The platelet P2Y12 receptor contributes to granule secretion through Ephrin A4 receptor.

Study Design
ADP activates platelets through the P2 purinergic receptors P2Y1 and P2Y12. Platelet lysates were analysed on the PTK PamChip after ADP treatment or other aggregation inducing steps. Strong phosphorylation was especially found in peptides representing Ephrin-receptor family members. Specifically blocking P2Y1, P2Y12 and Ephrin4, as a representative of the Ephrin-receptor family, apart or in combination, strongly interfered with these phosphorylations. ImmunobLOTS revealed agonist-induced EphA4 phosphorylation, that could be inhibited by various inhibitors, while time course experiments implied late stage involvement in platelet aggregation. Separate additions and combinations of inhibitors of P2Y12, P2Y1 and EphA4 confirmed that secretion as indicator of platelet activation was predominantly under control of P2Y12 and involved signaling through EphA4.

Key Findings

“Author Quote”
“To increase our insight in signalling pathways initiated by ADP, we applied protein tyrosine kinase (PTK) activity profiling using micro-arrays with peptides representing 144 phosphosites in signalling proteins”

Background
Physical clustering of Eph4 was known to induce tyrophosphorylation, to increase adhesion to collagen and fibrinogen binding/granule secretion and cytoskeletal rearrangements. The studies have suggested that ADP release and activation of P2Y12 and EphA4 form an autocrine loop that enhances platelet aggregation. Eph-receptors seem also involved in the release of contents of granules to contribute to thrombus stabilization. Knowledge of these processes will help to combat ischaemic stroke, myocardial infarction and vascular death.

Conclusion
In the PamChip® kinase assay tyrosine-kinase activity was measured in blood platelets under various conditions. The outcome of these experiments helped to formulate the hypothesis that the Ephrin–receptor family is involved in platelet function. This finding could assist in identifying processes involved in cardio-vascular diseases.