostatic consequences but unraveling its genetic determinants has been a significant challenge. In this issue of *Blood*, Sadler and colleagues report that rare nonsynonymous (protein-coding) variants in *VWF* show important and significant association with the severity of VWF deficiency.[1] The authors evaluated this by sequencing the coding regions of the VWF gene for unrelated persons with low VWF and von Willebrand disease (VWD), and normal subjects, followed by testing whether the presence of rare protein-coding variants is predictive of lower VWF antigen levels.

Sadler et al provide important new knowledge of the determinants of VWF antigen levels, which has emerged to be a complex trait, influenced by *VWF* and additional genes and acquired factors.[2-4] There are several noteworthy findings. First, Sadler and colleagues identified a relationship between the burden (or number) of rare, nonsynonymous *VWF* variants and the severity of VWF deficiency that was significant across all types of VWD. They noted that patients with type 3 VWD (who have the most severe VWF deficiency) had the greatest burden of such variants, although all groups, including those with type 1 or 2 VWD and low VWF, had more rare nonsynonymous variants than normal subjects. It is interesting that in none of the cases did the patients have more than 2 pathogenic or probable pathogenic variants. In Figure 3, Sadler et al provide a helpful illustration of the distribution of VWF antigen levels among normal subjects vs groups with VWF deficiency that do or do not carry rare, nonsynonymous *VWF* variants. It illustrates that the lowest VWF levels are in carriers of rare variants with known or predicted pathogenicity (based on high pathogenicity scores), and the less severe effects of carrying rare variants with low pathogenicity scores (see figure).



VWF factor levels among groups with different types of rare nonsynonymous *VWF* variants. The distributions of VWF antigen levels (vertical lines show medians) in normal subjects are compared with those of groups of subjects with VWD or low VWF who carry pathogenic variants, predicted pathogenic variants (1 + CADD >20), variants with low pathogenicity (1 + CADD <20), or no variants. CADD, combined annotation-dependent depletion. See Figure 3 in the article by Sadler et al that begins on page 3277.

Many rare protein-coding sequence variants are of recent origin,[5] and this may explain the considerable heterogeneity in rare *VWF* sequence variants that Sadler and colleagues found in their study. It is possible that additional determinants of VWF levels will be identified by looking beyond *VWF* for associations between VWF levels and rare protein-coding variants. Indeed, exploring for such relationships by a genome-wide approach has yielded important information for other human traits and diseases in 2 noteworthy studies.[6,7] Given the findings of Sadler et al and the considerable evidence that other genes influence VWF levels,[2,8] it would be particularly interesting to use whole-exome or -genome sequencing to investigate whether rare protein-coding variants (or other mutations) in those other genes would help predict the severity of VWF deficiency among persons with VWD or low VWF.

There are some intriguing questions about causation vs association that future studies could address, given the interesting findings of Sadler et al. For example, family studies of VWD and low VWF, that include index cases with multiple, rare, protein-coding *VWF* variants, would help determine whether the pathogenic or probable pathogenic variants are commonly coinherited with rare nonpathogenic variants that could be markers of a "disease allele." This determination may explain why rare protein-coding variants with low pathogenicity scores show significant association with low VWF levels, whereas variants with known or predicted pathogenicity are associated with even lower VWF levels. In addition, family studies would help address whether VWF levels are significantly lower when both copies of *VWF* contain protein-coding variants with known or probable pathogenicity. The copy numbers of some other *VWF* variants have already been established as

influencing VWF levels.[4] It would also be interesting to directly test several of the rare nonsynonymous *VWF* variants (with high vs low pathogenicity scores) that persons with VWD and low VWF carry, to determine their effects on VWF biosynthesis and secretion, as there is evidence that the more common VWF deficiencies are caused by reduced VWF synthesis and/or constitutive secretion.[3]

Do the new findings have implications for how physicians diagnose or treat VWD and low VWF? Laboratories that perform *VWF* sequencing for diagnostic purposes should incorporate the findings of Sadler et al when reporting on the presence of rare nonsynonymous variants. New guidance on VWD diagnosis was recently published that considered current evidence, real and potential benefits and harms, patient values and preferences, and practical issues that have impacts on diagnosis, treatment, and access to care.[9] The added value of testing persons with bleeding and confirmed VWF deficiency for rare nonsynonymous variants for purposes other than research awaits further exploration. It is noteworthy that Sadler and colleagues estimated that rare nonsynonymous variants in the *VWF* gene independently predict ~16% of VWF antigen levels, which is an important but lesser effect than ABO blood group.[2] Other inherited traits, age, and comorbidities also influence VWF levels in both health and disease.[2,10]

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THROMBOSIS AND HEMOSTASIS

Comment on Houghton et al, page 3284

Calf muscle pump dysfunction and VTE risk

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Chronic venous disease (CVD), which includes chronic venous insufficiency (CVI), a common chronic condition with one of its mechanisms involving calf muscle pump dysfunction, is a potential, but poorly appreciated, risk factor for venous thromboembolism (VTE). In this issue of *Blood*, Houghton et al identified patients in Olmsted County, Minnesota without a history of VTE who had undergone evaluation of calf muscle function by plethysmography (see figure) over a 17-year period.[1] Only those with plethysmographically determined normal venous outflow bilaterally (indicating no obstructive deep vein thrombosis [DVT]) were included. Patients with unilateral calf muscle pump dysfunction were more likely to develop DVT, and any calf muscle pump dysfunction was associated with higher mortality.

CVD (including CVI) is a multifaceted disease, with 3 basic pathophysiologic states existing in isolation or in any combination: valvular insufficiency, venous obstruction inside or external to the involved veins, and calf muscle pump dysfunction. The physiologic hallmark is venous hypertension that is manifested by diverse, but easily recognizable, clinical signs ranging in severity from small reticular veins, varicosities, and edema to hyperpigmentation, lipodermatosclerosis, and ulceration.[2] CVI may be primary or secondary to events such as previous DVT. When caused by a previous DVT, CVI is called postthrombotic syndrome. CVD is ubiquitous in the aging population, with a prevalence of clinically significant disease as high as 64%.[2,3]

Among patients with CVI, the risk of developing venous thromboembolic events, including DVTs and pulmonary emboli (PEs), is incompletely understood. Multiple observational studies found that the diagnosis of CVI is associated with VTE; as an example, among 1272 medical outpatients, CVI was present in 70% of DVT cases compared with 41.4% of controls (odds ratio, 4.45, 95% confidence interval [95% CI], 3.10-6.38).[4] Varicose veins, a clinical manifestation of CVD, are a well-established risk factor for VTE.[5] What is less clearly understood is the precise role of the 3 pathophysiologies in contributing to VTE risk, because CVD is a heterogenous diagnosis. For example, primary valvular reflux has only recently been identified as a novel risk factor for VTE. In a nested case-control