The studies by Graham et al in this issue of Blood reveal the importance of dense granule secretion for in vivo platelet accumulation following laser injury of cremaster arterioles. The most striking observation is that dense granule deficient mice (ruby-eye mice) show almost no platelet accumulation at injury sites, indicating that molecules released by dense granules are necessary for efficient thrombus formation. In VAMP-8−/− mice, thrombus formation is delayed and decreased (but not absent) following laser injury, suggesting that the v-SNARE Endobrevin/VAMP-8 is an important component of in vivo platelet granule secretion. The incomplete inhibition of platelet accumulation at sites of injury in VAMP-8−/− mice was somewhat surprising because VAMP-8 mediates the release of all platelet granules, hence an additive effect might have been expected. The results can be explained by functional redundancy of SNAREs, whereby VAMP-2 or -3 can mediate granule release in the absence of VAMP-8. The authors observe that VAMP-2 and VAMP-8 levels are comparable in mouse platelets, whereas VAMP-3 and VAMP-8 levels are comparable in human platelets. As VAMP-2 levels are greatly diminished in human platelets, this suggests that the major alternative granule v-SNARE in humans is VAMP-3.

The dense granule constituents responsible for mediating platelet accumulation in vivo were examined in vitro via aggregation studies of plasma-free washed platelets. Compared with wild-type, platelets from VAMP-8−/− and ruby-eye mice required 1.5- to 10-fold higher concentrations of thrombin for complete aggregation, while addition of 10-fold higher concentrations of thrombin for complete aggregation, while addition of exogenous ADP produced normal aggregation responses in low thrombin concentrations. The function of PKCa−/− mouse platelets (which have impaired dense granule biogenesis and defective secretion of both dense and α-granules) can also be restored by ADP in vitro. Thus, ADP is a key dense granule component, and its release during activation is likely required for efficient platelet accumulation at vascular injury sites. The thrombus formation defects observed in ruby-eye mice indicate that in vivo thrombin generation following laser injury cannot compensate for the absence of dense granule secretion. Although these studies provide important new insights into the role of platelet granule secretion in vivo, some questions do remain.

For example, which SNAREs and regulatory proteins control selective secretion from granules, including the recently discovered distinct populations of α-granules? What contribution does α-granule secretion make to thrombus formation in vivo? The availability of megakaryocyte-restricted gene knockout mice will undoubtedly shed light on some of these questions. Importantly, the current studies suggest that antithrombolytic therapies targeting granule secretion could attenuate acute thrombus formation and also reduce the development of chronic atherosclerotic lesions by inhibiting the release of inflammatory and immune-modulating factors from platelets.

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

REFERENCES


THROMBOSIS & HEMOSTASIS

Comment on Sutherland et al, page 1091

When 1 plus 1 equals 3 in VWD

Anne C. Goodeve UNIVERSITY OF SHEFFIELD

In this issue of Blood, Sutherland and colleagues describe an unusual in-frame deletion of exons 4-5 of the VWF gene associated with both dominantly inherited type 1 and with type 3 VWD.1

The mutation was initially identified in homozygous type 3 von Willebrand disease (VWD) patients, subsequently found in further compound heterozygous patients with type 3 VWD, and then sought in a previously studied cohort of type 1 VWD cases.2 It has thus been identified in 8 of 24 (33%) alleles of the type 3 VWD cohort of 12 apparently unrelated index cases (2 homozygotes and 4 compound heterozygotes) and additionally identified in 2 of 34 (6%) type 1 VWD index cases.

Most mutations identified in type 3 VWD are “null” mutations, resulting in a lack of expression due to non-sense, frameshift, splice site, and large deletion mutations. Large deletions typically result in a lack of von Willebrand factor (VWF) expression, and penetrance of bleeding symptoms in simple heterozygous individuals with such null mutations is very low. The deletion currently described is unusual in that a truncated VWF protein is produced, albeit in very low quantity, that has a dominant negative effect on expression of the second allele. The small group of heterozygous type 1 VWD patients with the mutation have mean VWF levels of around 25 IU/dL, and the mutation appears to result in bleeding in the majority of individuals investigated.

All but one family where the mutation was identified shared a common haplotype associated with the deletion comprising 25 SNP throughout VWF. A second related haplotype found in heterozygous form in only one type 3 family appeared to have arisen from a subsequent recombination 3’ to the deletion. The distribution of the deletion is not yet known; however, its presence in a number of presumed independent type 3 and type 1 VWD families suggests that it may be widespread in families of British origin, possibly including emigrant families. Analysis of the other 2 large
Alu-mediated VWF deletion followed by recombination in one family. The top portion of the figure shows the VWF gene intron-exon structure. Unequal homologous recombination between Alu Y repeats in introns 3 and 5 removes 8631 bp and results in an in-frame deletion of exons 4-5. The deletion is located on an ancestral haplotype (Hap 1), present in all but one of the alleles investigated by Sutherland et al. A subsequent recombination event (panels 2 and 3) results in generation of haplotype 2 (Hap 2). The recombination location is likely to lie between intron 5 and exon 8. Professional illustration by Debra T. Dartez.

type 1 VWD cohorts collected in Europe and Canada will help provide insight into the mutation’s distribution. If it is sufficiently widespread, then the mutation should be sought first in any mutation screening strategy for types 1 and 3 VWD, prior to VWF DNA sequence analysis. A specific long polymerase chain reaction (PCR) described by the authors, which produces a deletion-specific 1084 bp amplicon along with a wild-type product of 1694 bp, will facilitate this analysis.

Examination of the sequence surrounding the deletion breakpoint implicated unequal homologous recombination between 2 Alu Y repeat elements in introns 3 and 5 as the mutation mechanism. There are approximately 100 000 Alu repeats in the human genome, and these have been repeatedly involved in the generation of deletion mutations. As the authors highlight, this is at least the third example of an Alu-mediated deletion in type 3 VWD and in early reports of VWF deletions, mutation mechanism was not investigated so the mechanism is likely underrecognized. Alu repeats may be involved in both homologous and non-homologous recombination, as a core 26 bp repeat sequence is reported to be recombinogenic. A previously reported type 2 VWD patient (disease subtype not specified) had a large in-frame deletion of 31 kb (exons 26–34) associated with recombination between an Alu repeat in intron 25 and a short stretch of homologous sequence in intron 34. These examples suggest that many more large deletions, both in-frame and resulting in null alleles, will be identified as techniques for gene dosage (large deletion or duplication) analysis are developed. Many of these mutations are currently likely to be missed, where a heterozygous deletion is masked by the presence of an intact second allele. Initial analysis of the partial cloned sequence of VWF indicated that there were 14 Alu sequences identified, but there are likely to be many more than this in the entire gene. Close sequence similarity between repeat sequences can help promote unequal homologous recombination, resulting in deletion of the intervening sequence. In the current example, Alu Y sequences surrounding the breakpoint had over 80% sequence similarity.

The subsequent recombination event identified in one family by Sutherland et al is also of interest. SNP analysis suggests that the recombination site lies between intron 5 and exon 8. There may now be sufficient density of VWF SNP reported that the region involved could be identified and sequence features implicated in the recombination event could also be investigated.

Conflict-of-interest disclosure: A.C.G. received sponsorship from CSL-Behring for maintenance of ISTH-SSC on VWF website.

REFERENCES


VASCULAR BIOLOGY

Comment on Wei et al, page 1123

Functional redundancy of Ets1 and Ets2

Peter Oettgen BETH ISRAEL DEACONESS MEDICAL CENTER

In this issue of Blood, Wei and colleagues investigate the overlapping roles of Ets1 and Ets2 in the regulation of endothelial cell function and survival during embryonic angiogenesis.

Ets1 and Ets2 are known to regulate endothelial-restricted gene expression and endothelial function. For example, Ets1 has been shown to regulate the expression of several genes that regulate endothelial function and angiogenesis including VEGF, VEGF-R2, and Tie2. However, homozygous inactivation of Ets1 is associated with defects in T-cell function, but no defects in vascular development or angiogenesis. Similarly, while Ets2 is a critical regulator of trophoblast function during extraembryonic development, it is dispensable for development of the embryo proper. Mice with a homozygous hypomorphic mutant of Ets2, in which the conserved threonine 72 phosphorylation site is mutated, Ets2T72A/T72A mice, are viable and appear normal. In an elegant series of experiments, Wei et al uncover the overlapping roles of Ets1 and Ets2 during vascular development. First, they cross Ets1−/− mice with Ets2T72A/T72A mice to derive double mutant Ets1−/−; Ets2T72A/T72A mice. The double mutants are embryonal lethal and begin to develop vascular abnormalities at day E10.5, including a marked reduction in vascular complexity, defective branching, with the remaining vessels being dilated. To further validate that the phenotype would be investigated.
When 1 plus 1 equals 3 in VWD

Anne C. Goodeve