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# A novel $\beta$ 1-tubulin mutation associated with secretion-dependent platelet hyperactivity and prothrombotic risk *via* GPVI and CLEC-2 engagement

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## Background

While mutations in  $\beta$ 1-tubulin (TUBB1) have previously been linked to macrothrombocytopenia, their effect on platelet function remains poorly understood. We previously identified a novel *TUBB1* mutation, p.P160L, associated with thyroid dysgenesis, macroplatelets, and platelet hyperactivation (Stoupa A *et al.* EMBO Mol Med. 2018).

## Aim

To investigate the impact of the *TUBB1* p.P160L mutation on platelet morphology and function.

## Methods

A knock-in mouse model harboring the *Tubb1* p.P160L mutation was used to evaluate the hemostatic phenotype *in vivo* and *in vitro*. Wild-type (WT), heterozygous (HET), and homozygous (KI) mice were compared.

## Results

Morphological abnormalities and microtubule disorganization: Platelet counts were normal in HET and KI mice, but platelet size was increased. Electron microscopy revealed rounded platelets with disorganized microtubule structures. Microtubule sedimentation assays showed reduced TUBB1 incorporation into microtubules, suggesting that *Tubb1* p.P160L mutation impairs microtubule organization and platelet structure.

Enhanced platelet activation dependent on increased RhoA activity: Flow cytometry analysis revealed increased activation of integrin  $\alpha_{IIb}\beta_3$  in response to convulxin and rhodocytin, while responses to ADP and thrombin were unchanged. Inhibition of ADP and thromboxane  $A_2$  amplification pathways normalized integrin activation. P-selectin exposure was also elevated in response to convulxin and rhodocytin. RhoA activity was increased after rhodocytin activation in KI platelets. These results suggest that enhanced GPVI- and CLEC-2-dependent activation and secretion in p.P160L platelets were dependent on increased RhoA activity.

Prothrombotic phenotype: Under arterial flow conditions in a collagen-coated perfusion chamber, *thrombi* formed by HET and KI mice were larger than those from WT. This phenotype was reversed by inhibition of ADP and thromboxane  $A_2$ . Additionally, in an *in vivo* thrombosis model, arteriolar occlusion time was shortened in HET and KI mice, indicating a prothrombotic tendency.

## Summary

This study demonstrates for the first time that a mutation in TUBB1 disrupts microtubule organization and promotes secretion-dependent platelet hyperactivation *via* GPVI and CLEC-2 pathways, driven by increased RhoA activity. This results in a heightened thrombotic risk, revealing a novel functional consequence of *TUBB1* mutations.