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-β superfamily

- TGF-β1, β3
  - ALK-5 (LDAS)
  - ALK-6
  - ALK-3
  - BMP-2
  - Smad 2/3
  - Smad 1/5
  - Smad 4
  - Smad 4
  - Smad 1/5

- Endoglin (HHT1)
  - TβRII (MFS2)
  - TGF-β1, β3
  - BMPRII (PAH)
  - Smad 1/5

- Vascular Diseases associated
  - (HHT1)
  - (HHT2)
  - (PAH)
  - (MFS2)
  - (JPHT)
  - (JPHT)
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**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mutation confirmed</th>
<th>Number</th>
<th>Age</th>
<th>Endoglin level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median</td>
<td>Range (25-75%)</td>
<td>Median</td>
</tr>
<tr>
<td>HHT1</td>
<td><em>Endoglin</em></td>
<td>109</td>
<td>42.4</td>
<td>21.3</td>
</tr>
<tr>
<td>HHT2</td>
<td><em>ALK1</em></td>
<td>61</td>
<td>42.2</td>
<td>22.3</td>
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<tr>
<td>Control</td>
<td>None</td>
<td>84</td>
<td>37.9</td>
<td>38.9</td>
</tr>
</tbody>
</table>

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**

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

* 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

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

Could endoglin associate with eNOS and hsp90?
Endoglin Associates with eNOS in Human Endothelial Cells

**Endoglin Modulates the eNOS Activation Complex**

Human Endothelial Cells

Murine Endothelial Cells

![Graphs showing the modulation of eNOS activation complex by Endoglin.](image)
Increased Endothelium-Dependent Dilatation in Eng +/- Resistance Arteries

Endothelium-Dependent Dilatation

Smooth Muscle Contractility

Endothelium-Independent Dilatation

$^*P < 0.05$

Pressure on endothelial sites with reduced endoglin leads to uncoupling of eNOS activity and production of damaging superoxide and initiation of lesion.
eNOS-derived $O_2^-$ Production in Eng $^{+/+}$ and Eng $^{+/1-}$ 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

Blood Flow

Normal Myogenic Constriction

Pressure

Blood Flow

Impaired Myogenic Constriction

Pressure

Blood Flow
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. Karamanuchi 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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