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Editorial

Loss of APOLD1: a new vascular bleeding disorder?

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Running title: Bleeding due to loss of APOLD1

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The **APOLD1** gene encodes the apolipoprotein L domain-containing 1 (or the vascular early response gene, VERGE; MIM612456) that 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 display 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 shows 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).

The study by Stritt et al., in this issue of the Journal, 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 and findings are summarised in figure 1. The observed defects are typically associated in EC structures that highly express APOLD1 being cell-cell junctions and the Weibel-Palade bodies (WPB). APOLD1 depletion results in alterations of EC morphology with the formation of actin+ stress fibres and the loss of cell-cell junctions that increase EC permeability (Figure 1). In addition, WPB in EC reformat to autophagosomes-like organelles after APOLD1 depletion resulting in a spontaneous loss of the WPB stored proteins von Willebrand factor (VWF) and angiopoietin 2 (ANGPT2) that are subsequently enriched in the extracellular space (Figure 1). Increased autophagy flux, earlier described as regulator of VWF secretion (5), was the proposed mechanism for the spontaneous organelle release. Finally, these data were used to support 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 present with an unusually type of spontaneous and trauma-related bleeding defect as they have normal coagulation and platelet function test parameters and don’t respond to classical treatment with tranexamic acid or platelet transfusion. Interestingly, the use of vasodilators or aspirin worsened their bleeding tendency, and they present with microcirculatory symptoms such as livedo reticularis after use of desmopressin and Raynaud syndrome. Platelets from these carriers present with normal alpha granule counts that however store less VWF and VWF plasma antigen and activity levels were elevated or in the higher normal range. Platelet alpha granules express APOLD1. Functional studies using patient-derived EC were not performed to validate the two parts of this study, but this will probably be required to better understand the bleeding pathology. Of note is the fact that the
human phenotype contradicts with what was earlier found for Apold1 knockout mice (2,3) urging more studies.

A vascular bleeding disorder is present in Ehlers Danlos syndrome (EDS) and coincides with joint hypermobility as a result from abnormalities in 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 from defects in the interaction between defective collagen and platelets and VWF, though 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). These patients 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 clinical phenotypes than bleeding. Studies that have measured VWF levels in EC and plasma of HHT patients don’t exist and it is not known if 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 next generation sequencing studies that only 3.2% of 619 patients with inherited bleeding of unknown aetiology (having normal coagulation and platelet function test parameters) carry 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 aetiology remain undiagnosed and the clinical management for their bleeding tendency can be very challenging.

The vascular bleeding disorder detected in this study will be difficult to identify using available lab-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 lookup 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 TIER2 gene to the diagnostic-grade gene list of the ISTH 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 activity. The R49* variant is absent in the population variant database gnomAD (gnomad.broadinstitute.org). Remarkably, this database mention 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 TOPMed samples that includes a study of bleeding) contains > 50 subjects that are heterozygous for a nonsense or frameshift APOLD1 variant. This probably indicates that the R49* variant might cause a yet unexplored disease mechanism as this frequency seems high for a severe bleeding disorder or other patients exist with a very mild (even sub-clinical) phenotype. Additional genetic studies are warranted.

In conclusion, this study nicely combined fundamental, clinical, and genetic studies to characterize a novel vascular bleeding disorder. Some open questions remain that require additional studies. Especially additional gene-phenotype studies will be essential to understand this disorder. In addition, this study 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 aetiology.

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**References**

4. Stritt S, Nurden P, Nurden AT, et al. APOLD1 loss causes endothelial dysfunction involving cell junctions, cytoskeletal architecture, and Weibel-Palade bodies, while disrupting hemostasis. Haematologica. xxx


Figure legend
Schematic representation of healthy blood endothelial cells that are closely connected via tight and adherence junctions to prevent blood loss (upper panel). Endothelial cells contain Weibel-Palade bodies that store VWF and ANGPT2, amongst other proteins. Loss of APOLD1 results in dysmorphic endothelial cells with reduced cell-cell junctions, increased permeability, and potentially 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.