The L33P polymorphism of integrin β3 PSI domain displays enhanced thiol isomerase activity and upregulates the coagulation cascade

Brock Hoard[1,2], Eric Cerenzia[2,3], Preeti Bhoria[4], Xi Lei[4], Guangheng Zhu[4], Pingguo Chen[4,5], Heyu Ni[1-6]

[1]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, M5S 1A1, ON, Canada; [2]Department of Laboratory Medicine, LKSKI-Keenan Research Centre for Biomedical Science, St. Michael's Hospital, and Toronto Platelet Immunobiology Group, Toronto, M5B 1W8, ON, Canada;[3]Department of Physiology, University of Toronto, Toronto, ON, M5S 1A1, Canada;[4]Toronto Platelet Immunobiology Group and Department of Laboratory Medicine, Keenan Research Centre for Biomedical Science, St. Michael's Hospital, University of Toronto, Toronto, ON, Canada; [5]Canadian Blood Services Centre for Innovation, Toronto, ON, M5G 2M1, Canada; [6]Department of Medicine, University of Toronto, Toronto, ON, M5S 1A1, Canada.

Introduction

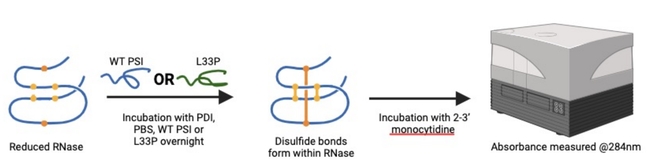
- Integrin PSI domain has recently been discovered to possess endogenous thiol-isomerase potential.[1]
- Within the PSI domain, a leucine-to-proline substitution at residue 33 (L33P) has been associated with increased risk of cardiovascular disease (CVD).[2]
- L33P has been associated with enhanced platelet adhesion, outside-in signaling, and subsequent aggregation during the first wave of hemostasis.[3,4,5]
- However, L33P's impact within the coagulation system (second wave of hemostasis) has never been adequately explored.
- The examination of this polymorphism's impact within coagulation will help identify therapeutic targets for patients as well as elucidate its mechanism of action.

Hypothesis

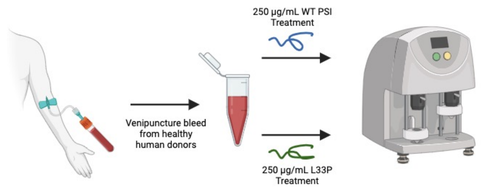
Integrin β3 L33P polymorphism increases thrombus formation via enhanced thiol isomerase activity and upregulation of coagulation.

Methods

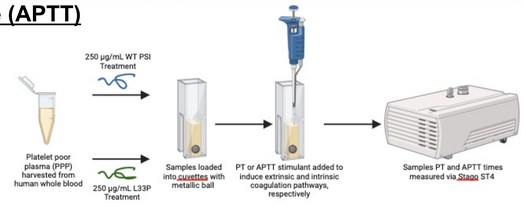
Reduced RNase Thiol Isomerase Activity Assay



Thromboelastography (TEG)



Prothrombin time (PT) and Activated Partial Thromboplastin Time (APTT)



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Results

L33P polymorphism displayed enhanced thiol isomerase activity via a reduced RNase assay

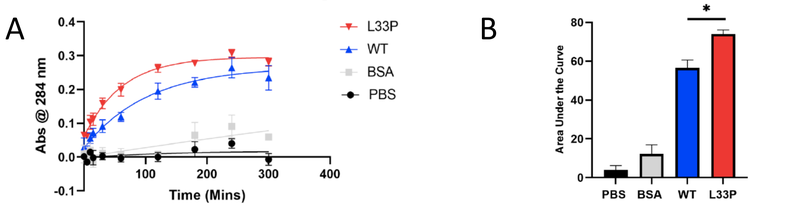

Figure 1. (A) 1x PBS, BSA, WT PSI and L33P PSI were incubated with 5 µg/mL reduced RNase. Then, 2-3' monocytidine was added to each individual reaction mixture. Reaction was carried out for a total of five hours, and absorbance was measured at 284 nanometres. **(B)** Area under the curve analysis. Mean ± SEM displayed. n=4, * = $p < 0.05$.

L33P polymorphism enhanced breakdown of human insulin β chain

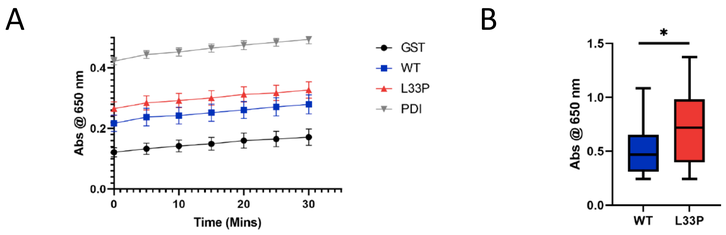

Figure 2. (A) Over a 30-minute reaction time course, 250 µg/mL PDI significantly enhanced β chain reduction, while 250 µg/mL L33P PSI domain slightly enhanced turbidity in comparison to 250 µg/mL WT PSI domain. **(B)** Within an overnight reaction condition(s), L33P PSI domain significantly enhanced β chain reduction turbidity relative to WT PSI domain. Mean ± SEM, n = 4. * = $p < 0.05$.

Recombinant human L33P PSI domain significantly increases clot rate in whole blood coagulation

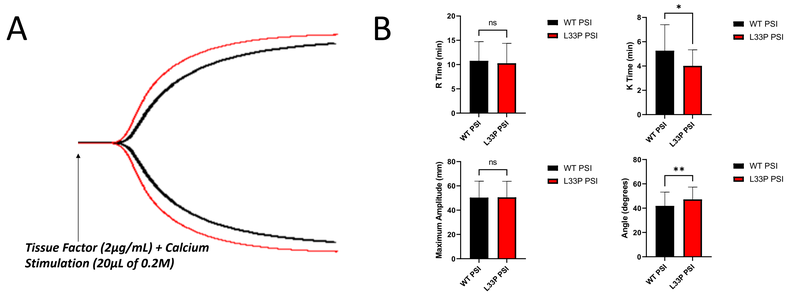

Figure 3. (A) Representative TEG blood coagulation tracing. **(B)** TEG parameters including cloth strength (maximum amplitude), time for clot to begin forming (R), rate of clot formation (Angle), and clot formation time (K). Error bars represent mean ± SE. Statistical analysis completed with two tailed t-test. n=5

Bacitracin inhibits coagulation and causes fibrinolysis in both WT PSI and L33P treatment groups

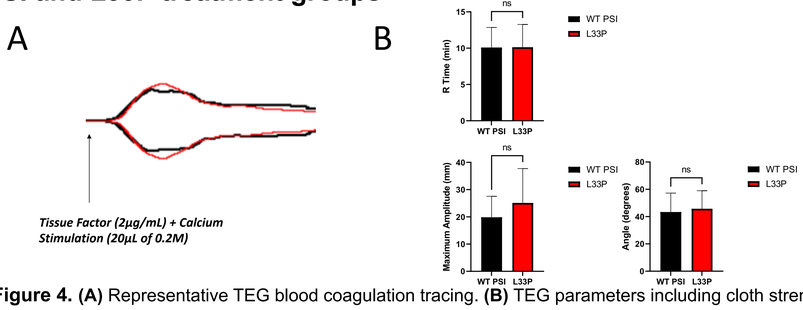

Figure 4. (A) Representative TEG blood coagulation tracing. **(B)** TEG parameters including cloth strength (maximum amplitude), time for clot to begin forming (R), rate of clot formation (Angle), and clot formation time (K). Error bars represent mean ± SE. Statistical analysis completed with two tailed t-test. n=4

Recombinant human L33P PSI domain significantly increased clot weight and decreased clot formation time

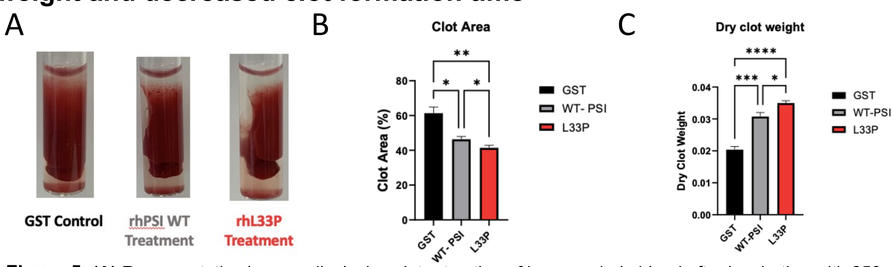

Figure 5. (A) Representative images displaying clot retraction of human whole blood after incubation with 250 µg/mL recombinant human WT PSI, L33P treatments or GST control. **(B)** A measurement of clot area in rhPSI clots in comparison to rhL33P clots using GST control. Mean ± SEM, n=3. **(C)** A measurement of human whole blood clot dry weight measured 16 hours post clot formation. Mean ± SEM, n=3, * = $p < 0.05$.

Recombinant human L33P PSI domain significantly enhances blood coagulation through the extrinsic pathway

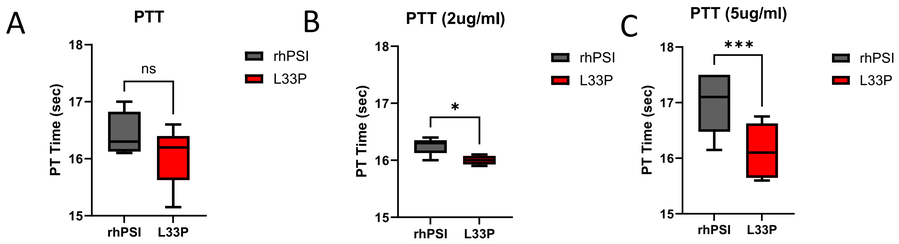

Figure 6. (A) PT time was recorded without external influence from 2 µg/mL Tissue Factor. No significant difference between recombinant WT PSI and L33P was discovered. **(B)** PT time was recorded after incubation with 2µg/mL recombinant Tissue Factor. **(C)** PT time was recorded after incubation with 5µg/mL recombinant Tissue Factor. Time to coagulation was recorded via diagnostica stago machine. Mean ± SEM displayed. n = 5, ran in duplicate. * = $p < 0.05$, *** = $p < 0.001$.

Recombinant human L33P PSI domain tends to enhance blood coagulation through the intrinsic pathway

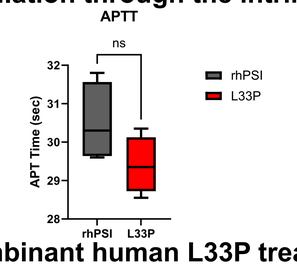

Figure 7. Briefly, 50 µL PPP was incubated with 250 µg/mL human recombinant hPSI domain or L33P. Coagulation was stimulated through the intrinsic pathway via HemoSil® APTT reagent and 0.02M CaCl. Statistical difference between recombinant PSI and L33P was discovered. Time to coagulation was recorded via diagnostica stago machine. Mean ± SEM displayed. n = 4, ran in duplicate. * = $p < 0.05$.

Recombinant human L33P treated plasma displays enhanced fibrin branching and formation

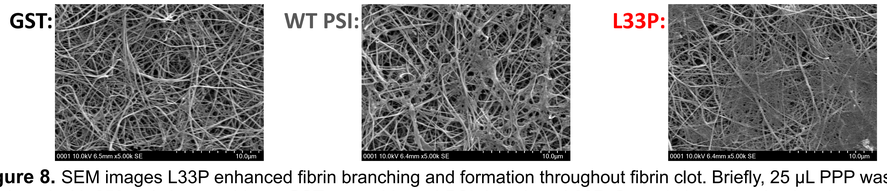

Figure 8. SEM images L33P enhanced fibrin branching and formation throughout fibrin clot. Briefly, 25 µL PPP was incubated with 250 µg/mL recombinant GST, recombinant hPSI domain or L33P. Coagulation was stimulated through addition of 0.4M CaCl. Representative SEM images of the center of clot.

Summary and Future Directions

- L33P displays enhanced thiol isomerase activity in comparison to WT PSI.
- L33P treated whole blood displays faster clot formation time and increased fibrin formation.
- L33P decreases both the extrinsic and intrinsic coagulation pathways activation time.
- Further studies will investigate L33P and PSI direct interactions with coagulation factors.