

satisfaction and opens new avenues of research toward a further understanding of red cell volume regulation, a number of questions remain unanswered. Whereas the finding that PIEZO1 protein is expressed in erythroblasts and is present in the membrane of the mature cell could account for dehydration of reticulocytes, as well as of red cells in HX, the mechanism of cell dehydration of erythroid cells remains to be defined. Furthermore, because the newly recognized function of PIEZO1 as a stretch-activated cation channel can be rationalized as an essential element in red cell volume regulation during repeated cycles of membrane deformation during passage through the microvasculature (panel A), it is not clear how its function is regulated in erythroblasts in the relatively static bone marrow environment.

Another unresolved question is how the various identified mutations in PIEZO1 account for the large phenotypic variability in the clinical expression between patients. Similarly, while the documented expression of PIEZO1 in fetal lymphatic vessel endothelium suggests a potential causative role for the protein in the pathogenesis of perinatal effusions, it not clear why only a subset of HX patients with mutations in PIEZO1 exhibits this clinical syndrome.

What then are the implications of these findings? One is that PIEZO1, as the newest member of transport proteins responsible for regulating cation content of red cells, will advance our mechanistic understanding of disordered volume regulation, not only in HX but also in a number of other red cell disorders, including sickle cells.^{7,8} It will indeed be exciting if PIEZO1 can be shown to play a key role in the well-documented deoxygenation-induced increase in cation permeability of sickle red cells; for this phenomenon is responsible for pathogenic cell dehydration, contingent on membrane deformation (panel B).⁹ The study by Adolfo and his colleagues¹ represents the first step in what should prove a long and productive journey toward a comprehensive understanding of red cell hydration pathways in health and disease.

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

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● ● ● THROMBOSIS & HEMOSTASIS

Comment on Carcao et al, page 3946

F8 gene and phenotype: single player in a team?

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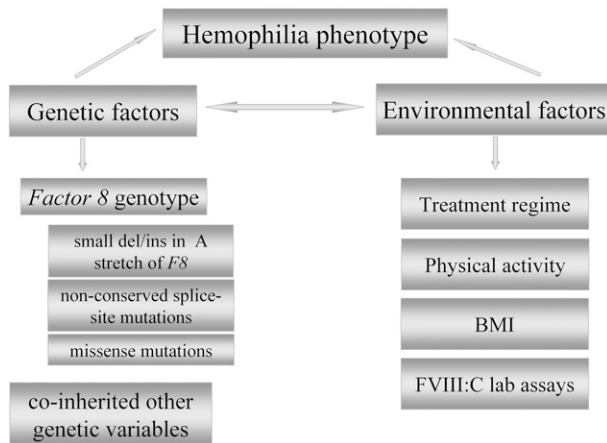
In this issue of *Blood*, Carcao et al demonstrate that the phenotypic variability in patients with severe hemophilia A correlates with the *F8* mutation, where patients with non-null mutations exhibit a milder bleeding phenotype compared with those with null mutations, although this difference is not likely to influence the treatment decision making.¹

Deficiency of blood coagulation FVIII results in hemophilia A, a serious bleeding disorder. The classification of hemophilia is based on the residual factor VIII activity; thus, the clinical presentation of the disease is primarily dependent on the severity of deficiency. In most cases, the bleeding tendency correlates well with the degree of factor deficiency. However, within the different groups of severity, considerable heterogeneity has been observed. A subset of 10% to 15% of patients with severe hemophilia A exhibit a mitigated disease phenotype, with significantly reduced frequencies of spontaneous bleeding, delay in the onset of the first bleeding and further in development of arthropathy, and lower consumption of factor concentrates.²⁻⁴ If it is possible to predict a patient's specific bleeding risk, individualized treatment regimens could be possible, minimizing the unnecessary burden and costs of prophylaxis in patients having a mild bleeding tendency.

Here, Carcao et al performed a detailed analysis on the correlation between phenotypic expression of severe hemophilia A and *F8* mutation in 621 previously

untreated patients. Since the introduction of prophylactic treatment, several parameters (bleeding frequency and concentrate consumption) characterizing the clinical phenotype of hemophilia are no longer applicable. Therefore, the authors postulate that the time from birth to the start of prophylaxis, and especially ages at first bleed and ages at first and second joint bleed, may represent the most suitable variables defining the bleeding phenotype of patients with severe hemophilia. They provide evidence that the type of *F8* mutation can serve as a determinant of bleeding tendency in severe hemophilia.

Patients bearing non-null mutations exhibit a milder clinical phenotype, a later age of disease diagnosis, and a later age of the first bleed, first joint bleed, and second joint bleed compared with those with null mutations. A review of literature shows limited data, and only a few studies demonstrate that small deletions/insertions within poly-A runs of exon 14 of *F8*, nonconserved splice-site mutations, and some missense mutations have a mitigating effect on the bleeding phenotype (see figure).⁵⁻⁷



Possible factors affecting the variability of hemophilia phenotype. BMI, body mass index; del/ins, deletions/insertions; FVIII:C, factor VIII activity. Adapted from Pavlova and Oldenburg.⁹

The milder phenotype in these patients may be a result of the presence of residual amounts of endogenous FVIII activity that are undetectable with current routine FVIII activity measurement assays.

The resulting small amount of endogenous FVIII protein not only is associated with a mitigated phenotype of severe hemophilia A but also might protect against inhibitor development. Although the results of Carcao et al show statistical significance, the longest time difference between the groups of patients with null and non-null mutation is only 2.3 months for the age of first joint bleed. This finding raises the question of whether *F8* mutation can affect clinical decisions such as the choice and time of onset of prophylaxis and the application of individualized dose regimens in children with severe hemophilia A. The authors demonstrate that the relatively minor differences in the ages at first bleed and ages at first and second joint bleed, ranging between 1.2 and 2.3 months within the groups with null and non-null mutation, although contributing for the milder clinical phenotype, might not significantly influence the treatment decision, at least at early childhood.

In addition, considerable interpatient phenotypic heterogeneity among patients with the same *F8* mutation suggests that there are other factors than *F8* mutation accounting for mitigating hemophilia phenotype.⁸ Although it is believed that the *F8* mutations are the strongest predictor of patient's bleeding tendency, the carefully performed investigations by Carcao et al revise the presence of other factors as genetic alterations and polymorphisms in other

hemostatic genes, differences in inflammatory and immune response, and limitations in laboratory diagnostic, as well as environmental factors that alone or together could shape the individual bleeding phenotype in patients with severe hemophilia (see figure). Thus, the authors speculate that differences in the treatment regimens appear to be the largest modifier of hemophilia phenotype rather than *F8* mutation or other genetic variations in coagulation.

Why some individuals with severe hemophilia bleed more than others is still poorly understood. The study of Carcao et al is a prospective one, based on a relatively high number of subjects, and spread a light in finding markers characterizing the clinical phenotype even before the first bleeding occurs. On the basis of the findings of this study, it is reasonable to assume that *F8* mutation, as a genetic marker for the heterogeneity of hemophilia, most likely does not play this role alone but is, rather,

a team player together with other factors in this complex process. Thus, identifying factors attenuating the bleeding phenotype in patients with hemophilia could allow us not only to predict the bleeding pattern but also to influence the treatment decisions, such as time of starting the prophylaxis, tailoring it according to bleeding pattern, and they could also suggest potential alternative targets for preventive and therapeutic intervention.

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● ● ● TRANSPLANTATION

Comment on Boelens et al, page 3981

Better BMT for Hurler syndrome—on the level?

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In this issue of *Blood*, Boelens et al report transplantation outcomes for the largest cohort assembled to date of patients with Hurler syndrome, demonstrating key associations with survival and outlining approaches that result in higher levels of α -L-iduronidase, the enzyme missing in this devastating disorder.¹