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A new thrombomodulin-related coagulopathy

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Individuals with bleeding disorders of unknown aetiology exhibit bleeding diatheses of varying severity. Challenges persist in identifying the molecular basis of the bleeding experienced by many of these individuals, however, the advent and accessibility of advanced sequencing technologies has enabled the identification of deleterious mutations in a number of genes encoding proteins necessary for optimal haemostatic function¹. On occasion, the connection between genotype and bleeding is not immediately apparent, but further investigation can ultimately yield unexpected insights into the biology underlying key haemostasis pathways.

One such example is an autosomal dominant bleeding disorder characterised by dysfunctional thrombomodulin (TM) activity, that has been termed 'thrombomodulin-associated coagulopathy' (TM-AC).² TM is a transmembrane glycoprotein receptor best characterised for its activity on endothelial cells, where it captures thrombin generated at the fringes of the injured vessel site and re-directs its proteolytic activity towards protein C (PC) and thrombin activatable fibrinolysis inhibitor (TAFI). Thrombin bound to TM vastly enhances the rate of activation of both zymogens compared to thrombin alone³. Once generated, activated protein C (APC) degrades procoagulant activated cofactors factor V (FVa) and factor VIII (FVIIIa) to limit further thrombin generation. In contrast, activated TAFI (TAFIa) exerts its antifibrinolytic activity by cleavage of basic C-terminal lysine residues in fibrin, limiting fibrin-mediated enhancement of tissue-plasminogen activator (t-PA) plasminogen activation. Consequently, the role of TM in regulating thrombin activity places it at the nexus of coagulation and fibrinolytic regulatory pathways^{4,5}.

TM-AC was first described in a family exhibiting abnormal bleeding that could not be attributed to known coagulation disorders by routine laboratory analyses. Genetic analysis, however, found that these individuals were heterozygous for a novel TM gene variant (*THBD* p.Cys537Stop), which introduces a premature stop codon resulting in truncation of TM within the transmembrane region² (Figure 1). Interestingly, each affected individual possessed plasma soluble TM (sTM) levels >100 fold higher than that normally observed. The mechanism by which the TM Cys537Stop is more readily released from the cell surface is still not fully understood, but may be associated with decreased membrane stability or increased susceptibility to proteolysis from membrane or plasma proteases^{2,6}. Tissue factor-driven thrombin generation was minimal in plasma from individuals with TM-AC, but could be corrected by co-incubation with anti-TM or anti-protein C antibodies. Therefore, elevated sTM plasma levels cause thrombin generated in response to injury to bias towards activation

of its TM-dependent anticoagulant substrates, rather than activation of the procoagulant substrates necessary for efficient clot formation.^{2,7} Subsequent independent studies have identified the same TM mutation in a number of families⁷⁻⁹ and all affected individuals are characterised by excessive post-traumatic bleeding following surgery, dental extraction or childbirth.^{2,7} Subsequently clots made from the plasma of individuals with *THBD* p.Cys537Stop were found to be less susceptible to clot lysis *in vitro* due to increased TAFI activation caused by raised plasma sTM levels⁸. The potential relationship between enhanced antifibrinolytic activity in moderating bleeding caused by excessive APC generation is not known.

The study by Westbury *et al* builds on these previous findings by identifying a novel TM mutation (pPro496Argfs*10) that results in similar bleeding symptoms to those observed in individuals with TM-AC harbouring the *THBD* p.Cys537Stop allele. The *THBD* pPro496Argfs*10 mutation results in a stop-gain that causes synthesis of a TM variant truncated at the membrane-proximal C-terminal region of the extracellular domain (Figure 1). This truncated TM variant is therefore presumably no longer membrane-localised and is instead secreted directly into the bloodstream, resulting in plasma sTM levels >100-fold higher than normal. Despite the distinct mechanisms leading to elevated sTM levels, plasma from individuals with the *THBD* pPro496Argfs*10 mutation also exhibited limited tissue factor-dependent thrombin generation as a result of re-direction of thrombin towards sTM-enhanced plasma APC generation. As in previous studies in TM-AC plasma, delayed clot lysis was also observed.

Incredibly, the study also identified individuals in the same family who had co-inherited a stop-gain mutation (p.Arg114*) in the gene that encodes TAFI (*CPB2*), resulting in expression of a TAFI variant truncated at Arg114 before the catalytic domain. In addition, one family member possessed the *CPB2* p.Arg114* mutation in the absence of TM-AC. This truncated TAFI variant was not secreted, resulting in a ~50% reduction in plasma TAFI levels in individuals carrying the *CPB2* p.Arg114* variant. This represents the first reported case of genetic TAFI deficiency in humans and presents an unique opportunity to evaluate the effect of human TAFI deficiency on normal haemostasis and the potential role that accelerated TAFI activation might have in regulating bleeding in individuals with TM-AC. Previous studies have demonstrated that TAFI-deficient mice do not exhibit any haemostatic abnormalities¹⁰ and therefore it is not entirely surprising that the individual in this family with partial TAFI deficiency (but not TM-AC) was clinically asymptomatic, despite shortened clot lysis times compared to

clots from plasma taken from unaffected individuals. Lysis of clots generated from the plasma of individuals who co-inherited both *THBD* pPro496Argfs*10 and *CPB2* p.Arg114* alleles exhibited prolonged clot lysis times compared to unaffected individuals, but importantly, there was no evidence that the clinical bleeding scores from individuals with TM-AC were affected by partial TAFI deficiency.

The study by Westbury *et al* therefore helps to further characterise the pathophysiological role of unregulated sTM shedding on APC and TAFI generation in TM-AC. They demonstrate that partial TAFI deficiency significantly increases the rate of clot lysis *in vitro*, but does not significantly impact upon haemostasis in affected individuals, either in the presence or absence of co-inherited TM-AC. This discrepancy may arise from APC suppression of thrombin generation of fibrin deposition *in vivo*, limiting substrate for TAFIa to act upon, a limitation not applicable to the *in vitro* fibrinolytic assay used. Nevertheless, the identification of asymptomatic individual(s) with partial TAFI deficiency provides encouragement that therapeutic strategies to attenuate TAFI activity for the treatment of thromboembolic disease¹¹ are unlikely to result in an increased risk of bleeding. Although individuals with TM-AC do not display other overt symptoms beyond abnormal bleeding, it is possible that accelerated sTM shedding or secretion from the vasculature of individuals with TM-AC could also attenuate important non-anticoagulant properties of TM that are largely dependent on TM cell membrane localisation. These activities include PAR1 anti-inflammatory signalling by APC, which is mechanistically linked to protein C activation by the thrombin-TM complex on the endothelial cells¹², inhibition of leukocyte-endothelial cell interactions¹³, and TM regulation of complement on the endothelial cell surface¹⁴. Finally, prior studies indicate that the identification of suitable haemostatic agents to control bleeding in individuals with TM-AC is often challenging⁷, and in keeping with other rare bleeding disorders, bespoke therapies for individuals with TM-AC are currently unavailable. Further efforts are required to identify optimal strategies for the treatment of individuals with bleeding disorders arising from novel mechanisms of haemostatic disruption that will continue to emerge in the post-genomic era.

AUTHOR CONTRIBUTION:

All author's contributed to writing and reviewing the final manuscript.

CONFLICT OF INTEREST DISCLOSURE:

The authors declare that they have no conflicts of interest to declare.

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***THBD* gene variants linked to
Thrombomodulin-associated coagulopathies**

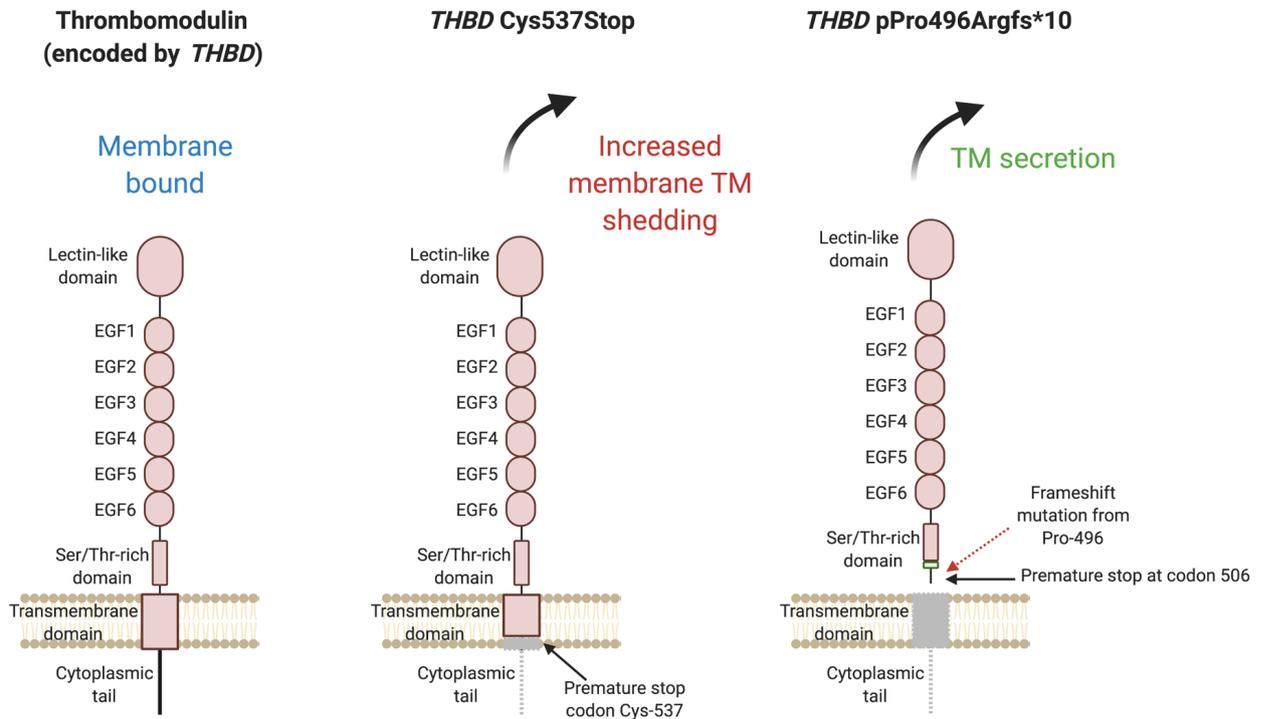


Figure 1: TM mutations causing thrombomodulin-associated coagulopathy. The TM structure consists of an N-terminal lectin-like domain, 6 consecutive epidermal growth factor (EGF) -like domains, a Ser/Thr-rich domain, a transmembrane domain and a cytoplasmic tail. To date, two TM mutations have been demonstrated to cause TM-AC. *THBD* Cys537Stop arises from a premature stop codon at Cys537 resulting in truncation of TM within the transmembrane domain, whereas *THBD* pPro496Argfs*10 introduces a stop-gain mutation causing truncation of TM at the membrane-proximal C-terminal region of the extracellular domain