Platelet GPIba containing PT-VWD mutations binds the VWF-C4 domain and competes with aIIbß3 integrin

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BACKGROUND: We previously identified G14, a peptide inhibitor with therapeutic potential in platelet-type von Willebrand disease (PT-VWD) - a rare bleeding disorder caused by gain-of-function mutations in platelet glycoprotein Ib alpha (GPIbα^{GOF}: Gly233Val, Met239Val), enhancing its interaction with von Willebrand factor (VWF). G14 disrupted and outcompeted GPIbα^{GOF}-VWF (full length) interaction (Fig. 1A, B). To investigate its mechanism, we examined whether G14 interfered with GPIbα^{GOF} binding to the VWF-A1 domain. Surprisingly, G14 did not affect this interaction suggesting an alternative binding site between the two proteins (Fig. 1C).

AIM: To identify the alternative interaction site for GPIbαGOF on VWF.

METHODS: HIS-tagged C3-C5 and C4 VWF domains were expressed and purified from BL21 *E. coli* for protein pulldown assays. GPIbα^{GOF} was used as prey with G14 and αIIbβ3 acting as competitive inhibitors. Eluates were probed via western blotting. PIPE-Site software was used to predict the site of interaction between GPIbα^{GOF} and VWF. Binding was predicted using AlphaFold3.

RESULTS: G14 was identified using a GPIbα^{GOF}-mediated aggregation assay, where GPIbα^{GOF}-coated latex beads aggregate with VWF (Fig. 1D). G14 inhibits this aggregation (Fig. 1E). Under the same conditions, VWF adopts a globular conformation (Fig. 1F). G14 was designed using our inhouse AI, which identified interaction sites for GPIbα^{GOF} at the VWF-A1 and C4 domains (Fig. 2A, B). Supporting this, GPIbα^{GOF} interacts with the VWF C3-C5; precisely C4, with G14 subsequently disrupting these interactions (Fig. 2C-F). As C4 also mediates αIIbβ3 integrin binding, we introduced αIIbβ3 after incubating GPIbα^{GOF} with VWF. αIIbβ3 competes with and partially displaces GPIbα^{GOF}, presumably via the RGD domain (Fig. 2G).

CONCLUSIONS: GPIbα^{GOF} binds to VWF-C4 domain and competes with αIIbβ3 for this site (Fig. 2H). This work supports new insights into the physiological interaction between VWF and platelet as well as the pathology of platelet disorders, particularly PT-VWD.

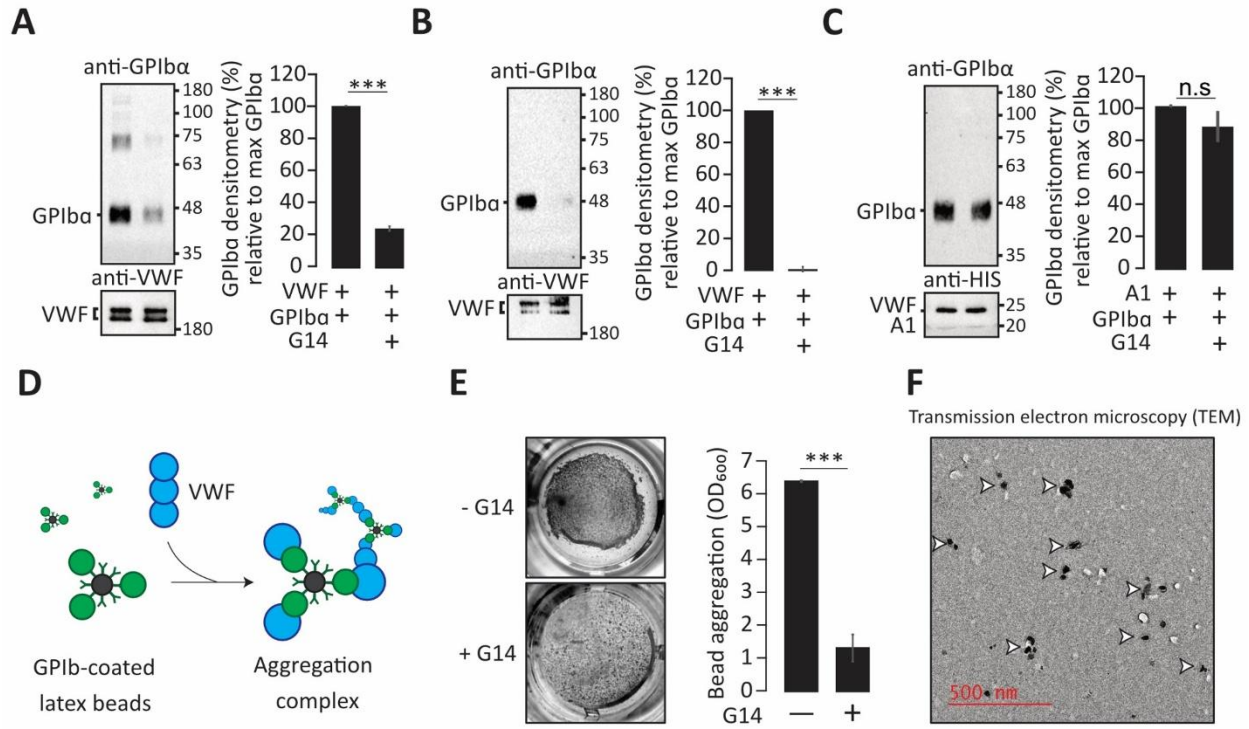

Figure 1: Indirect data suggests that GPIbα^{GOF} has an alternate binding site on full-length VWF independent of the VWF-A1 domain. (**A**) The G14 peptide disrupts the protein-protein interaction (PPI) between full-length, recombinant VWF and GPIbα^{GOF}. (**B**) GPIbα^{GOF} and full-length VWF were incubated for 15 minutes on ice. Subsequent addition of G14 to this PPI results in the peptide outcompeting the PPI. (**C**) The G14 peptide does not interfere with the GPIbα^{GOF}-VWF A1 PPI. (**D**) A schematic underlying the principle of the aggregation assay. (**E**) An aggregation assay demonstrates that GPIbα^{GOF} interacts with full-length VWF, which is disrupted by the presence of G14. (**F**) TEM reveals that recombinant full-length VWF (250 ng/mL in the presence of uranyl acetate) is in a globular conformation under the same conditions as the aggregation assay. White arrows indicate globular VWF particles. Scale bar = 500 nm. All experiments were performed in biological and technical triplicate. All errors are derived from the standard deviation, with significance assessed using a two-tailed unpaired T-test. *** indicates a P-value less than or equal to 0.001. “n.s” refers to a P-value that is greater than or equal to 0.05.

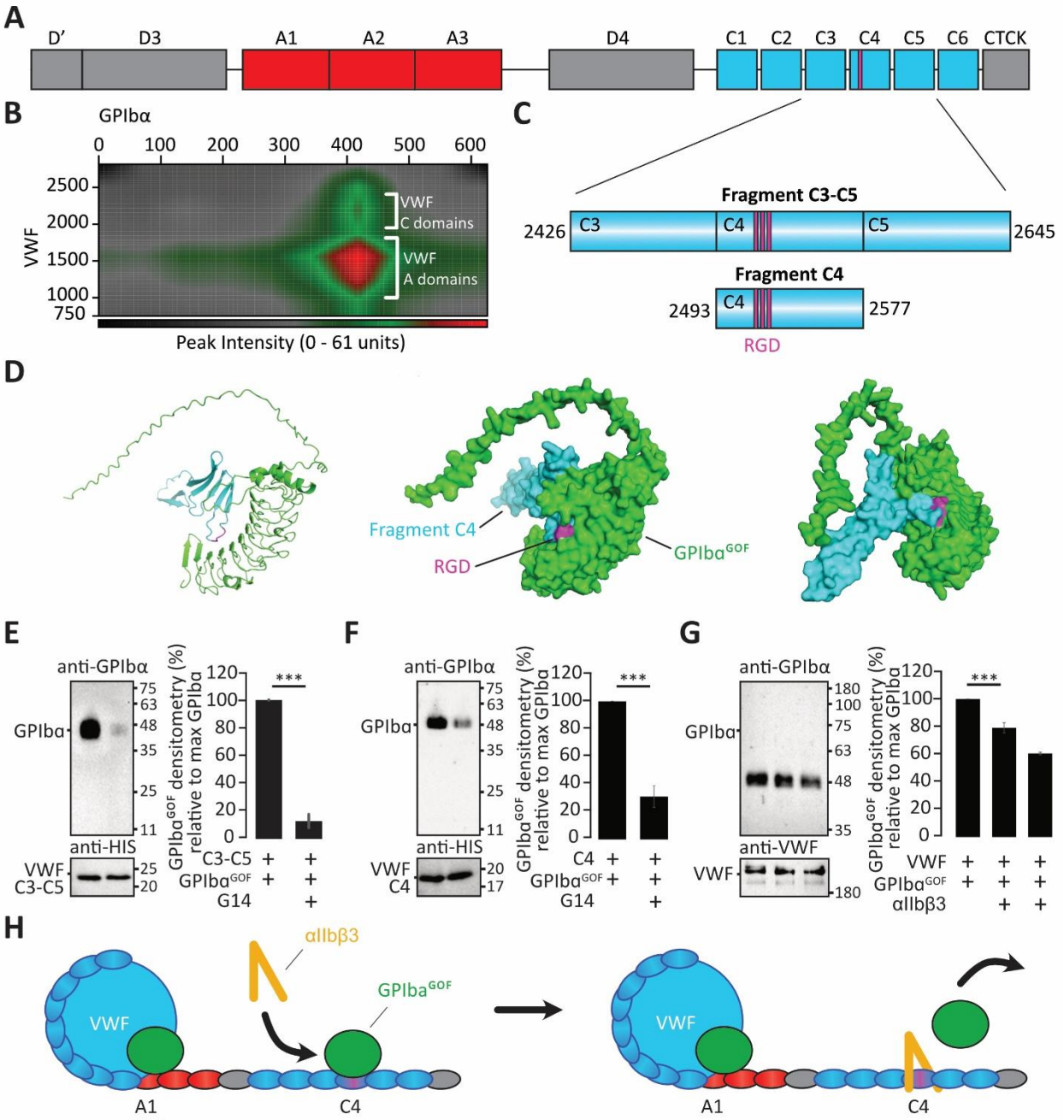

Figure 2: GPIbα^{GOF} interacts with VWF-C4, which can be disrupted by G14 or αIIbβ3 integrin. (**A**) A schematic of the domains in full-length VWF. The three "A" domains are highlighted in red, whereas the six "C" domains are highlighted in blue. (**B**) PIPE predicts that GPIbα^{GOF} interacts with two independent sites on full-length VWF (an A domain and a C domain) as shown by red colours, whereas green and grey zones have little or no predicted contribution to the PPI. (**C**) A schematic of the C3-C5 and C4-VWF fragments used in this study. A magenta colour illustrates the approximate location of the RGD domain. (**D**) GPIbα^{GOF} (green) is predicted to dock with the C4 protein fragment (blue) via AlphaFold3. The RGD domain contained within the C4 fragment is highlighted in magenta (**E**) GPIbα^{GOF} interacts with a protein fragment containing HIS-tagged C3-C5 domains of VWF, with G14 disrupting this PPI. (**F**) The glycoprotein also interacts with HIS-tagged C4, with G14 disrupting the interaction. (**G**) αIIbβ3 integrin competes with GPIbα^{GOF} for full-length VWF. Lane 1 represents a control, in which GPIbα^{GOF} interacts with full-length VWF in the absence of αIIbβ3 integrin. Lane 2 represents a disrupted interaction in which αIIbβ3 integrin was added to the reaction mix after a preincubation between GPIbα^{GOF} and full-length VWF. Lane 3 depicts a preincubation between αIIbβ3 integrin and full-length VWF, with subsequent addition of GPIbα^{GOF}. (**H**) A schematic depicting GPIbα^{GOF} interacting with both the A1 and C4 domains on full-length VWF. When αIIbβ3 integrin is added to the reaction, it outcompetes and displaces GPIbα^{GOF}.All experiments were performed in biological and technical triplicate. All errors are derived from the standard deviation, with significance assessed using a two-tailed unpaired T-test. *** indicates a P-value less than or equal to 0.001.