A novel mutation in the DRY motif of the $P2Y_{12}$ receptor results in chronic bleeding in a patient

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Introduction: Platelets play a central role in the development of arterial thrombosis in heart disease. ADP is regarded as a central mediator of haemostasis and thrombosis mediating its actions through two G protein-coupled receptors (GPCRs), the $P2Y_1$ and $P2Y_{12}$ receptors ($P2Y_{12}R$). As part of the Genotyping and Phenotyping of Platelets (GAPP) consortium we have identified a number of mutations in receptor genes that could contribute to bleeding tendency in patients, including mutations in the $P2Y_{12}R$ (Daly et al., 2009 and Nisar et al., 2011). Recently we have identified a patient, with a chronic bleeding disorder expressing a novel missense mutation (R122C) in their $P2Y_{12}R$. Importantly this mutation is found within the DRY motif of this receptor which in other GPCRs plays a critical role in regulating conformational states. We therefore examined the consequences of this mutation upon $P2Y_{12}R$ function in both the patient's platelets and cell lines.

Methods: Platelet function was assessed by measuring platelet aggregation responses in platelet rich plasma (PRP) whilst functional responses to the $P2Y_{12}R$ were assessed by measuring VASP phosphorylation. In cell line studies HA-tagged wild type or R122C-$P2Y_{12}R$ were expressed in HEK293, human 1321N1 astrocytoma or CHO cells. Receptor trafficking was studied using ELISA and confocal microscopy, to quantify and visualise receptor trafficking, respectively. Protein interactions were investigated by co-immunoprecipitation and immunoblotting. $P2Y_{12}R$ activity was assessed as previously described (Daly et al., 2009).

Results: Aggregation responses to all doses of ADP (1-20 uM) were abnormal in the R122C patient, with secondary aggregation being consistently absent, whilst ADP-dependent VASP phosphorylation was reduced from 83% in a control sample to 10% in the patient following ADP stimulation demonstrating $P2Y_{12}$ dysfunction in the patient's platelets. Initial cell line studies indicated that versus wild type receptor the R122C variant expressed poorly at the cell surface and had significantly compromised ADP responses. Further studies revealed that although the R122C variant could express at the cell surface this mutant displayed a high degree of agonist-independent constitutive internalization versus the wild type. Further study revealed that following its surface expression and internalization the R122C variant unlike the wild type $P2Y_{12}R$ trafficked to lysosomes.

Conclusions: We have identified a novel $P2Y_{12}R$ defect associated with patient bleeding. Further our studies demonstrate that as with other GPCRs the DRY motif of the $P2Y_{12}R$ is critical in maintaining the receptor in a basal non-activated state.

References

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