Vascular Biology and HHT

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The genes associated with HHT1 and HHT2 are implicated in vascular homeostasis

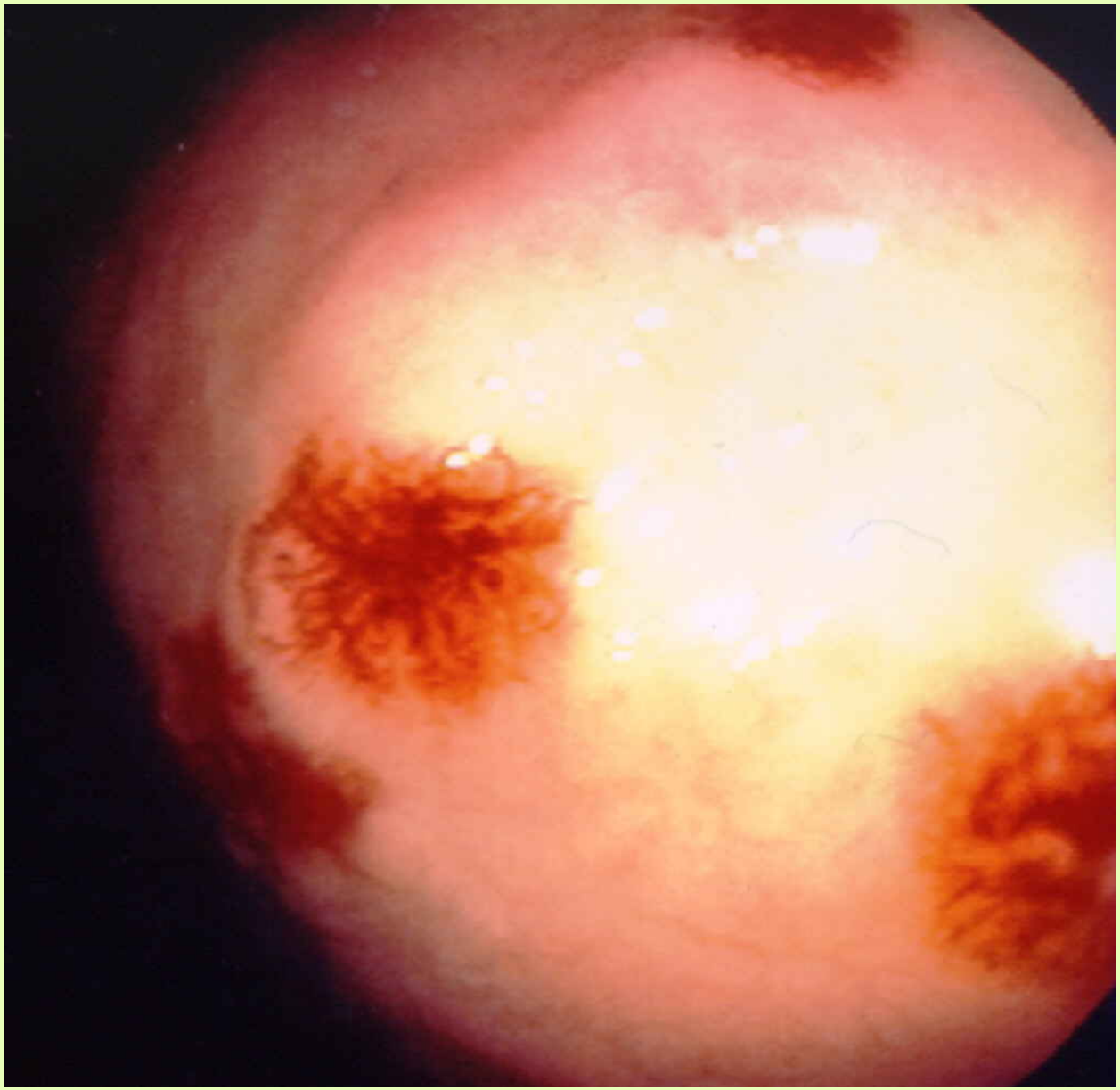
- **HHT is characterized by focal loss of capillaries, dilated vessels and arteriovenous malformations**

- **Endoglin and ALK1 are expressed primarily in endothelial cells**

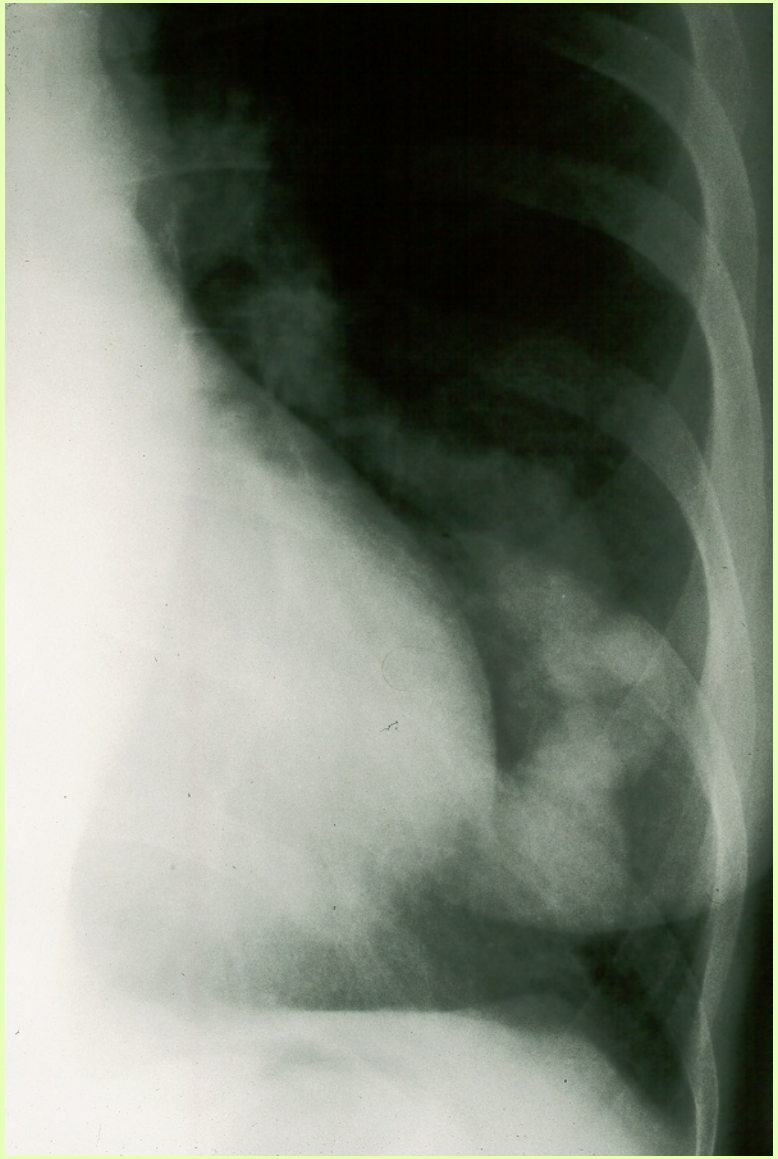
- **Mice with a single copy of these genes (*Eng* $^{+/-}$ and *Alk1*$^{+/-}$*)* can develop clinical signs of HHT**

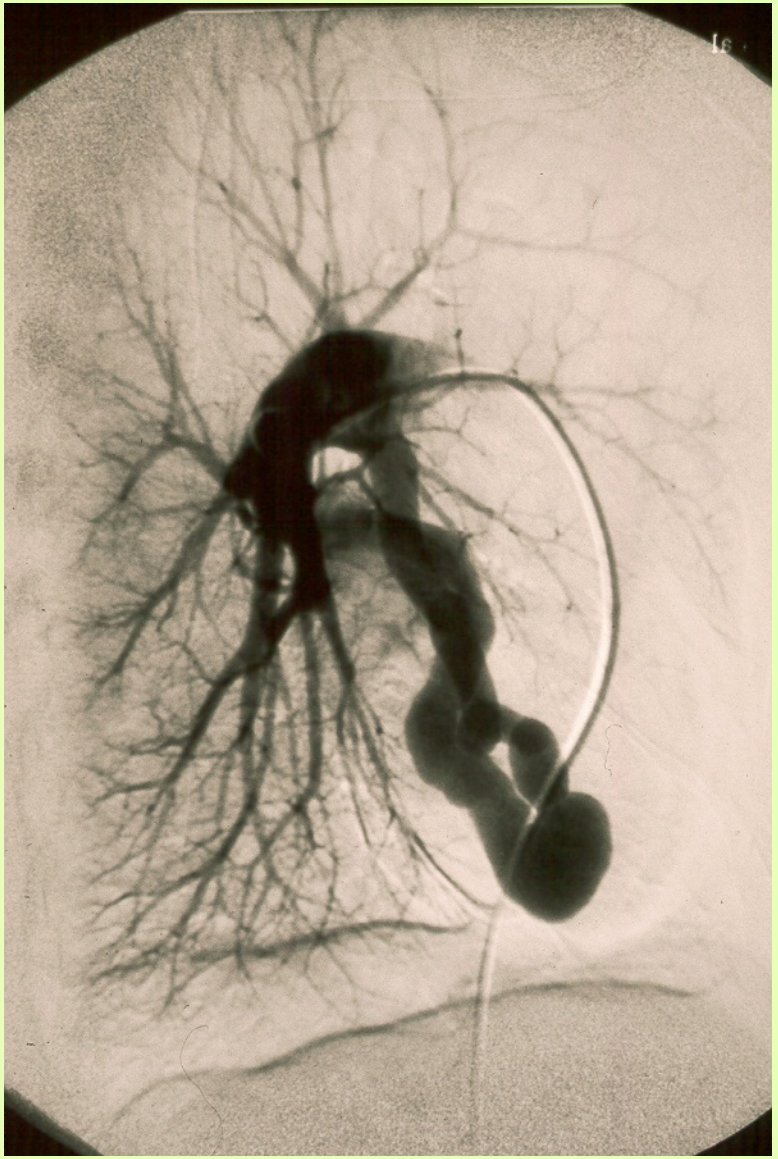
- **Mice lacking *Endoglin* or *Alk1* genes die at mid-gestation of cardiovascular defects**

Gastrointestinal telangiectases

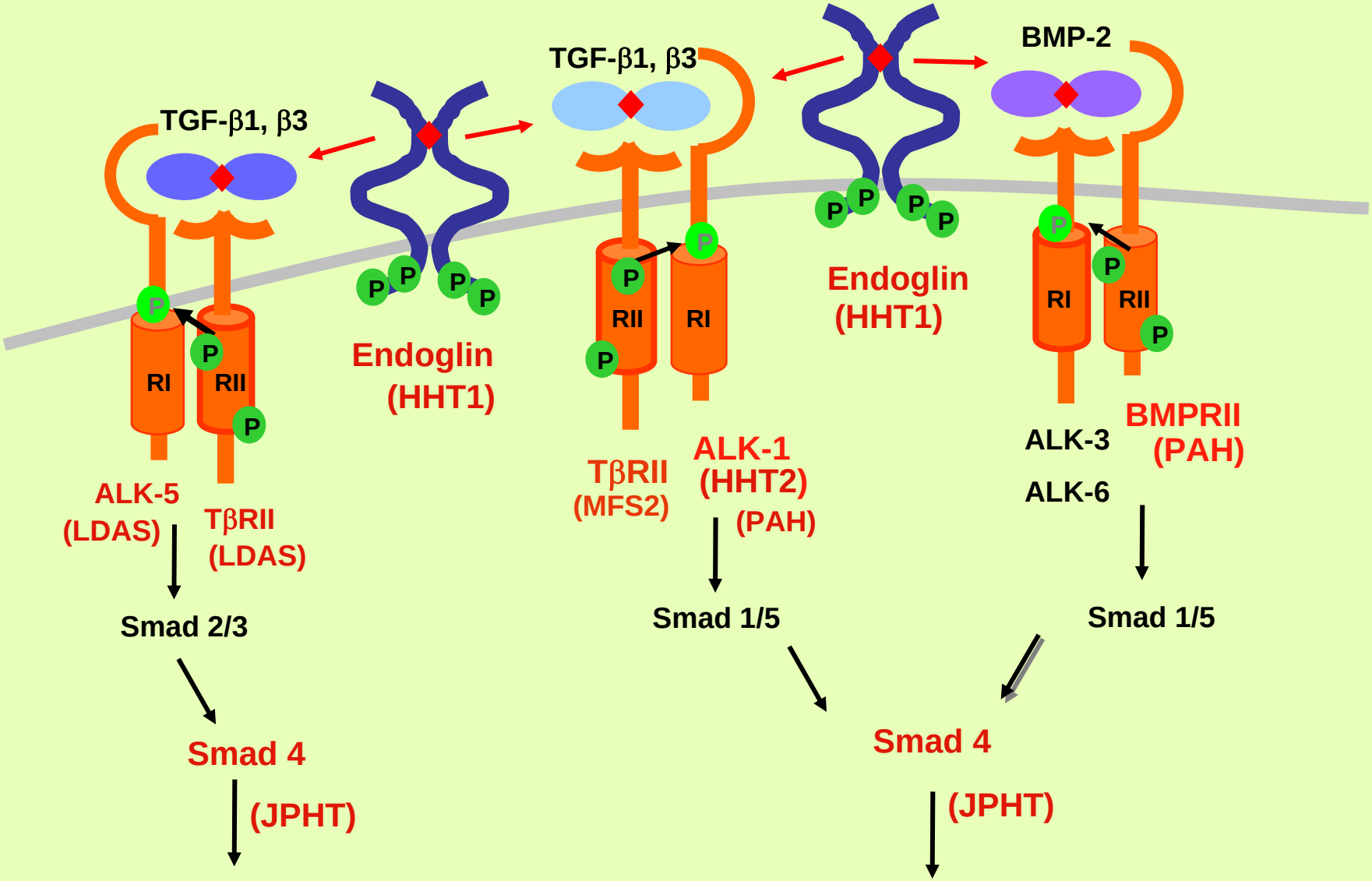


Pulmonary Arteriovenous Malformations





Vascular Diseases associated with TGF-ß superfamily



Mechanisms of HHT pathogenesis

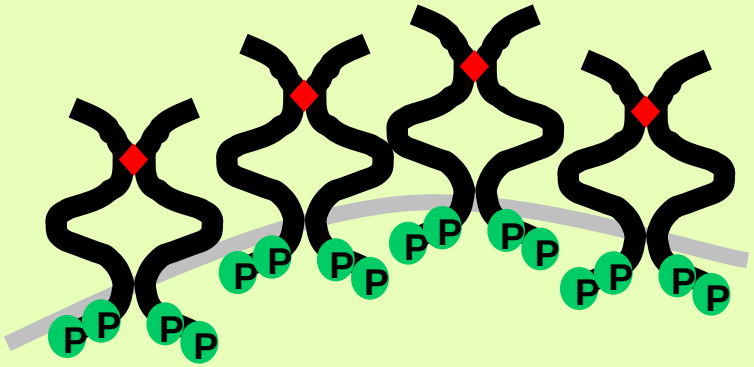
- **Haploinsufficiency in endoglin or ALK1**

- **Impaired endothelial Nitric Oxide synthase (eNOS) activation leading to superoxide production**
- **Enhanced vasodilation and impaired myogenic response**

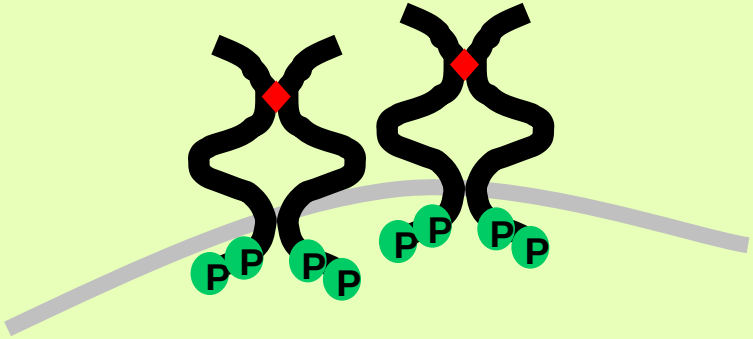
- **Dysfunctional TGF-β/endoglin/eNOS pathway**

Haploinsufficiency is associated with HHT

Non-affected individual



HHT1 patient



Endothelial cells or activated monocytes

Endoglin levels are reduced in blood monocytes of HHT1 patients

Group	Mutation confirmed	Number	Age		**Endoglin level** (%)	
			Median	Range (25-75%)	**Median**	Range (25-75%)
HHT1	*Endoglin*	109	42.4	21.3	**48.0***	19.0
HHT2	*ALK1*	61	42.2	22.3	**91.0**	37.0
Control	None	84	37.9	38.9	**88.5**	29.5

The two-sided pair normal approximation obtained from the Two-Sample Wilcoxon test is reported: * $P<0.001$ for HHT1 relative to HHT2 and control groups.

Abdalla and Letarte J Med Genet 43:97, 2006

Levels of endoglin and ALK1 are selectively reduced in endothelial cells of HHT1 and HHT2 newborns

Family type	Newborn Status	#	Endoglin level (%)		#	ALK1 level (%)	
			Median	Range (25-75%)		Median	Range (25-75%)
HHT1	***ENG* mutation**	30	**45.0***	13.0	7	94.0	18.0
HHT1	No *ENG* mutation	18	98.0	17.0	3	92.0	23.0
HHT2	***ALK1* mutation**	8	98.5	25.5	4	**60.5^**	24.5
HHT2	No *ALK1* mutation	6	106.5	31.0	3	96.0	3.0

* The two-sided pair normal approximation (Two-Sample Wilcoxon test) for comparison to the group without *Eng* mutation ($P < 0.001$).
The t-test was used for comparison to the groups with and without an *ALK-1* mutation ($P = 0.0002$ in both cases).
^Distribution of ALK1 levels for this group was $P = 0.03$ (by T-test), when compared to the combined HHT1 groups or to the last group.

Abdalla and Letarte J Med Genet 43:97, 2006

How can haploinsufficiency in ENG or ALK1 lead to dilated vessels and AVMs?

• We speculated that the production of Nitric Oxide (NO) by eNOS may be altered in HHT, where vessels are dilated.

• NO regulates vascular tone.

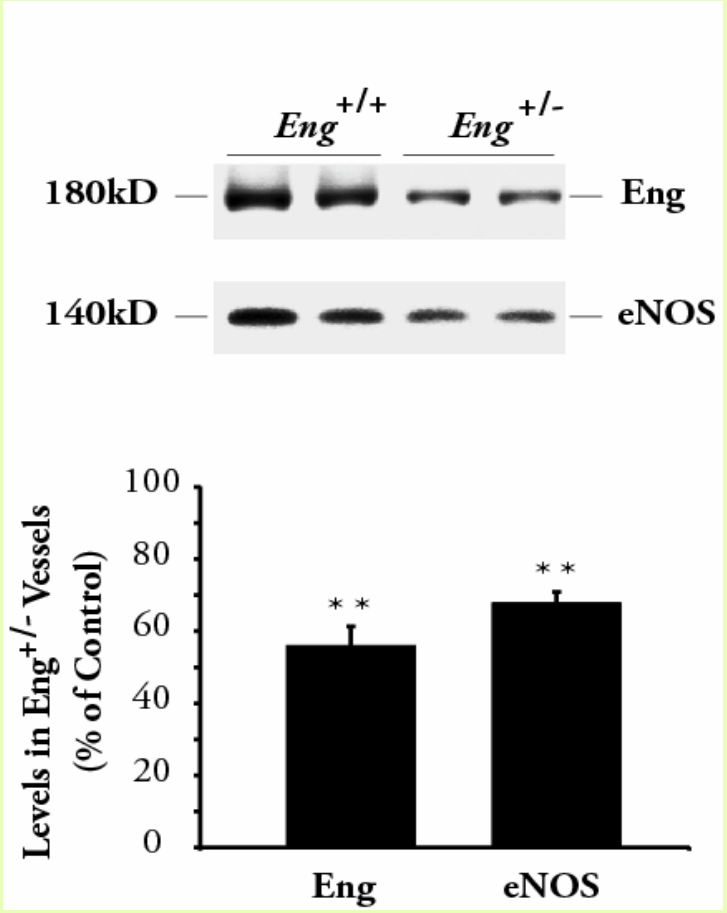
• Reduced NO levels are often associated with cardiovascular disease.

• Modeling of the hemodynamics of the microcirculation predicts that loss of local vasomotor control may cause AVMs. (*Quick, CM. et al. 2001*)

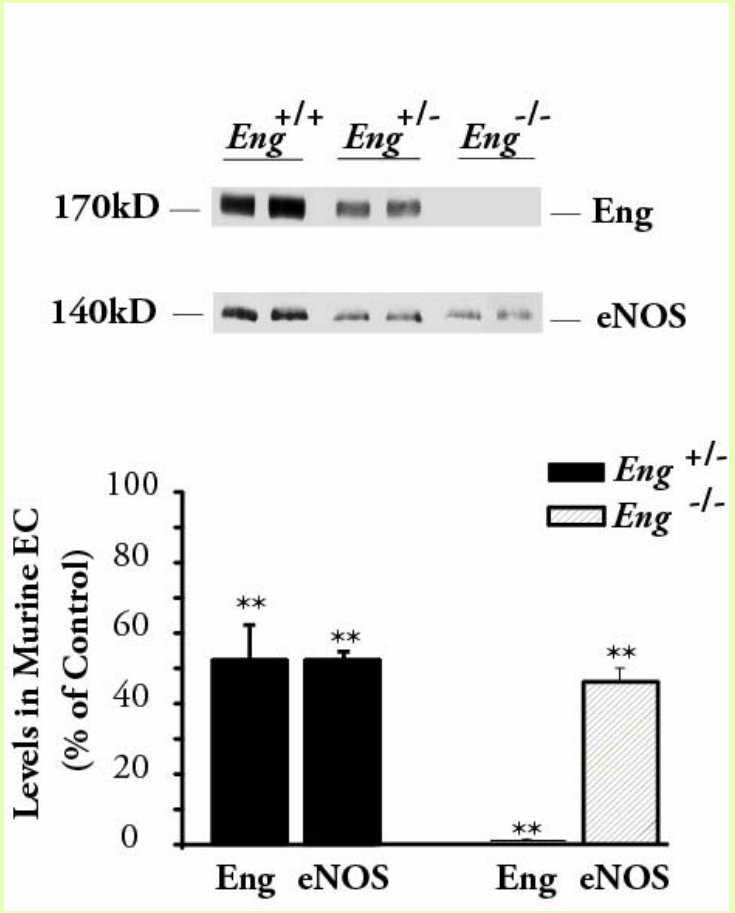
•We hypothesized that:
•Endoglin may modulate eNOS activation and thereby contribute to the local regulation of vascular tone and integrity.

eNOS levels are reduced in Eng $^{+/-}$ mice

Blood Vessels

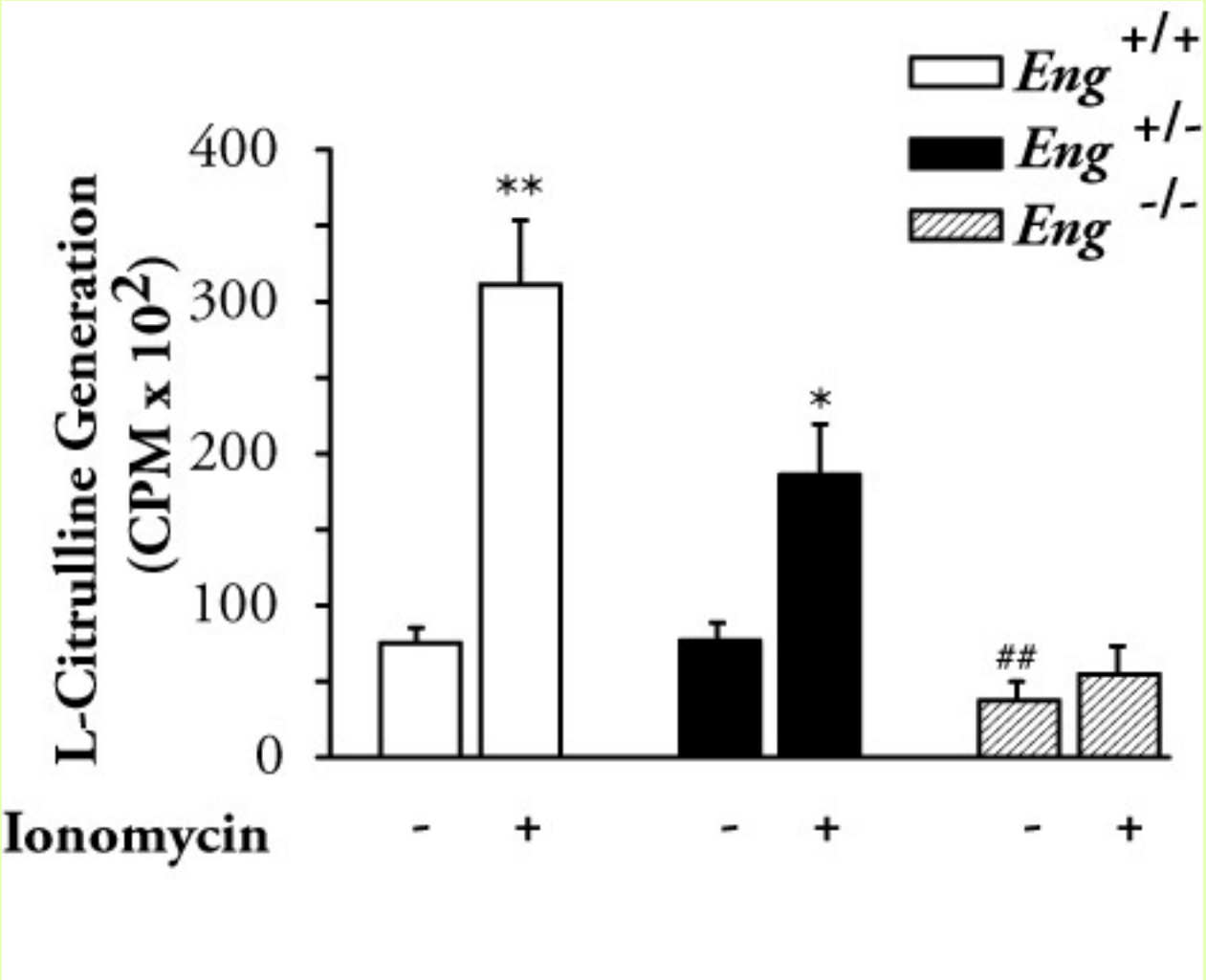


Endothelial Cells



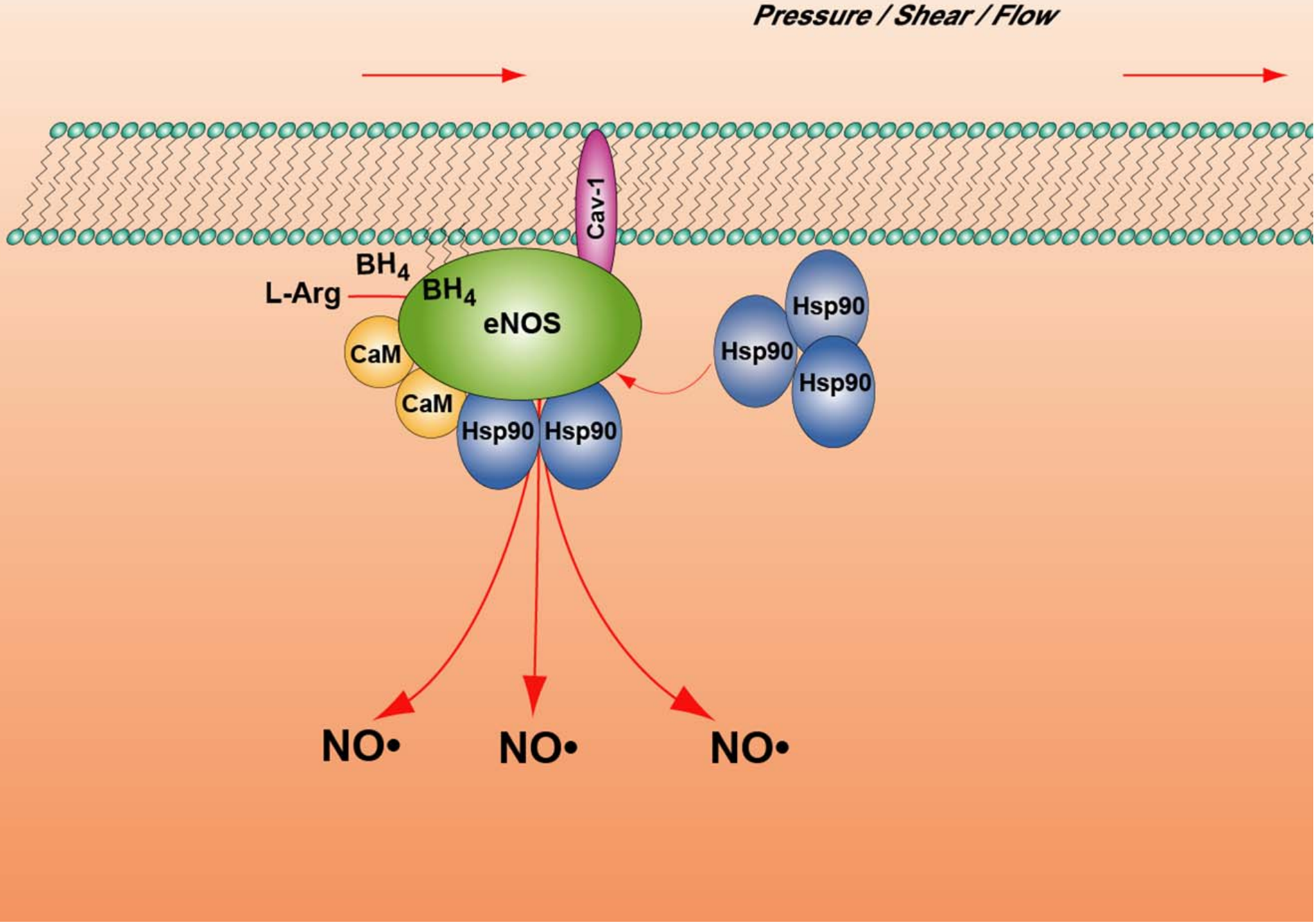
Toporsian et al, Circ. Res. 2005; 96:684-692

NO production is impaired in Eng $^{+/-}$ and Eng $^{-/-}$ endothelial cells

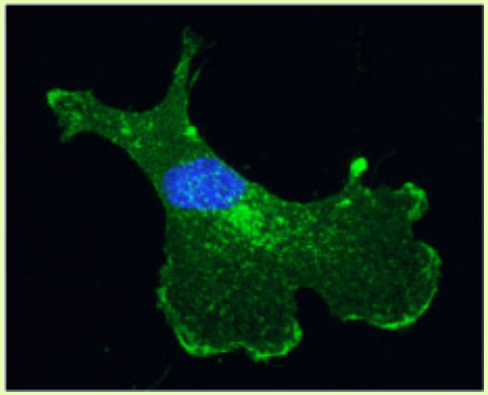


Toporsian et al, Circ. Res. 2005; 96:684-692

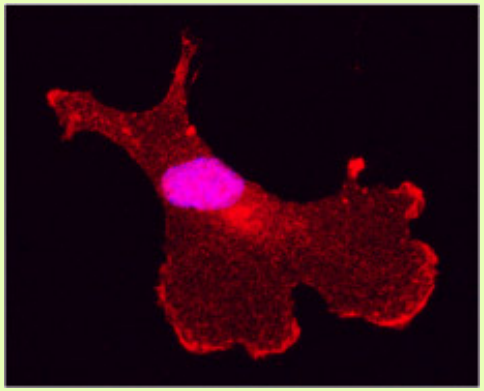
Could endoglin associate with eNOS and hsp90?



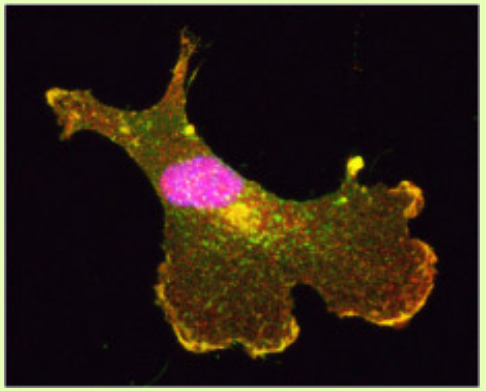
Endoglin Associates with eNOS in Human Endothelial Cells



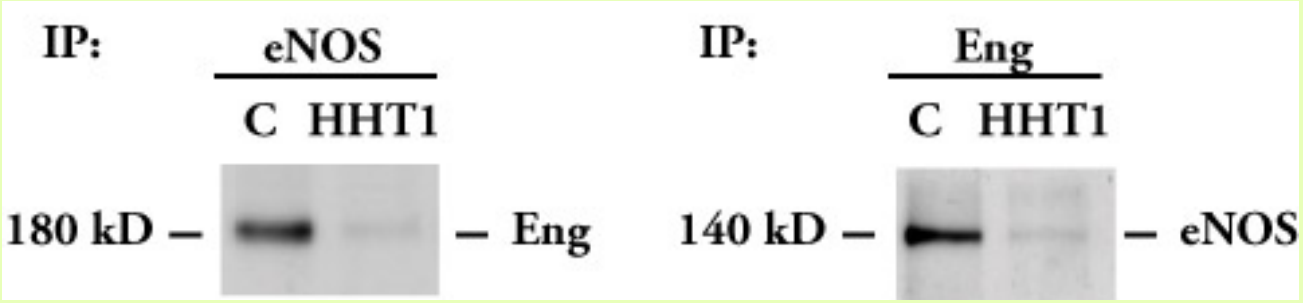
Eng



eNOS



Eng / eNOS Merge



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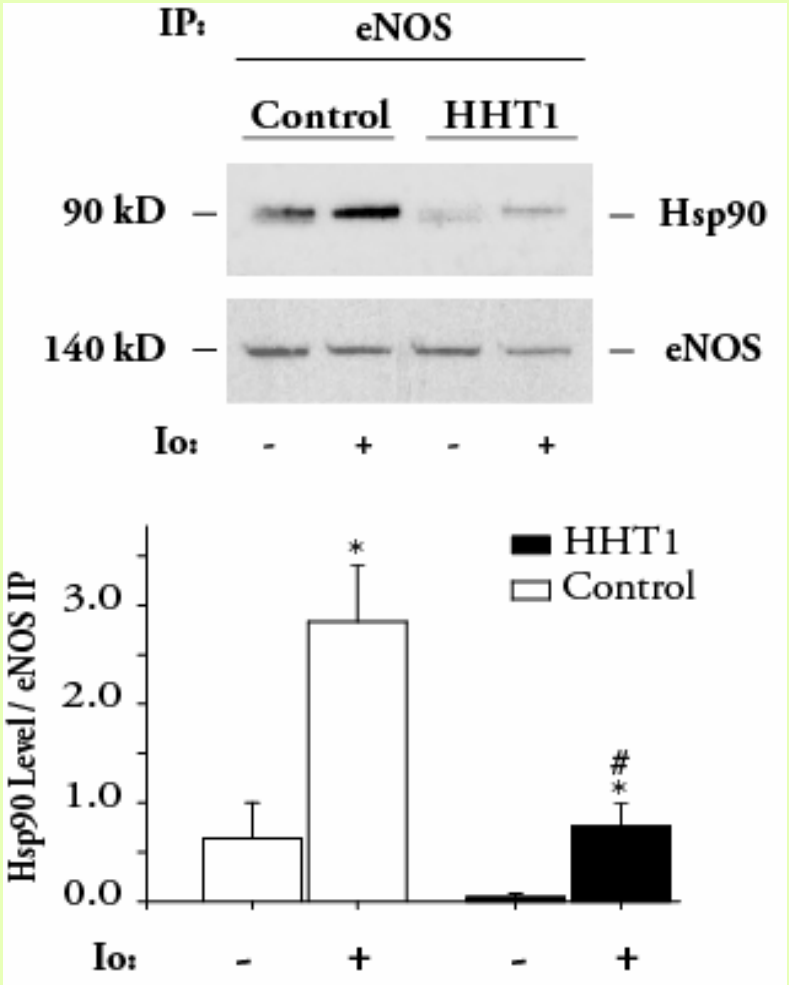
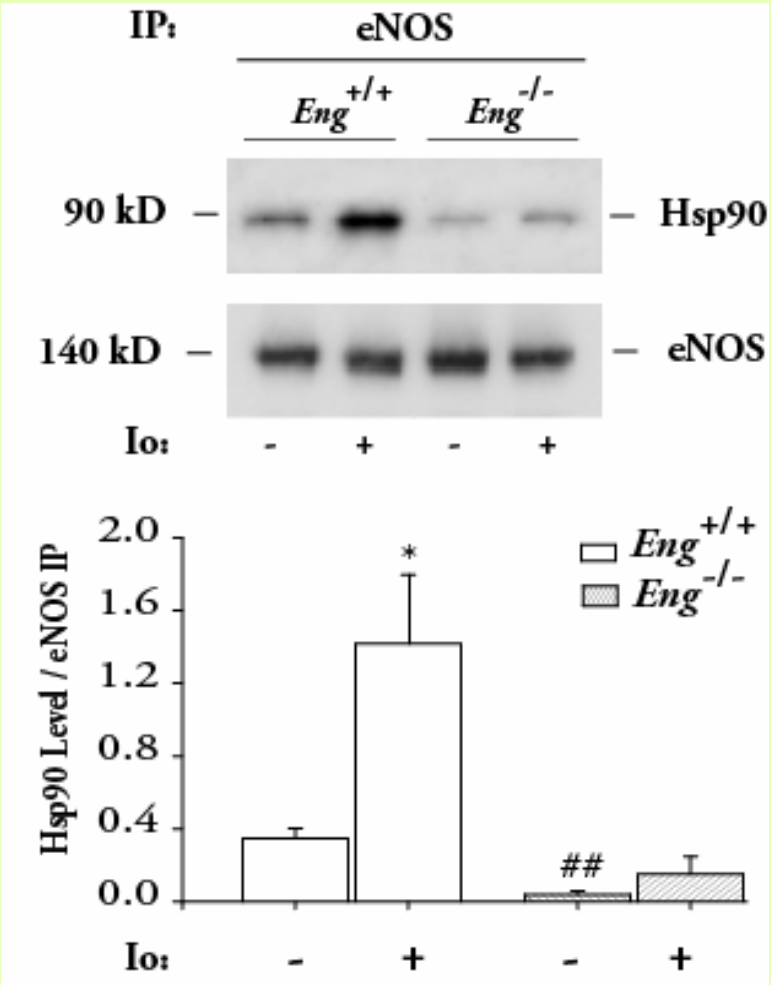
Endoglin Modulates the eNOS Activation Complex

Human Endothelial Cells

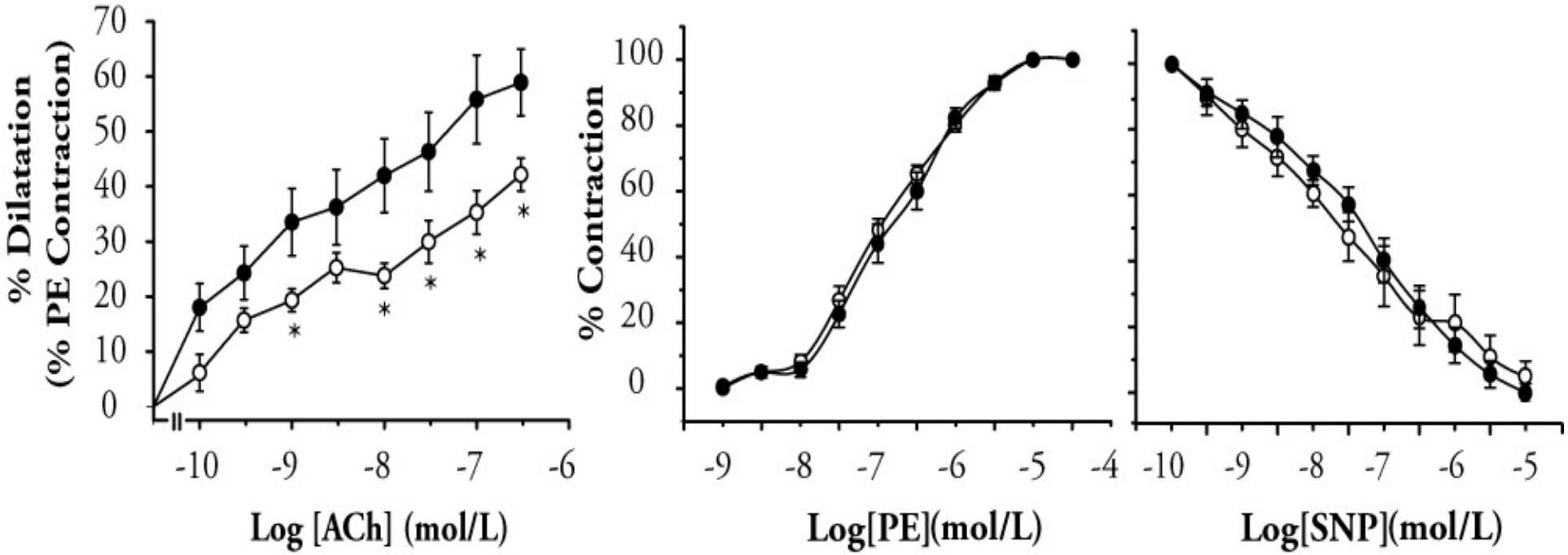


Murine Endothelial Cells



Increased Endothelium-Dependent Dilatation in Eng $^{+/-}$ Resistance Arteries

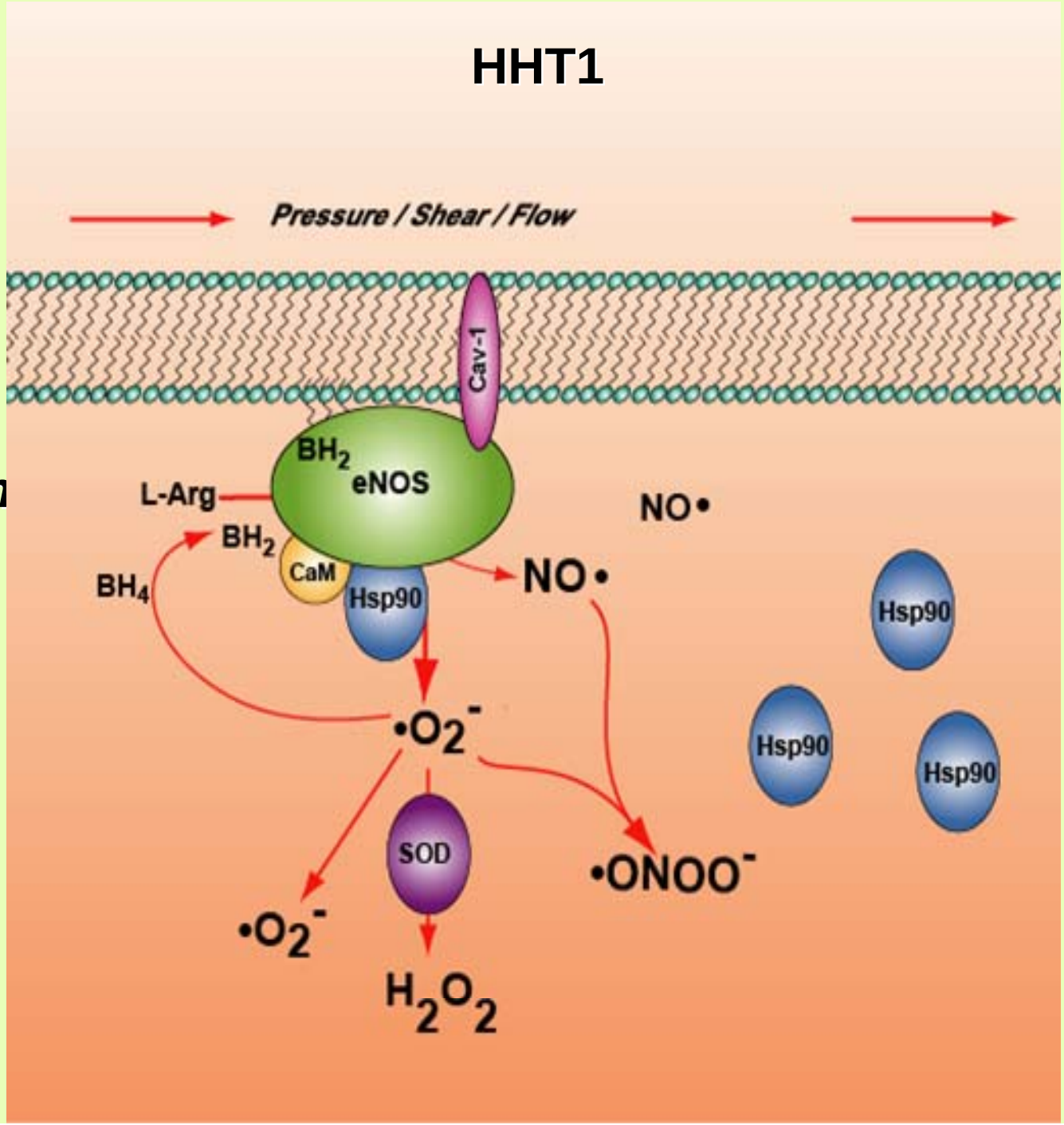
Endothelium-Dependent Dilatation | **Smooth Muscle Contractility** | **Endothelium-Independent Dilatation**



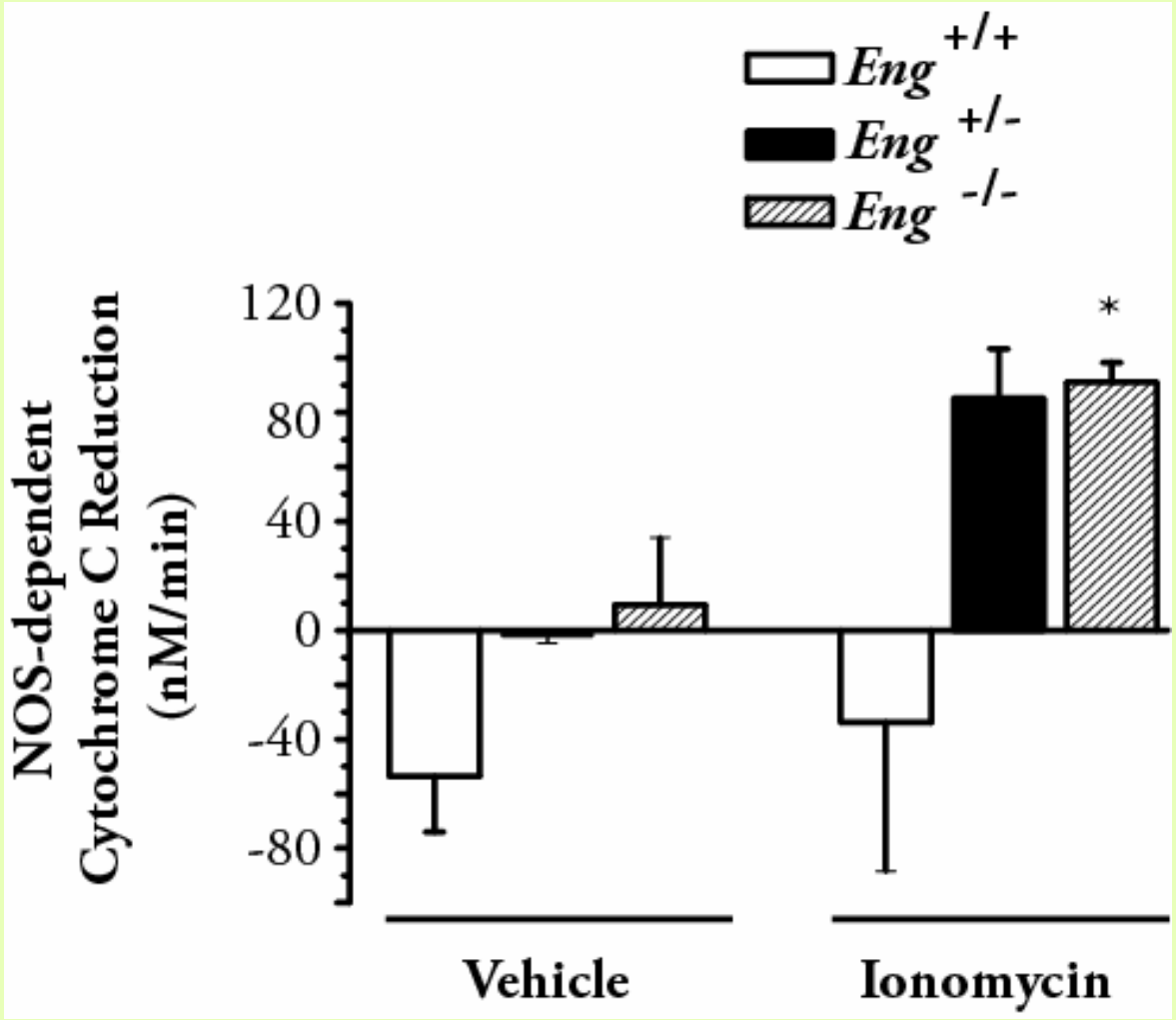
$^{*}P < 0.05$

Toporsian et al, Circ. Res. 2005; 96:684-692

Pressure on endothelial sites with reduced endoglin leads to uncouplin of eNOS activity and production of damaging superoxide and initiation of lesion

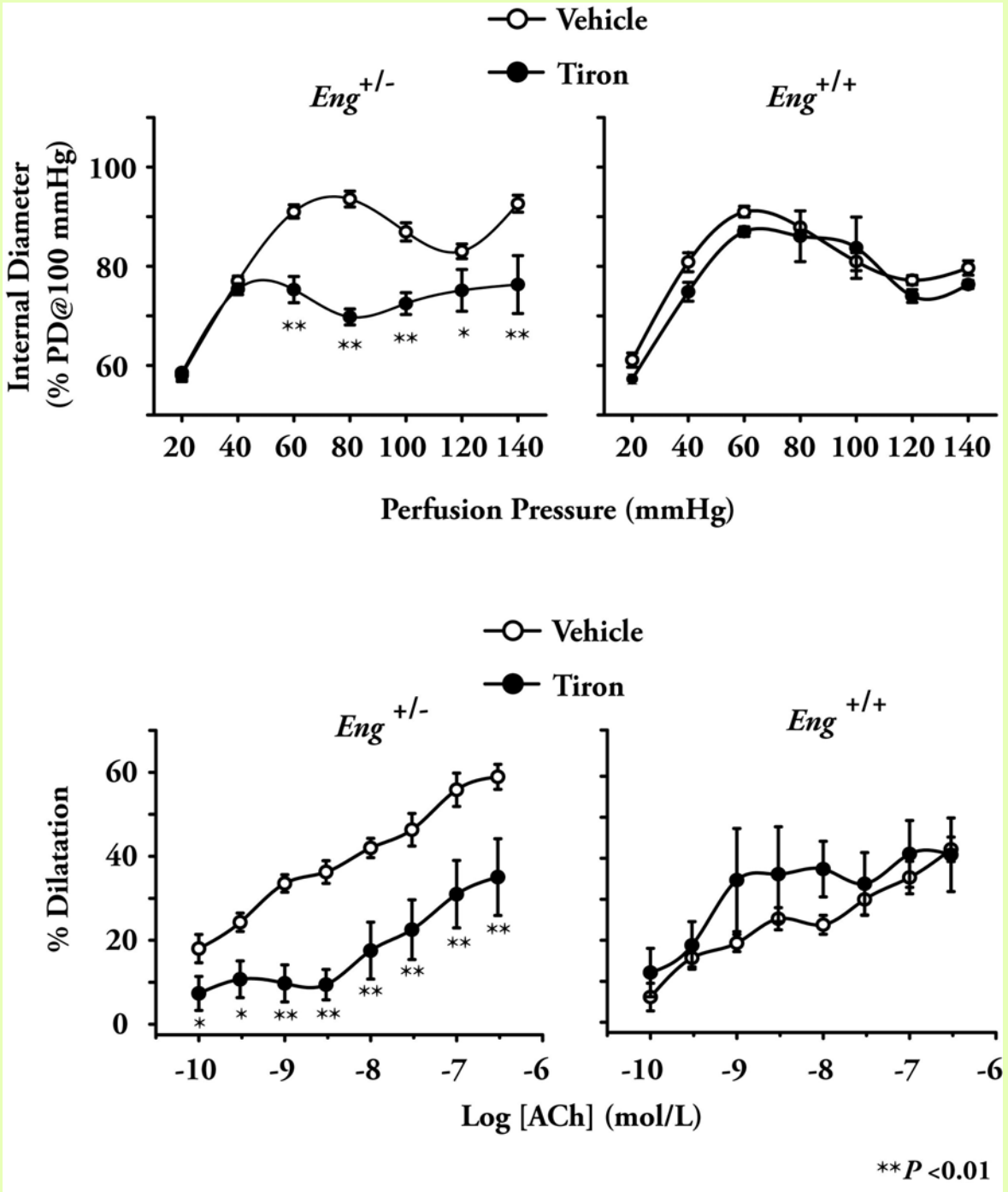


eNOS-derived O_2^- Production in Eng $^{+/-}$ and Eng $^{-/-}$ Endothelial Cells



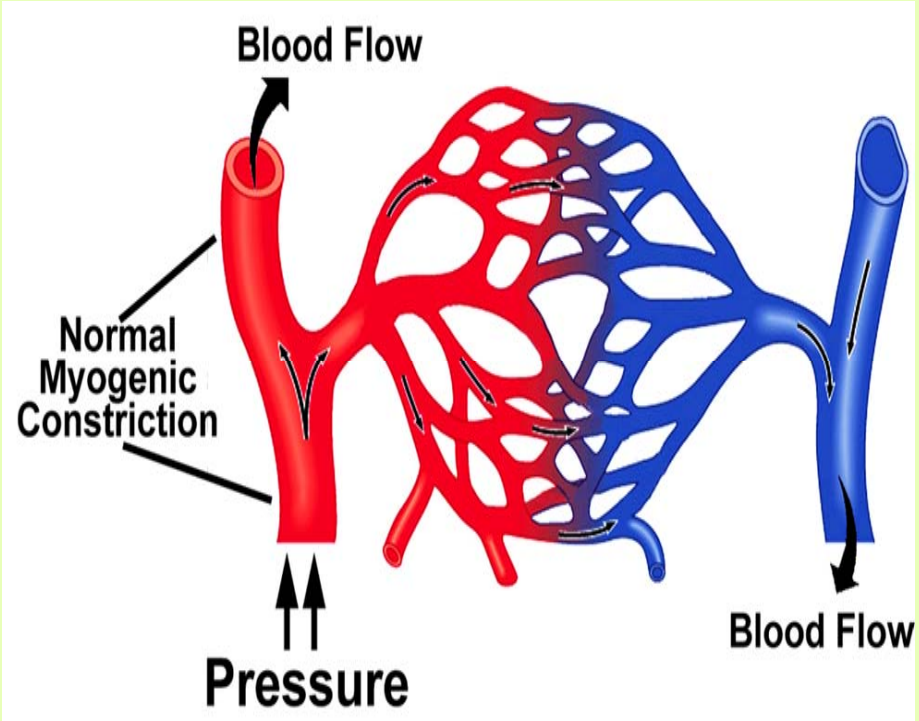
Toporsian et al, Circ. Res. 2005; 96:684-692

Reversal with the anti-oxidant Tiron of abnormal myogenic response and acetylcholine-induced dilatation

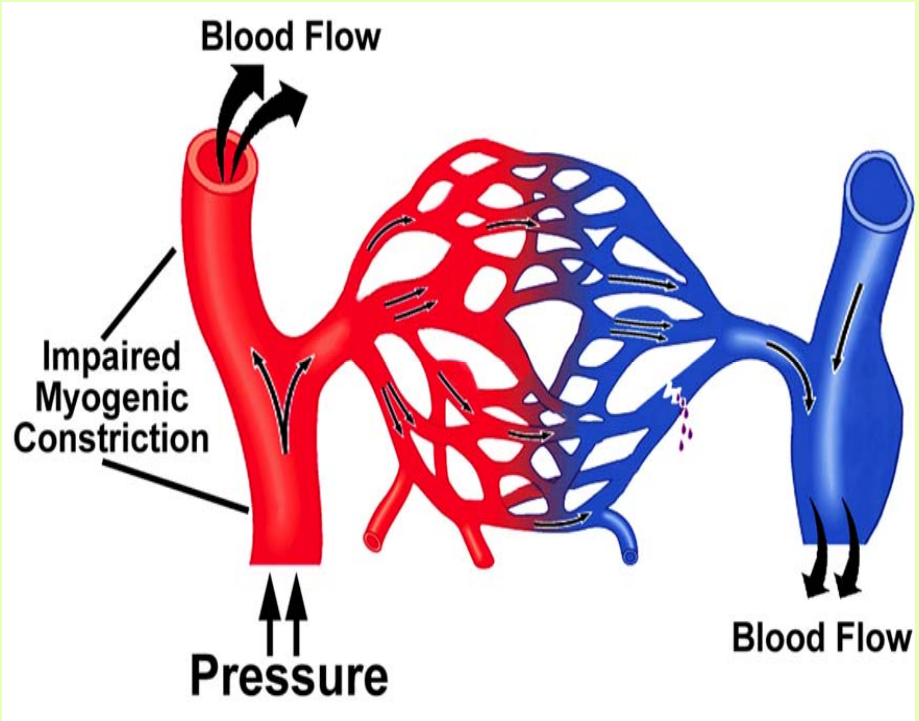


Model of HHT Pathogenesis

Normal



HHT



Conclusions on endoglin and eNOS functional association

- **Novel role for endoglin in the local regulation of vascular tone**

- **Endoglin resides in caveolae where it associates with and stabilizes eNOS, and enhances eNOS-Hsp90 association during Ca^{2+} activation**

- **In HHT1, eNOS activity is "uncoupled" generating superoxide instead of nitric oxide**

- ***Eng*$^{+/-}$ resistance arteries display impaired eNOS-dependent vasodilatation and myogenic reactivity which are restored by superoxide scavengers**

- **Can we link the role of endoglin in eNOS regulation to its function in the TGF-β1/β3 receptor complex?**

How studying a different disease gave us clues about mechanisms of HHT?

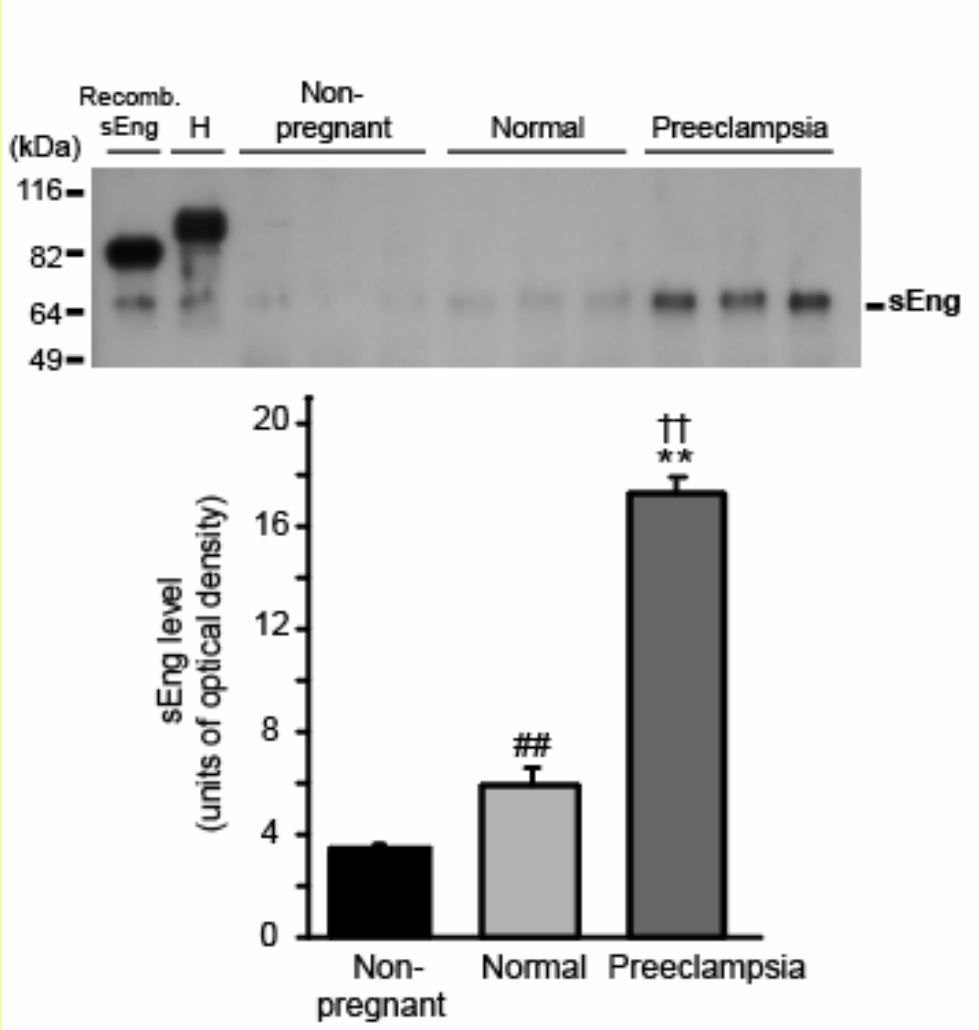
Endoglin is upregulated in placenta during preeclampsia

What is pre-eclampsia?

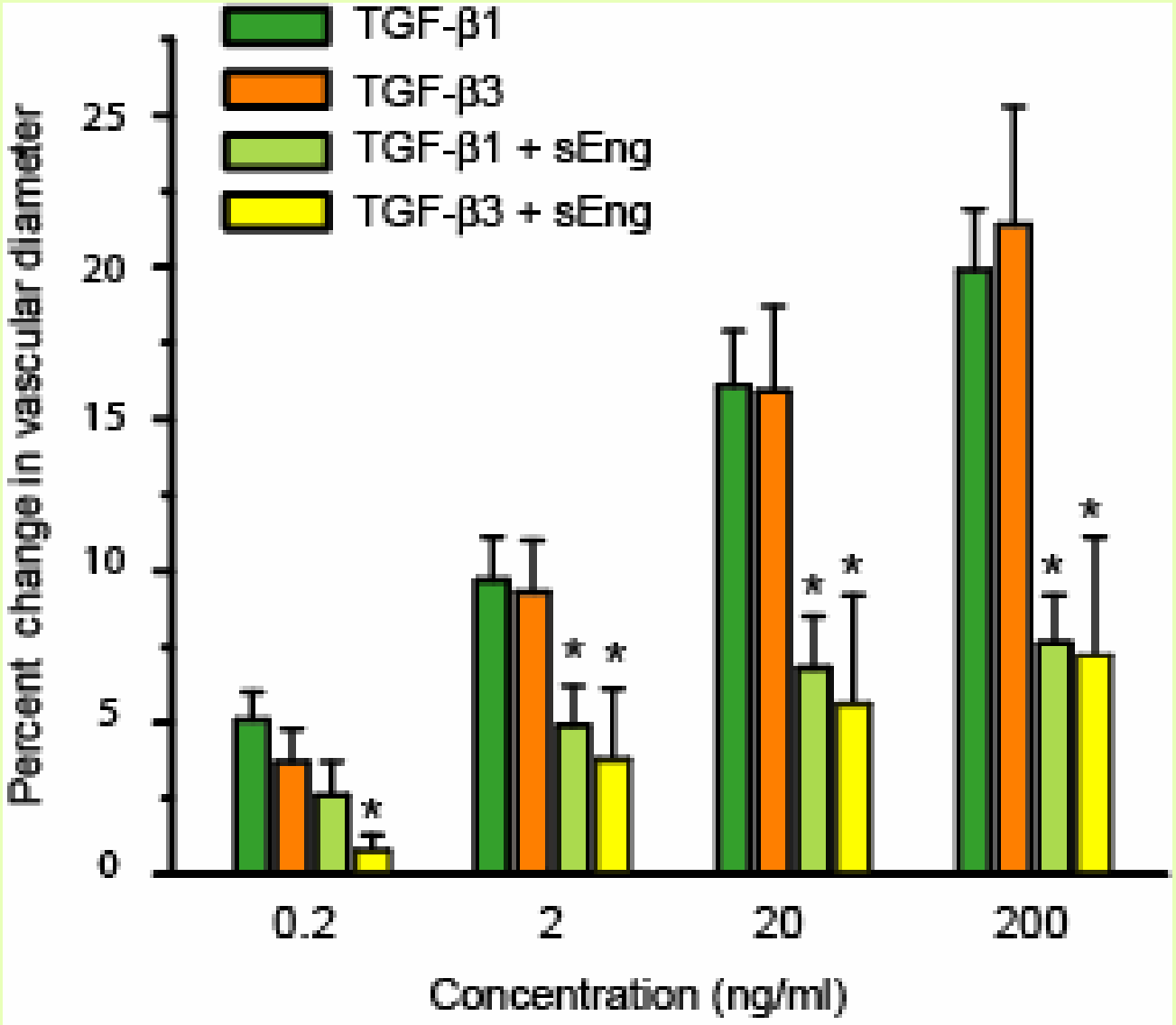
- **Associated with 5% of pregnancies worldwide**
- **A pregnancy-specific syndrome that causes hypertension and proteinuria in the third trimester**
- **Clinical manifestations reflect endothelial dysfunction, resulting in vasoconstriction**

- **High levels of circulating sVEGF-RI of placental origin were found in pre-eclamptic patients (*A. Karamanchi et al*)**
- **We now report a soluble form of endoglin (sEng) circulating at increased levels and causally related to the pathogenesis (*S. Venkatesha, M. Toporsian et al. Nat Med June 4 2006)***

Increased levels of Soluble Endoglin (sEng) in the sera of pre-eclamptic women



TGF-β1 and -β3 induce vasodilation via an endoglin-dependent mechanism



Soluble endoglin (sEng) contributes to the pathogenesis of pre-eclampsia

- **Endoglin expression much higher in the placenta of pre-eclamptic women**
- **Elevated levels of a placenta derived 65kDa sEng in sera of pre-eclamptic women**
- **Recombinant sEng can induce pre-eclampsia in mice**
- **Recombinant sEng blocks:**

  -TGF-β1 binding and Smad2 signaling in endothelial cells,

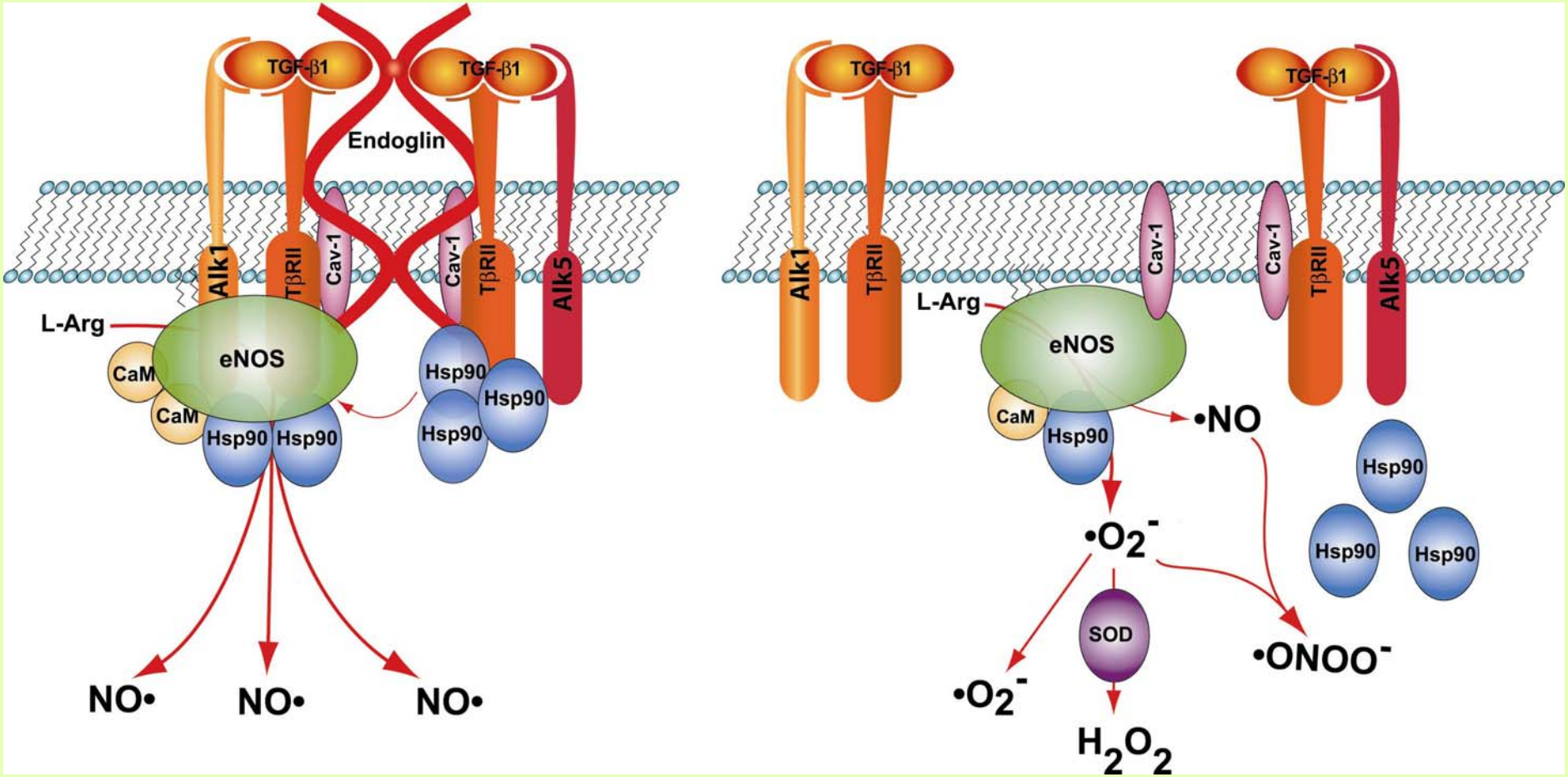
  -TGF-β1 effects on eNOS activation and vasodilation

  -capillary formation

Model of pathogenesis of HHT

Normal

HHT



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