Towards a novel pathophysiological model for PT-VWD: evidence, insight, and integration

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Introduction:

Platelet-type von Willebrand disease (PT-VWD) is a paradoxical bleeding disorder. Despite the increased platelet-VWF interactions caused by gain-of-function (GOF) mutations in platelet glycoprotein Ibα (GPIbα), patients experience excessive bleeding suggesting impaired clot stabilization. Prior intravital microscopy (IVM) studies from our group showed platelet plugs can form in PT-VWD mice (GPIbα^{Gly233Val}) treated with the antithrombotic anti-GPIbα antibody 6B4; however, these plugs readily disintegrate implicating dysfunction in secondary hemostasis. Recent data (unpublished) suggests that recombinant GPIbα$^{Gly233Val,\ Met239Val}$ not only interacts with the canonical VWF-A1 domain but also the noncanonical VWF-C4 domain, potentially disrupting αIIbβ3-VWF interactions.

Aim: To present a hypothetical model of PT-VWD pathophysiology integrating prior evidence and novel recent observations.

Methods:

We previously integrated biochemical, biophysical, computational, and ultrastructural approaches to assess PT-VWD pathophysiology (Table 1). PPIs were assessed using pull-down assays with full-length VWF and engineered VWF domain fragments (A1, C3–C5, and C4). Peptide G14 was used as a highly specific competitive inhibitor. Computational interaction mapping was performed using the Protein–Protein Interaction Prediction Engine (PIPE) and AlphaFold3 (AF3). Functional assays included latex-bead aggregation (AGG) using recombinant VWF, complemented by transmission electron microscopy (TEM) assessed VWF conformation. Competitive binding experiments (PPI assays) evaluated interactions among mutant GPIbα, VWF, and αIIbβ3. Affinities were measured using circular dichroism spectroscopy (CD-Spec). Flowcytometry (FLOW) assessed platelet activation and fibrinogen binding, and IVM-monitored platelet plug formation in murine PT-VWD.

Results:

Our findings support a theoretical model (Figure 1) with these elements: (1) pathogenic GPIbα interacts with globular or extended VWF via the C4 domain (supported by PIPE, AF3, AGG, TEM, PPI assays, CD-Spec) competing with and inhibiting normal αIIbβ3 binding to VWF-C4 (PPI assays); (2) potentially impairing platelet activation (FLOW), (3) leading to decreased fibrinogen binding and recruitment (FLOW); (4) resulting in disintegration of the initial platelet plug *in vivo* (IVM). Taken together, these data suggest that pathogenic GPIbα engages the noncanonical VWF-C4 domain, interfering with αIIbβ3–VWF interactions potentially leading to destabilized platelet plugs, ultimately impeding stable clot formation.

Conclusions:

The theoretical model presented here reconciles enhanced platelet–VWF interactions with paradoxical bleeding, providing new insights into PT-VWD pathophysiology and thus potential new diagnostic and therapeutic targets.

Table 1: Assays used to construct the hypothetical PT-VWD model.

Assay (abbreviation)	Assay (full name)	Purpose
PIPE	Protein-Protein Interaction Prediction Engine	To predict novel interaction sites between two proteins
AF3	AlphaFold3	To predict structural-based interactions between two proteins
AGG	Aggregation assay	To demonstrate that pathogenic GPIbα interacts with globular VWF
TEM	Transmission electron microscopy	To demonstrate the VWF in the AGG assay is globular, not elongated
PPI assays	Protein-protein interaction assays	To assess the interplay between pathogenic GPIbα, VWF, and αIIbβ3
CD-Spec	Circular dichroism spectroscopy	To determine the binding affinity between pathogenic GPIbα and VWF C4
FLOW	Flowcytometry	To assess platelet receptor expression and fibrinogen binding
IVM	Intravital microscopy	To assess platelet plug formation in PT-VWD mice

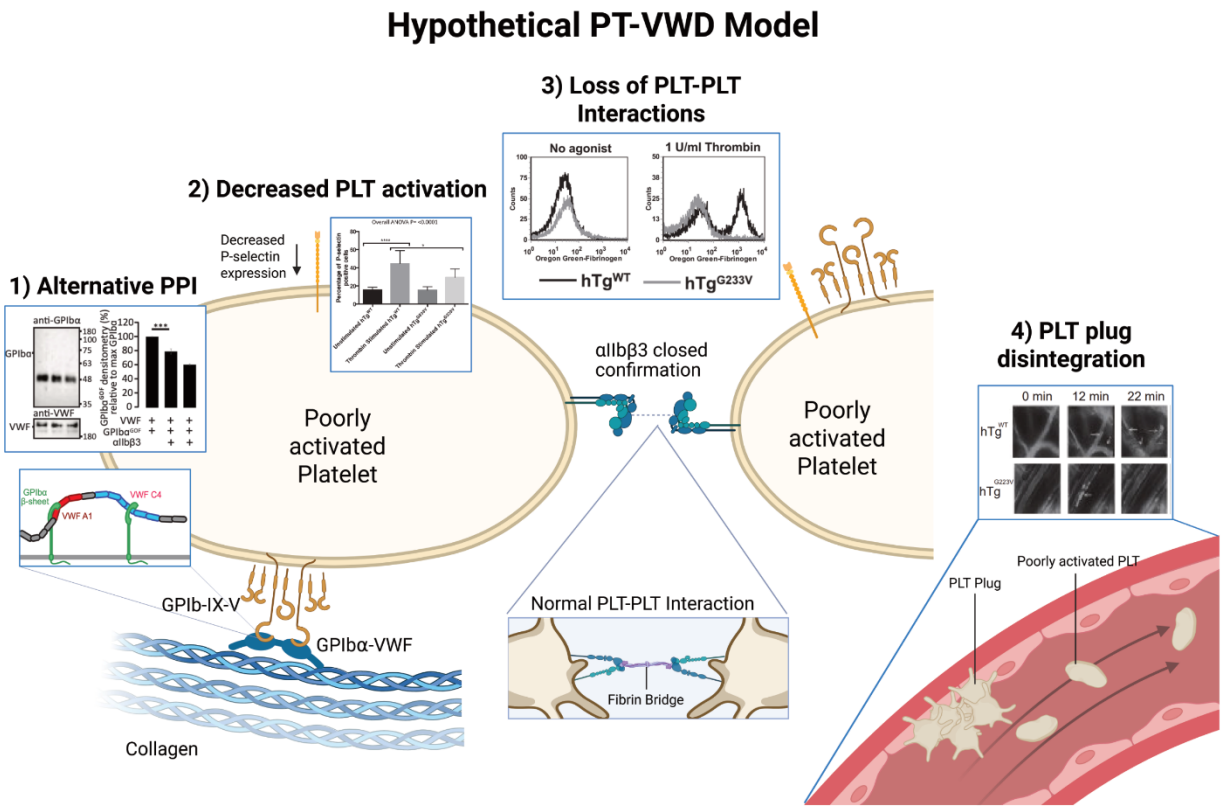

Figure 1: A hypothetical model of PT-VWD pathophysiology linking pathogenic GPIbα mutations to impaired clot stabilization. This schematic summarizes a proposed mechanism whereby mutant GPIbα (e.g., G233V) in PT-VWD aberrantly interacts with both canonical (VWF-A1) and noncanonical (VWF-C4) domains of VWF (1). These alternative interactions lead to interference with normal αIIbβ3–VWF binding, contributing to decreased platelet (PLT) activation and reduced P-selectin expression (2). As a consequence, αIIbβ3 remains in a closed conformation, limiting PLT–PLT interactions (3), and ultimately leading to disintegration of initially formed PLT plugs in vivo (4). This model integrates previous and novel data to explain how gain-of-function GPIbα mutations in PT-VWD produce less stable platelet plug and thus impair clot formation.