Apolipoprotein A-IV is a novel endogenous inhibitor of thrombosis: the roles of its polymorphisms on platelet function

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Introduction

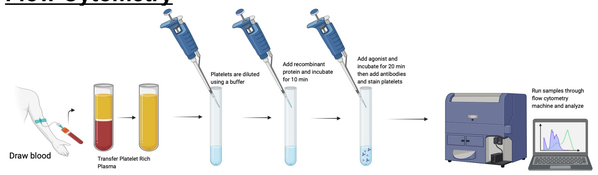
- Our group has recently identified apolipoprotein A-IV (apoA-IV) as a novel endogenous inhibitor of thrombosis through the competitive blockade of the platelet αIIbβ3 integrin[1].
- Several studies demonstrate that plasma apoA-IV levels inversely correlate with cardiovascular disease (CVD)[2-4].
- The two most common polymorphisms of wildtype (WT) apoA-IV, C-terminus mutations Q360H and T347S, have been linked to an increased risk of CVD[2,5-7].
- However, the underlying mechanisms have not previously been explored.
- A better understanding of apoA-IV and its polymorphisms may explain the observed clinical risk of CVD in the polymorphism patients and may provide insight for future patient treatment and therapeutic development.

Hypothesis

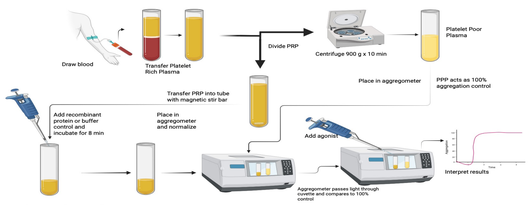
ApoA-IV polymorphisms Q360H and T347S have a weaker inhibitory effect on platelet function and against thrombosis, increasing one's risk of thrombotic cardiovascular events.

Methods

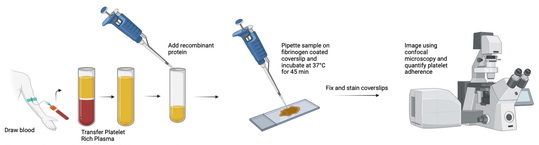
Flow Cytometry



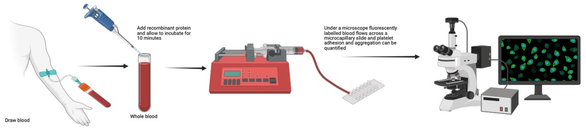
Light Transmission Aggregometry



Platelet Spreading



Perfusion Chamber



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Results

FITC-labeled Q360H and T347S bind less to platelets than WT ApoA-IV *in vitro*

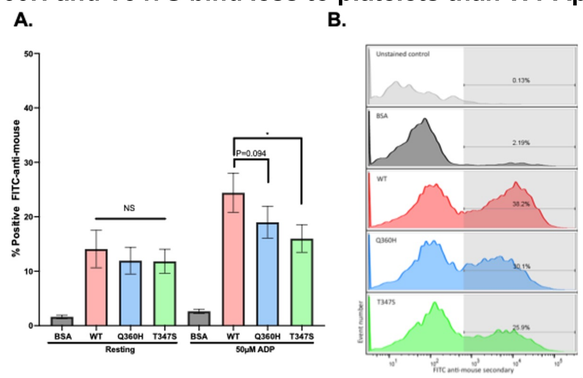

Figure 1: (A) Human PRP incubated with 100 μg/ml apoA-IV ApoA-IV or BSA as a control. Difference between mean percent FITC positive cells for BSA and apoA-IV in resting condition and active (using 50μM ADP) conditions, * represents $p < 0.05$, n = 4. **(B)** Representative histogram showing the FITC distribution of activated platelets in each condition.

Q360H and T347S inhibited activation of human platelets less than WT *in vitro*

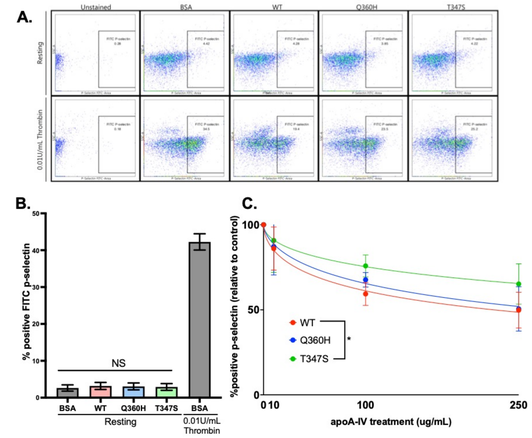

Figure 2: Human PRP incubated with ApoA-IV or BSA as a control. **(A)** Representative dot plots showing gating strategy of P-selectin positive population in resting and activated groups, using 0.01 U/ml of thrombin. **(B)** Percent positive platelets for P-selectin on resting platelets treated with 250 μg/ml apoA-IV show no significant difference. **(C)** ApoA-IV shows dose-dependent inhibition of percent positive platelets for P-selectin, * represents $p < 0.05$, n = 5.

Q360H and T347S inhibited human PRP and gel-filtered platelets aggregation less than WT *in vitro*

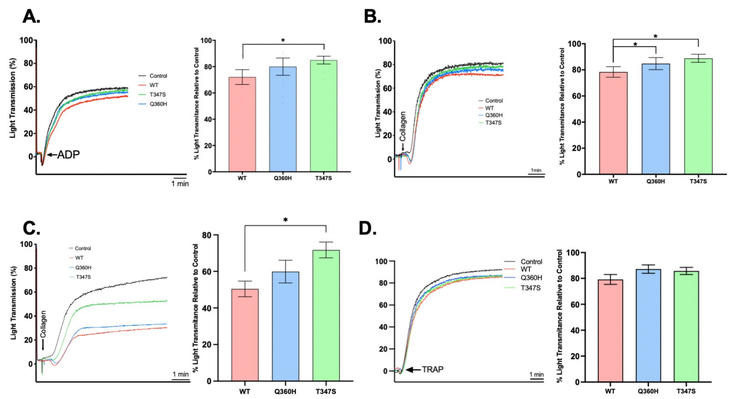

Figure 3: Human PRP or GFP was combined with 160μg/ml apoA-IV or a buffer control. **(A)** Human PRP was activated using 2.5μM ADP, * represents $p < 0.05$, n = 8. **(B)** Human PRP was activated using 1.25μg/ml type IV collagen, * represents $p < 0.05$, n = 8. **(C)** Human GFP was activated using 2.5μg/ml of type IV collagen, * represents $p < 0.05$, n = 9. **(D)** Human GFP was activated using 4μM of thrombin receptor-activating peptide (TRAP), n = 4.

Q360H and T347S impaired human platelet spreading less than WT *in vitro*

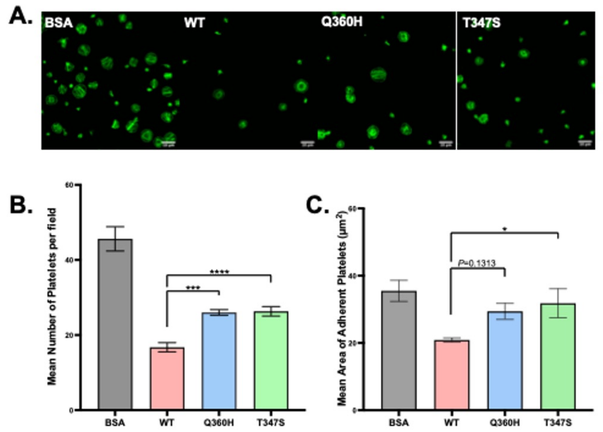

Figure 4: Human PRP was spread over immobilized fibrinogen. Platelets were labeled with Alexa 488-phallodin and imaged at distinct areas for each experiment with a confocal microscope. **(A)** Representative images of spread platelets in each treatment were taken. **(B,C)** A significant difference in platelet spreading between WT and both Q360H and T347S was shown through mean number of platelets per field, and between WT and T347S through the mean surface area of each platelet from all images acquired, *, ***, **** represents $p < 0.05$, $p < 0.001$, $p < 0.0001$ respectively, n = 6.

Ex vivo Thrombosis formation was inhibited by Q360H and T347S less than WT at high shear (1800s^{-1})

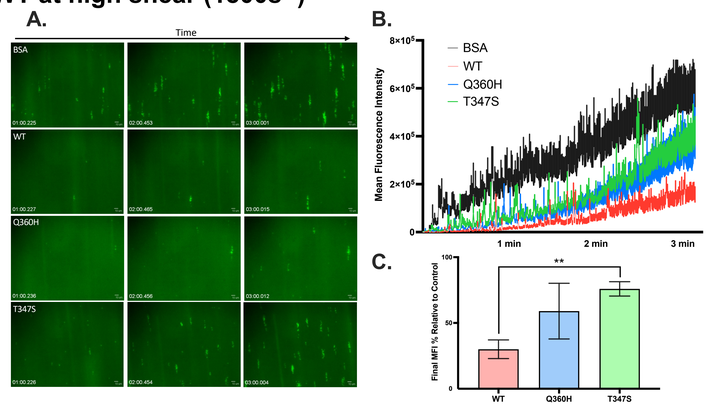

Figure 5: Heparinized human whole blood labeled with DiOC$_6$ and perfused across a collagen-coated microcapillary chamber at 1800s-1 with 160μg/ml of BSA or apoA-IV for 3 minutes. **(A)** Representative images of platelet adhesion over the course of 3 minutes in each treatment group. **(B)** Thrombus mean florescent intensity over time. **(C)** A significant difference in platelet adhesion shown between WT and T347S, $p < 0.01$, n = 5.

Summary and Future Directions

- ApoA-IV polymorphisms Q360H and T347S showed decreased binding to activated platelets and attenuated inhibitory effect on platelet activation in comparison to WT apoA-IV.
- ApoA-IV polymorphisms Q360H and T347S showed impaired inhibition of ADP, collagen and thrombin receptor activation peptides (TRAP)-induced platelet aggregation, and platelet spreading in comparison to WT apoA-IV.
- ApoA-IV polymorphisms Q360H and T347S showed decreased inhibitory effect on thrombosis in an ex vivo perfusion chamber model under high shear (1800s^{-1}) in comparison to WT apoA-IV.
- Future studies will investigate the biomolecular affinity difference of apoA-IV an its polymorphism to the αIIbβ3 integrin.