The effect of novel GPIIb mutations causing Glanzmann thrombasthenia on the synthesis, intracellular transport, surface expression and function of the fibrinogen receptor

Summary of PhD thesis

Gergely Losonczy MD

Debrecen, 2007

Two patients suffering from severe recurrent hemorrhagic symptoms since early childhood were investigated. The coagulation screening tests and platelet count were normal while bleeding time and PFA-100 closure times were highly prolonged in both cases. Platelet aggregation occurred in neither of the patients in response to any of the physiological agonists. The absence of clot retraction and the lack of fibrinogen receptor (GPIIb/IIIa) on the platelet surface indicated type I Glanzmann thrombasthenia in the first case. Clot retraction was partially retained and the platelets displayed strongly reduced but well detectable number (2556/platelet) of surface expressed fibrinogen receptor (GPIIb/IIIa) indicating type II disease in the second patient. In the GPIIb gene a novel homozygous deletion (1618delC) causing frameshift and premature termination of protein synthesis (STOP533) was identified in the first patient. In the second case three novel heterozygous GPIIb gene mutations were identified. The 1772insG mutation caused frameshift and premature termination of protein synthesis (STOP575). On the same allele the C339G (L116V) mutation was detected. C2437A (H782N) mutation was found on the other allele. The effects of the novel GPIIb mutations on the synthesis, complex formation, intracellular transport, surface expression and function of the GPIIb/IIIa complex were studied by expression experiments using BHK (baby hamster kidney) cells co-transfected with wild type or mutant GPIIb constructs and with wild type GPIIIa. As a consequence of the STOP533 and STOP575 nonsense mutations part of the thigh domain and the whole calf module were lost. Both mutations induced nonsense-mediated decay of GPIIb mRNA in platelets and when expressed in BHK cells the truncated GPIIb proteins were unable to form complex with GPIIIa. The results suggest that complex formation of GPIIb and GPIIIa does not only rely on the β-propeller domain but also requires the integrity of the thigh domain. Normal synthesis, maturation, intracellular transport, surface expression and fibrinogen binding ability indicated that L116V was not a causative mutation. The H782N mutation situated in the calf-2 domain interfered with the transport mechanism delivering pro-GPIIb/IIIa complex from the endoplasmic reticulum to the Golgi, hampered its maturation and surface expression suggesting that calf-2 domain plays an important role in the intracellular trafficking of the GPIIb/IIIa complex.