# Disturbed hemodynamics activates aberrant endothelial Notch signaling *via* mechanosensitive Ets-1 in varicose veins

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## Background

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

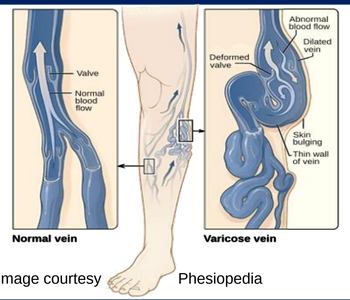



Image courtesy Phesiopedia

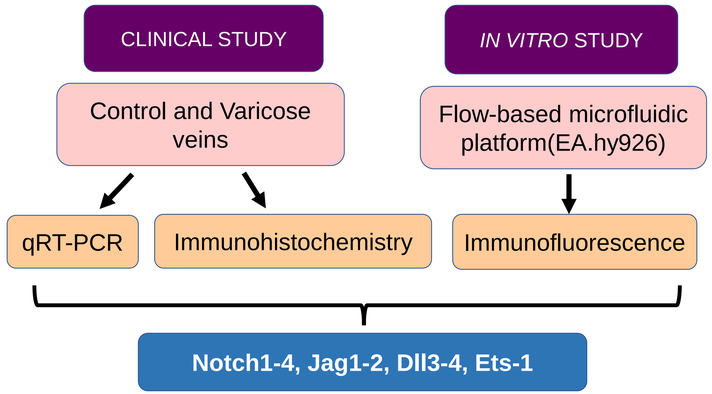
## Hypothesis

- We hypothesize that Notch signaling is deregulated in varicose veins.
- We propose that mechanosensitive Ets-1 plays a central role in the induction of Notch cascade.

## Objectives

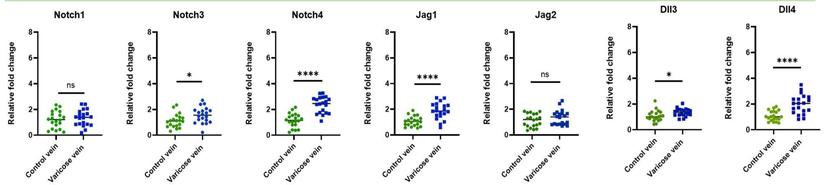
- To analyze mRNA and protein expression of Notch receptors and their ligands in human varicose veins.
- To examine the expression pattern of mechanosensitive Ets-1 in varicose veins at mRNA and protein level.
- To delineate Ets-Notch signaling in endothelial cells exposed to disturbed flow.

## Work flow



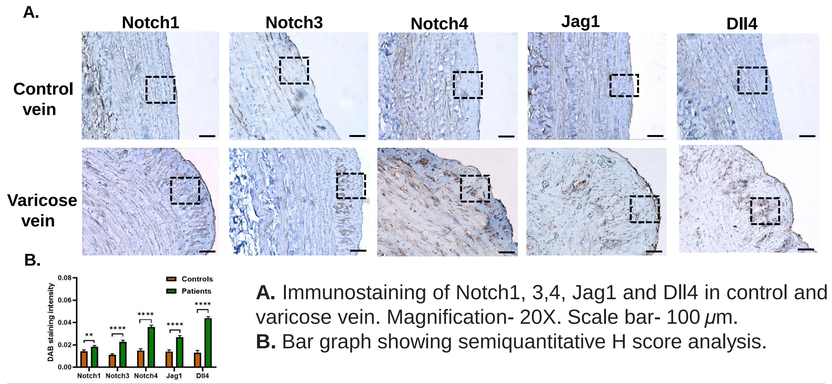

## Results

### Upregulation of Notch signaling in human varicose veins



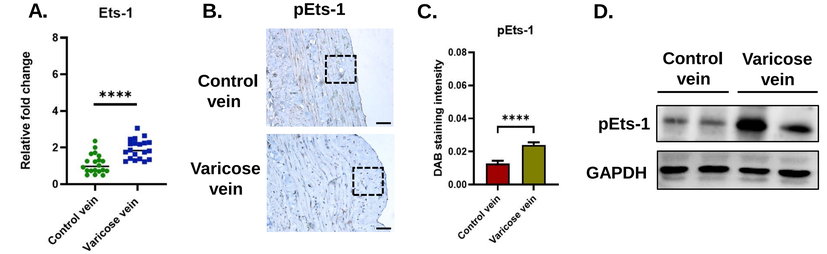

Scatter plots representing mRNA fold changes of Notch1,3,4, Jag1,2 and Dll3 in 20 human varicose and control saphenous veins. (* $p < 0.05$, **** $p < 0.0001$, ns not significant).

### Notch4 and its ligand Dll4 is overexpressed in the neointima of varicose veins

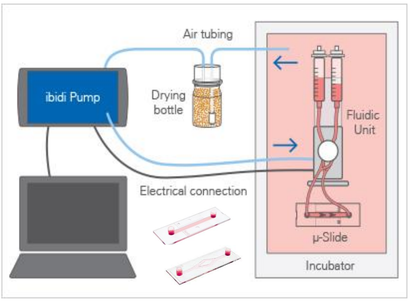



**A.** Immunostaining of Notch1, 3,4, Jag1 and Dll4 in control and varicose vein. Magnification- 20X. Scale bar- 100 $\mu$m.
**B.** Bar graph showing semiquantitative H score analysis.

### Phosphorylated Ets-1 is elevated at mRNA and protein levels in varicose veins



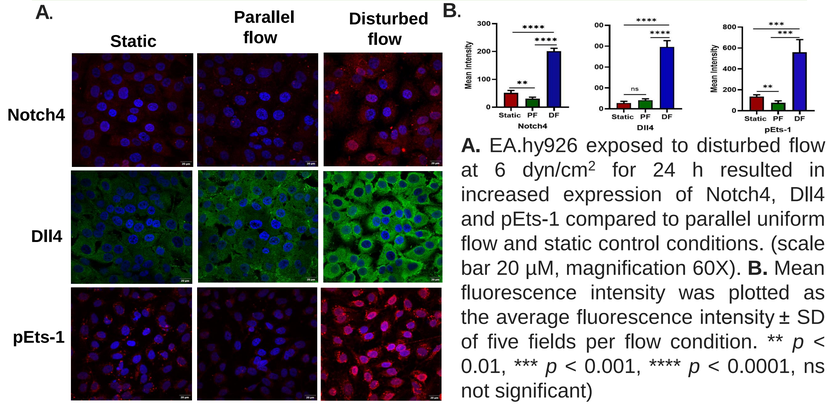

**A.** Scatter plot representing mRNA fold change of Ets-1 in 20 human varicose and control saphenous veins.( **** $p < 0.0001$) **B.** Immunostaining showing nuclear localization of phospho Ets-1 in varicose veins. **C.** Bar graph representing semiquantitative H score analysis **D.** Western blot showing overexpression of phospho Ets-1 in varicose veins.




**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.

Image courtesy: Ibidi, Germany

### Disturbed Venous Flow Induces pEts1-Notch4 signaling in Vein Endothelial Cells




**A.** EA.hy926 exposed to disturbed flow at 6 dyn/cm$^2$ for 24 h resulted in increased expression of Notch4, Dll4 and pEts-1 compared to parallel uniform flow and static control conditions. (scale bar 20 µM, magnification 60X). **B.** Mean fluorescence intensity was plotted as the average fluorescence intensity ± SD of five fields per flow condition. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns not significant)

## 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 Ets-1.
- Targeting Ets-1 rather than downstream Notch components may serve as an efficient strategy for varicose vein small molecular therapeutics.

## Future work

- Validation of Notch4,Dll4 and Ets-1 at a protein level in vitro and in clinical samples.
- Inhibitor assays to target Ets-1 in preventing Notch pathway in the presence of ensuing disturbed flow..

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