Differential endosomal sorting of a novel P2Y$_{12}$ purinoreceptor mutant identified in a patient with a mild bleeding disorder

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P2Y$_{12}$ receptors play an essential role in ADP-induced platelet activation, the regulation of which is critical for controlling haemostasis and thrombosis. Following a screen of patients with a mild bleeding disorder, a patient carrying a heterozygous mutation in the PDZ binding motif of P2Y$_{12}$ (P341A) was identified. P2Y$_{12}$ receptor recycling was significantly compromised in platelets from this patient. Here, using human cell line models, we investigated wild type (WT)-P2Y$_{12}$ and P341A-P2Y$_{12}$ mutant receptor recycling. Treatment with ADP resulted in delayed Rab5-dependent internalization of P341A-P2Y$_{12}$ when compared with WT-P2Y$_{12}$. Whilst WT-P2Y$_{12}$ rapidly recycled back to the membrane via Rab4 and Rab11 recycling pathways, limited P341A receptor recycling was observed, which was dependent upon Rab11 but not Rab4 activity. Intracellular compartmentalization of P341A was observed with localisation in Rab7-positive endosomes. Whilst P341A degradation was negligible, considerable receptor expression was observed in the trans-Golgi network (TGN). Rab7 GTPase activity is known to regulate the recruitment of retromer complex proteins to endosomes, which transports cargo to the TGN. Here we identified that P341A, not WT-P2Y$_{12}$, co-localized with the retromer cargo-recognition complex; depletion of which blocked limited recycling of P341A-P2Y$_{12}$ and promoted degradation. This study has identified key points of divergence in the endocytic traffic of P341A-P2Y$_{12}$ versus WT-P2Y$_{12}$. Given that these pathways are retained in human platelets, this research may help explain the compromised receptor function in the platelets of the P341A-P2Y$_{12}$ expressing patient.