Loss of APOLD1: a new vascular bleeding disorder?

Kathleen Freson

Department of Cardiovascular Sciences, Center for Molecular and Vascular Biology, University of Leuven, Leuven, Belgium

Correspondence: K. Freson
Kathleen.freson@kuleuven.be

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The *APOLD1* gene encodes apolipoprotein L domain-containing 1 (or vascular early response gene, VERGE; MIM612456), which was identified in 2004 as an endothelial cell early response protein induced after ischemia and expected to regulate endothelial cell signaling and vascular function.[1] Remarkably, only 18 PubMed hits are retrieved to date using the search term 'APOLD1', illustrating the yet unexplored function of this protein. Apold1 knockout mice displayed reduced edema formation but no changes in infarct size or neurological deficits after experimental stroke.[2] This could be explained by the notion that endothelial cells (EC) that stably express VERGE show enhanced permeability while increased VERGE expression has been associated with a breakdown of the blood-brain barrier. Another study with these mice showed that Apold1 deficiency results in a prothrombotic phenotype, accompanied by increased vascular tissue factor activity in the injured carotid arteries and increased platelet aggregation towards collagen.[3]

A study by Stritt *et al.*, published in this issue of *Haematologica*, now provides evidence for a role of APOLD1 deficiency in a human vascular bleeding disorder.[4] Detailed endothelial morphology and functional studies were performed after siRNA-mediated APOLD1 depletion in human dermal blood EC. The findings are summarized in Figure 1. The observed defects were typically associated in EC structures that highly express APOLD1, i.e., cell-cell junctions and Weibel-Palade bodies (WPB). APOLD1 depletion resulted in alterations of EC morphology with the formation of actin-positive stress fibers and the loss of cell-cell junctions which increased EC permeability (Figure 1). In addition, WPB in EC reformatted to autophagosome-like organelles after APOLD1 depletion, resulting in a spontaneous loss of proteins stored in the WPB, including von Willebrand factor (VWF) and angiopoietin 2 (ANGPT2), which were subsequently enriched in the extracellular space (Figure 1). Increased autophagy flux, earlier described as a regulator of VWF secretion,[5] was the proposed mechanism for the spontaneous organelle release. Finally, the data were used to support the discovery of a novel autosomal dominant bleeding disorder found in a pedigree that presented with a heterozygous *APOLD1* R49* nonsense variant detected by whole exome sequencing. The four carriers of this variant presented with an unusual type of spontaneous and trauma-related bleeding defect as they have normal coagulation and platelet function test parameters and do not respond to classical treatment with tranexamic acid or platelet transfusion. Interestingly, the use of vasodilators or aspirin worsened their bleeding tendency, and they developed microcirculatory symptoms, such as livedo reticularis after the administration of desmopressin and Raynaud syndrome. Platelets from these carriers had normal α granule counts but the granules stored less VWF than normal and VWF plasma antigen and activity levels were elevated or in the higher normal range. Platelet α granules express APOLD1. Functional studies using patient-derived EC were not performed to validate the two parts of the study and such investigations will probably be required to better understand the bleeding pathology. It is worth noting that the human phenotype contrasts with that found earlier for Apold1 knockout mice[2,3] urging more studies.

A vascular bleeding disorder is present in Ehlers-Danlos syndrome (EDS) and is found together with joint hypermobility as a result of abnormalities in the collagen of the vessel subendothelial layer and connective tissues caused by genetic defects in different collagen-coding genes.[6] The cause of bleeding in these patients can be due to loss of vessel wall integrity but also defects in the interaction between defective collagen and platelets and VWF, although these latter interactions have not been thoroughly evaluated in EDS patients. If hypermobility is obvious, these patients can be identified by clinicians. Vascular bleeding disorders due to defects in EC integrity are also present in patients with capillary malformation-arteriovenous malformation (CM-AVM) and hereditary hemorrhagic telangiectasia (HHT) due to genetic variants in *RASA1* and *ENG/ACVRL1/SMAD4/GDF2*, respectively.[7,8] Patients with these conditions are typically identified by the presence of vascular malformations of the brain causing cerebral hemorrhage. Therefore, EDS, CM-AVM and HHT are typically diagnosed based on the presence of more specific

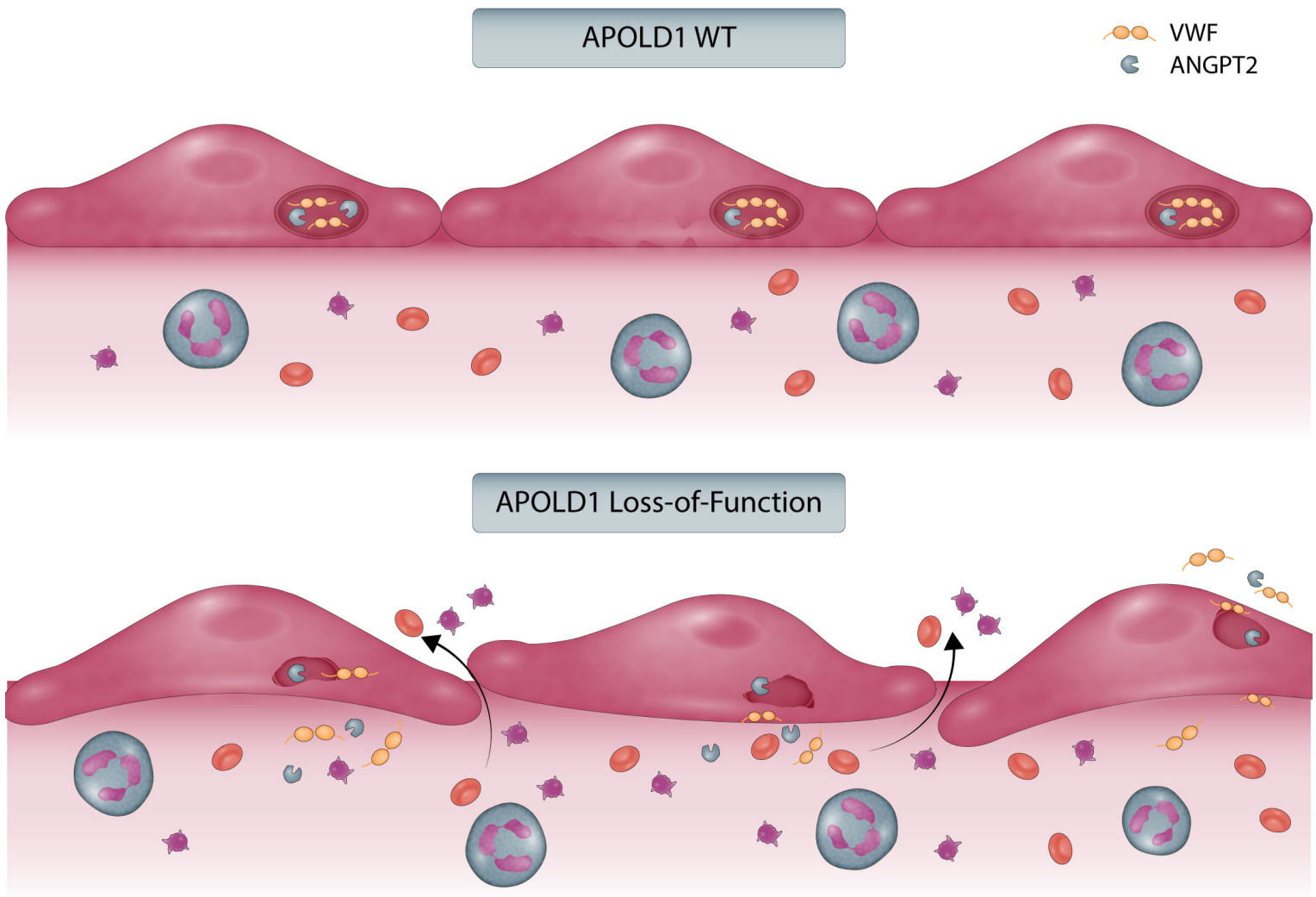

Figure 1. Schematic representation of vascular endothelial cells in a healthy subject and in patient with loss of APOLD1. Healthy blood endothelial cells are closely connected via tight and adherens junctions to prevent blood loss (upper panel). Endothelial cells contain Weibel-Palade bodies that store VWF and ANGPT2, among other proteins. Loss of APOLD1 results in dysmorphic endothelial cells with reduced cell-cell junctions and increased permeability, potentially leading to a bleeding disorder (lower panel). The Weibel-Palade bodies in these cells resemble autophagosomes and spontaneously release their content, resulting in elevated extracellular levels of VWF and ANGPT2. APOLD1: apolipoprotein L domain-containing 1; WT: wild-type; VWF: von Willebrand factor; ANGPT2: angiopoietin 2.

clinical phenotypes than bleeding. No studies have measured VWF levels in EC and plasma of HHT patients and it is not known whether their EC contain normal WPB. Other types of vascular bleeding disorders in humans have not yet been described.

Vascular bleeding disorders are difficult to identify as they are typically missed in the current diagnostic workup due to a lack of efficient laboratory-based screening methods that use patient-derived EC. We know from a next-generation sequencing study that only 3.2% of 619 patients with inherited bleeding of unknown etiology (having normal coagulation and platelet function test parameters) carried genetic variants in known genes for EDS and inherited coagulation and platelet disorders.[9] Three of these patients had a genetic variant in a known EDS gene. However, most of these patients with inherited bleeding of unknown etiology remain undiagnosed and the clinical management of their bleeding tendency can be very challenging.

The vascular bleeding disorder detected in the study by Stritt *et al.* will be difficult to identify using available laboratory-based assays unless high plasma VWF and ANGPT2 levels are specifically associated with this type of bleeding that occurs in the presence of microcirculatory defects. Over the last decade, diverse groups have used exome and genome sequencing to detect novel genes for bleeding[10] and a look for variants in *APOLD1* would be of great importance to validate the findings of this study and enhance our understanding of genotype-phenotype correlations for this gene. This gene can be added as a TIER2 gene to the diagnostic-grade gene list of the International Society of Thrombosis and Haemostasis to enhance knowledge in the scientific community.[11] The heterozygous *APOLD1* R49* nonsense variant results in a premature stop codon and the generation of a shorter APOLD1 protein that lacks three transmembrane domains and the coiled-coil domain. Platelets from the patients express 50% APOLD1 protein levels and the shorter protein was not detected, pointing to a loss of function. The R49* variant is absent in the population variant database gnomAD (*gnomad.broadinstitute.org*). Remarkably, this database mentions that the pLI score for *APOLD1* is 0, meaning that this gene is not protected against nonsense or frameshift variants. Indeed, gnomAD V2.1.1 (excluding samples in TOPMed, which includes a study of bleeding) contains data on more than 50 subjects who are heterozygous for a nonsense or frameshift *APOLD1* variant. This suggests that the R49* variant might cause a yet

unexplored disease mechanism, as this frequency seems high for a severe bleeding disorder, or that other patients exist with a very mild (even sub-clinical) phenotype. Additional genetic studies are warranted.

In conclusion, the study by Stritt *et al.* nicely combined basic, clinical, and genetic research to characterize a novel vascular bleeding disorder. Some open questions remain that require additional studies. In particular, further gene-phenotype investigations will be essential to understand this disorder. The study also nicely illustrates our need for better laboratory assays to identify bleeding defects caused by defective EC and potentially explain patients with inherited bleeding of unknown etiology.

Disclosures

No conflicts of interest to disclose.

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