Aberrant mechanosensitive signaling pathway induce venous endothelial dysfunction in varicose veins

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Background

- Varicose veins are characterized by hemodynamic instability due to valvular incompetence and factors such as orthostatism.
- How altered biomechanical cues get translated into abnormal venous wall remodeling is unelucidated.

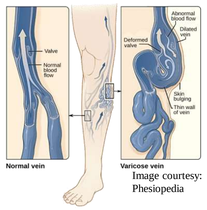


Image courtesy: Phesiopedia

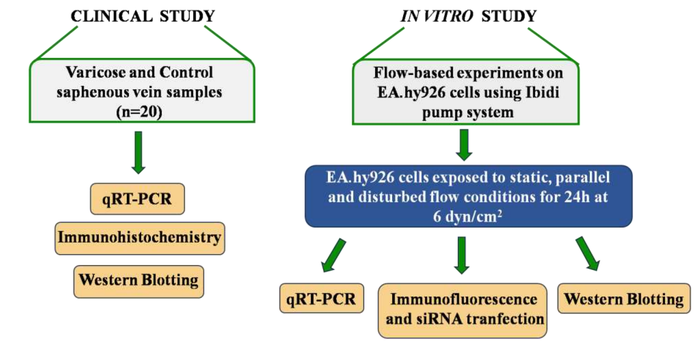
Hypothesis

- We hypothesize that Notch signaling is deregulated in varicose veins.
- We propose that mechanosensitive ETS1 plays a central role in the induction of Notch cascade.

Objectives

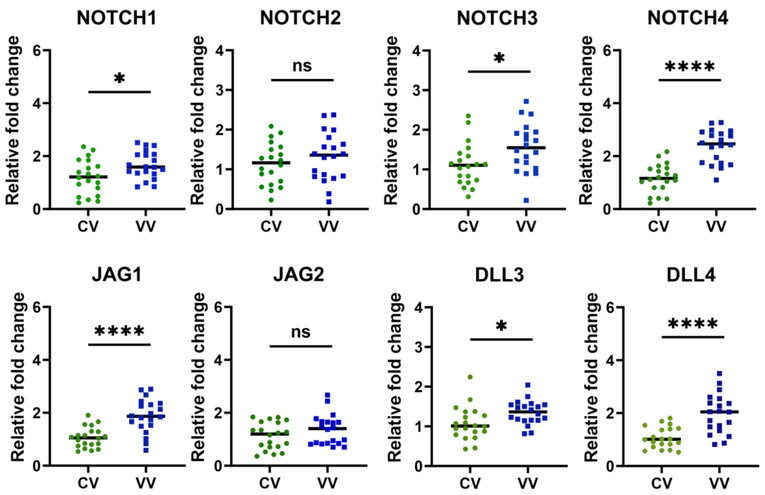
- To analyze mRNA and protein expression of Notch receptors and their ligands in human varicose veins.
- To examine the expression pattern of mechanosensitive ETS1 in varicose veins at mRNA and protein level.
- To delineate ETS1-Notch signaling in endothelial cells exposed to disturbed flow.

Work flow



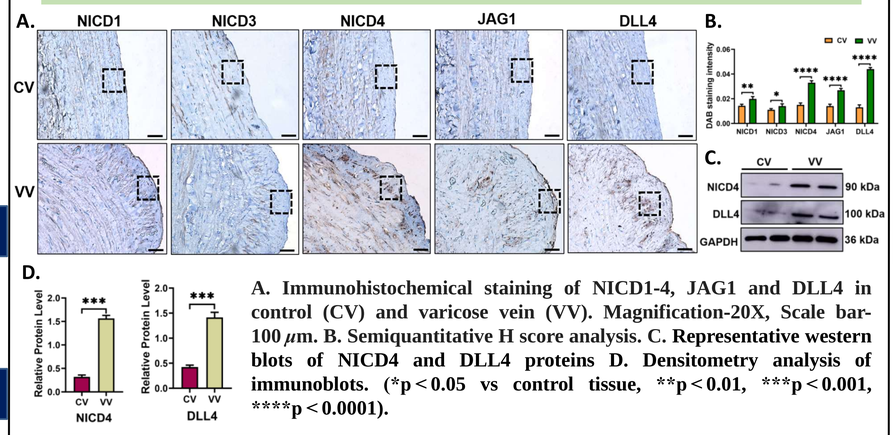
Results

Upregulation of Notch signaling in human varicose veins



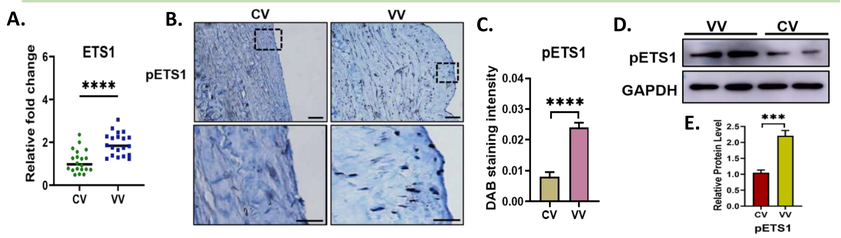
Scatter plots representing mRNA fold changes of NOTCH1-4, JAG1-2, DLL3-4 in 20 human varicose (VV) and control saphenous veins (CV). (* $p < 0.05$, **** $p < 0.0001$, ns not significant).

NOTCH4 and its ligand DLL4 is overexpressed in the neointima of varicose veins



A. Immunohistochemical staining of NICD1-4, JAG1 and DLL4 in control (CV) and varicose vein (VV). Magnification-20X, Scale bar-100 μm. B. Semiquantitative H score analysis. C. Representative western blots of NICD4 and DLL4 proteins D. Densitometry analysis of immunoblots. (*$p < 0.05$ vs control tissue, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).

Phosphorylated ETS1 is elevated at mRNA and protein levels in varicose veins



A. Scatter plot representing mRNA fold change of ETS1 in 20 human varicose (VV) and control saphenous veins (CV). B. Immunostaining showing nuclear localization of phosphoETS1 in varicose veins. C. Bar graph representing semiquantitative H score analysis. D. Western blot showing overexpression of phosphoETS1 in varicose veins. E. Bar graph showing densitometry analysis. (*$p < 0.001$, ****$p < 0.0001$).**

In vitro flow-based Ibidi pump system setup for shear stress experiments on endothelial cells

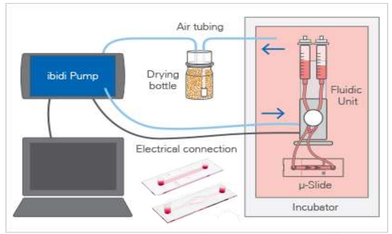
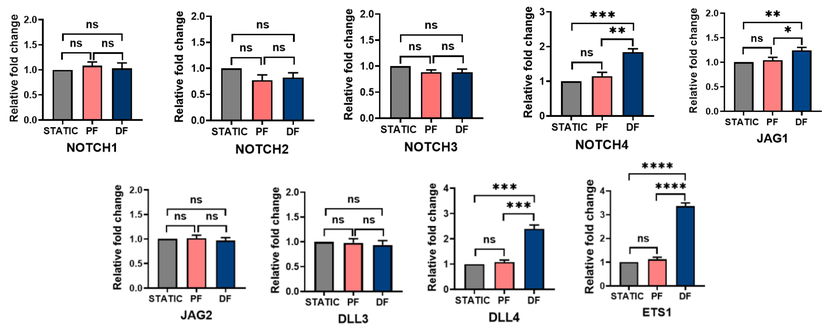


Image courtesy: Ibidi, Germany

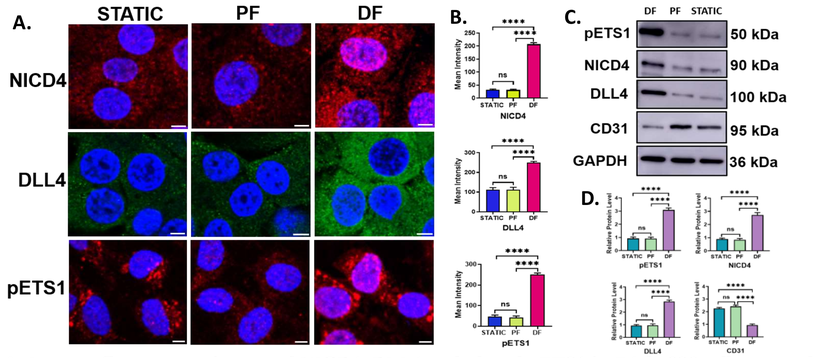
Schematic diagram representing flow-based microfluidic platform: The ibidi Pump System consists of two main components: the ibidi Pump and the Fluidic Unit. The ibidi Pump applies pressurized air (in blue) to the reservoirs of the Fluidic Unit. Fluidic Unit performs valve-switching operations on the Perfusion Set (fluidic reservoirs and tubing) to generate unidirectional flow in a channel µ -slide.

Elevated mRNA expression of NOTCH4, DLL4 and ETS1 in vein endothelial cells under disturbed flow



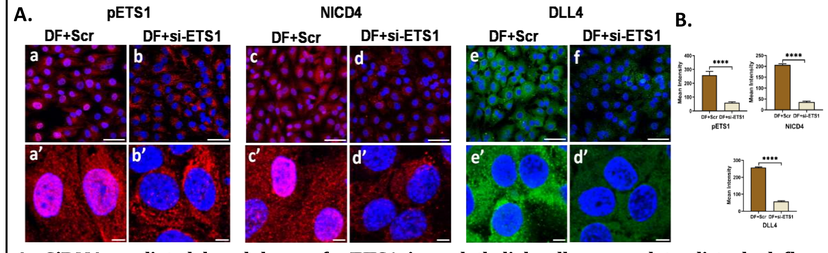
mRNA level expression of Notch receptors1-4, ligands Jag1-2, Dll3-4 and ETS1 upon exposure of endothelial cells to static, parallel and disturbed fluid flow for 24h (n=3). *$p < 0.05$ vs control tissue, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. ns nonsignificant difference.

Disturbed venous flow induces pETS1-NOTCH4 signaling in vein endothelial cells



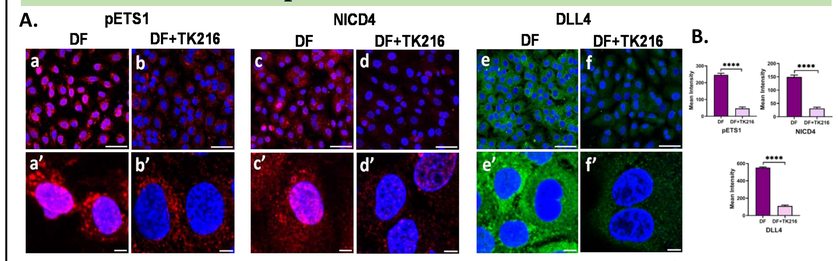
A. Immunofluorescence images of NICD4, DLL4 and phosphoETS1 in EA.hy926 cells exposed to Static, Parallel(PF) and Disturbed flow(DF). High magnification, Scale bar-20 µM. B. Mean fluorescence intensity. C. Representative western blots of pETS1, NICD4, DLL4 and CD31 proteins. D. Densitometry analysis of immunoblots. (**$p < 0.0001$, ns non- significant)**

Knockdown of ETS1 downregulated NOTCH4/DLL4 protein expression



A. SiRNA mediated knockdown of pETS1 in endothelial cells exposed to disturbed flow significantly downregulated the expression profile of pETS1, NICD4 and DLL4 protein. (a-f scale bar 50 µM, magnification 40×, a'-f' scale bar 20 µM and high magnification) B. Mean fluorescence intensity analysis. (**$p < 0.0001$)**

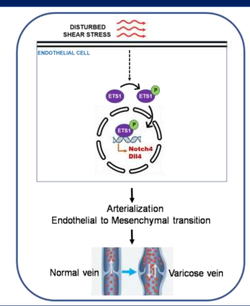
Inhibition of ETS1 by TK216 significantly reduced the overexpression of NICD4 and DLL4



A. ETS1 inhibition by 1µm TK216 significantly reduced the overexpression of pETS1, NICD4 and DLL4 in endothelial cells exposed to disturbed flow for 24 h. (a-f scale bar 50µM, magnification-40×, a'-f' scale bar 20 µM, high magnification). B. Mean fluorescence intensity analysis. ****$p < 0.0001$.

Discussion & Conclusion

- Our study provides evidence for the role of disturbed fluid shear stress-mediated NOTCH4/DLL4 expression in the pathogenesis of varicose veins, presumably through ETS1.
- Targeting ETS1 rather than downstream Notch components may serve as an efficient strategy for varicose vein small molecular therapeutics.



Future work

- To delineate the molecular mechanism by which fluid flow activate ETS1.

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