Cure HHT: Review of Recent Findings in the Field of Hereditary Hemorrhagic Telangiectasia Research

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PRIMARY RESEARCH ARTICLES

2014

1. **Ardelean, D.S. et al. Anti-VEGF Therapy Reduces Intestinal Inflammation in *Endoglin* Heterozygous Mice Subjected to Experimental Colitis.** *Angiogenesis*. 2014. 28: 641-659. doi: 10.1007/s10456-014-9421-x

   Senior Investigator: M. Letarte
   Molecular Structure and Function Program, Hospital for Sick Children, Toronto, ON, Canada.

   Authors show that induction of colitis in endoglin-deficient mice results in pathological gastrointestinal hypervascularization associated with increased gut inflammation and high VEGF expression compared to wild-type mice, suggesting that endoglin regulates both angiogenesis and inflammation in this model. Authors further demonstrate that treatment with VEGF-blocking antibodies reduces pathological angiogenesis and overall markers of gut inflammation.

2. **Ardelean, D.S. et al. Endoglin and Activin Receptor-Like Kinase 1 Heterozygous Mice Have a Distinct Pulmonary and Hepatic Angiogenic Profile and Response to anti-VEGF Treatment.** *Angiogenesis*. 2014. 17: 129-146. doi: 10.1007/s10456-013-9383-4

   Senior Investigator: M. Letarte
   Molecular Structure and Function Program, Hospital for Sick Children, Toronto, ON, Canada.

   Authors report that anti-VEGF therapy rescues reduced lung microvascular density phenotype of endoglin- and Alk1-heterozygous mice by reducing abnormal expression of TSP-1 in endoglin-deficient mice, and by decreasing abnormal expression of Ang-2 in Alk1-deficient mice. Authors further report that VEGF inhibition has similarly distinct effects on angiogenic signaling density in the livers of endoglin- vs. Alk1-heterozygous mice despite both resulting in reduced hepatic microvascular density. Authors therefore propose that the mechanisms by which endoglin and Alk1 disrupt angiogenic signaling are different.

3. **Aristorena, M. et al. Expression of Endoglin Isoforms in the Myeloid Lineage and their Role During Aging and Macrophage Polarization.** *J. Cell Sci.* 2014. 127: 2723-2735. doi: 10.1242/jcs.143644

   Senior Investigator: C. Bernabéu
   Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas (CSIC), 28040 Madrid, Spain; Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), 28040 Madrid, Spain; and Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), 28040 Madrid, Spain.

   Authors report that expression of the alternate spliceform of endoglin, S-endoglin, is upregulated in macrophages of aged animals as well as upon exposure to oxidative stress. S-endoglin expression is associated with reduced macrophage survival and proliferative capacity, suggesting that S-endoglin is an early marker of macrophage senescence.

4. **Chen, W. et al. De Novo Cerebrovascular Malformation in the Adult Mouse After Endothelial *Alk1* Deletion and Angiogenic Stimulation.** *Stroke*. 2014. 45: 900-902. doi: 10.1161/strokeaha.113.003655

   Senior Investigator: H. Su
   Center for Cerebrovascular Research, Department of Anesthesia and Perioperative Care, University of California San Francisco, San Francisco, CA, USA.

   Authors report that cerebral VEGF stimulation results in brain arteriovenous malformations, vascular hyperdensity, and increased endothelial cell proliferation in Alk1-deficient mice, but that this does not occur in unstimulated Alk1-deficient mice or in VEGF-treated wild-type mice. Endothelial Alk1 deletion also results in arteriovenous malformations in the intestine, lung, and skin wounds, but no vascular malformations are observed when Alk1 is deleted in pericytes.

5. **Choi, E.-J. et al. Novel Brain Arteriovenous Malformation Mouse Models for Type 1 Hereditary Hemorrhagic Telangiectasia.** *PLoS One*. 2014. 9(2): e88511. doi: 10.1371/journal.pone.0088511

   Senior Investigator: H. Su
   Center for Cerebrovascular Research, Department of Anesthesia and Perioperative Care, University of California San Francisco, San Francisco, CA, USA.

   Using several transgenic mouse models of inducible and tissue-specific endoglin deletion, the authors report that deletion of endoglin in smooth muscle cells produces arteriovenous malformations in the brain, spinal cord, and intestines. Induction of global endoglin deletion in adult animals produces cerebral arteriovenous lesions following VEGF stimulation, but deletion of endoglin in macrophages does not result in the appearance of arteriovenous malformations.

6. **Garrido-Martin, E.M. et al. Common and Distinctive Pathogenetic Features of Arteriovenous Malformations in Hereditary Hemorrhagic Telangiectasia 1 and Hereditary Hemorrhagic Telangiectasia 2 Animal Models – Brief Report.** *Arterioscler. Thromb. Vasc. Biol.* 2014. 34 (10): 2232-2236.
doi: 10.1161/atvbaha.114.303984

   Senior Investigator: S.P. Oh
   Department of Physiology and Functional Genomics, College of Medicine, University of Florida, Gainesville, FL, USA; and Lee Gil Ya Cancer and Diabetes Institute, Gachon University of Medicine and Science, Incheon, Republic of Korea.

   Authors report that in mice lacking endothelial expression of either endoglin or Alk1, skin wounding induces the appearance of subdermal arteriovenous malformations which arise by a more dynamic process in mice lacking endoglin than in mice lacking Alk1. Wound-induced arteriovenous malformations do not occur when either endoglin or Alk1 is deleted in smooth muscle cells.

7. **Han, C. et al. VEGF Neutralization Can Prevent and Normalize Arteriovenous Malformations in an Animal Model for Hereditary Hemorrhagic Telangiectasia 2.** *Angiogenesis.* 2014. 17(4): 823-830.
doi: 10.1007/s10456-014-9436-3

   Senior Investigator: S.P. Oh
   Department of Physiology and Functional Genomics, College of Medicine, University of Florida, Gainesville, FL, USA; and Lee Gil Ya Cancer and Diabetes Institute, Gachon University of Medicine and Science, Incheon, Republic of Korea.

   Using a dorsal window chamber model that permits real-time observation of vascular growth and remodeling, authors show that in Alk1-deficient mice, arteriovenous malformations form following skin wounding from de novo connections between arteries and veins. VEGF mimics the effect of skin wounding to induce arteriovenous malformations, and malformations that occur in Alk1-deficient mice in response to lipopolysaccharide injection are prevented by VEGF inhibition.

8. **Kawasaki, K. et al. Genetic Variants of *Adam17* Differentially Regulate TGFβ Signaling to Modify Vascular Pathology in Mice and Humans.** *Proc. Nat. Acad. Sci.* 2014. 111(21): 7723-7728.
doi: 10.1073/pnas.1318761111

   Senior Investigator: RJ Akhurst
   Helen Diller Family Comprehensive Cancer Center (HDFCCC) and Department of Anatomy, University of California, San Francisco, CA, USA; and Department of Anatomy, and Institute of Human Genetics, University of California, San Francisco, CA, USA.

Authors report that polymorphisms in ADAM17 generate hypomorphic variants capable of modulating TGFβ signaling by influencing the extent of Smad2 activation. Inhibition of ADAM17 enhances TGFβ signaling to increase the number of circulating endothelial cells, and polymorphic variants of ADAM17 are associated with increased incidence of pulmonary arteriovenous malformations in HHT1, but not HHT2, patients.

9. **Liu, Z. et al. Endoglin is Dispensible for Vasculogenesis, but Required for Vascular Endothelial Growth Factor-Induced Angiogenesis.** *PLoS One*. 2014. 9(1): e86273. doi: 10.1371/journal.pone.0086273

   Senior Investigator: P. ten Dijke
   Department of Molecular Cell Biology, Cancer Genomics Centre, Centre for Biomedical Genetics, Leiden University Medical Center, Leiden, The Netherlands.

   In comparative study of embryoid bodies derived from endoglin-deficient or wild-type cells, authors find that endoglin does not affect endothelial cell differentiation from mesodermal progenitors, but does impair vessel organization during developmental angiogenesis. Using several in vitro angiogenesis assays, authors show that endoglin is required for endothelial cell sprouting in response to VEGF signaling, but that endoglin knockdown does not directly affect VEGF/VEFR2 ligand-receptor interactions and is not necessary for downstream Erk activation.

10. **Moulinet, T. et al. High Prevalence of Arterial Aneurysms in Hereditary Hemorrhagic Telangiectasia.** *Int. J. Cardiol.* 2014. 176(3): 1414-1416. doi: 10.1016/j.ijcard.2014.08.16

    Senior Investigator: P. Kaminsky
    Département de Médecine Interne et Immunologie Clinique, Pôle des Spécialités Médicales, Centre Hospitalier Universitaire de Nancy, Hôpitaux de Brabois, 54500 Vandoeuvre-lès-Nancy, France; and Université de Lorraine, Vandoeuvre-lès-Nancy, France.

    In a retrospective review of HHT and non-HHT patients, authors find that incidence of thoraco-abdominal arterial aneurysms in HHT1 and HHT2 patients is several-fold higher than that found among control patients.

11. **Murphy, P.A. et al. Constitutively Active Notch4 Receptor Elicits Brain Arteriovenous Malformations Through Enlargement of Capillary-Like Vessels.** *Proc. Nat. Acad. Sci.* 2014. 111(50): 18007-18012. doi: 10.1073/pnas.1415316111

    Senior Investigator: R.A. Wang
    Laboratory for Accelerated Vascular Research, Department of Surgery, Division of Vascular Surgery, University of California, San Francisco, CA, USA.

    Authors show that forced expression of constitutively active Notch4 or Notch1 in the vasculature – but not in arteries alone – induces cranial arteriovenous malformations. In mice

expressing constitutive Notch4 in vascular endothelium, arteriovenous malformations form via remodeling of preexisting microvessels, and are associated with an increase in endothelial cell surface area but not with detectible increases in endothelial cell number or proliferation.

12. **Shen, F. et al. Endoglin Deficiency Impairs Stroke Recovery.** *Stroke.* 2014. 45: 2101-2106. doi: 10.1161/strokeaha.114.005115

    Senior Investigator: H. Su
    Center for Cerebrovascular Research, Department of Anesthesia and Perioperative Care, University of California San Francisco, San Francisco, CA, USA.

    Using mouse models of stroke, authors find that endoglin-deficient mice exhibit increased cerebral infarct volume, reduced macrophage infiltrate, lower post-ischemic angiogenesis, and delayed functional recovery following induction of cerebral ischemia compared to wild-type controls. Authors further report that single nucleotide polymorphisms in endoglin are associated with poorer surgical outcomes in human patients with brain arteriovenous malformations.

13. **Tørring, P.M. et al. Long Non-Coding RNA Expression Profiles in Hereditary Haemorrhagic Telangiectasia.** *PLoS One.* 2014. 9(3): e90272. doi: 10.1371/journal.pone.0090272

    Senior Investigator: K. Brusgaard
    Department of Clinical Genetics, Odense University Hospital, Odense, Denmark; and Human Genetics, Institute of Clinical Research, University of Southern Denmark, Odense, Denmark.

    Authors compare the long non-coding RNA expression profiles of telangiectasial and non-telangiectasial samples obtained from HHT1 and HHT2 patients, and identify 617 which have significantly altered expression in telangiectasial tissue. Subsequent analysis reveals that many of the genomic regions located near differentially expressed long non-coding RNAs are associated with genes broadly related to blood vessel development.

14. **Tual-Chalot, S. et al. Endothelial Depletion of Acvrl1 in Mice Leads to Arteriovenous Malformations Associated with Reduced Endoglin Expression.** *PLoS One*. 2014. 9(6): e98646. doi: 10.1371/journal.pone.0098646

    Senior Investigator: H.M. Arthur
    Institute of Genetic Medicine, Newcastle University, Newcastle, United Kingdom.

    Authors show that endothelial-deletion of *Acvrl1* (Alk1) in neonatal mice results in reduced arterial endothelial cell identity, vascular hyperbranching, and arteriovascular malformations in the retinal vasculature, as well as development of fatal pulmonary hemorrhage. Furthermore, *Acvrl1* deletion is associated with significant downregulation of endoglin gene expression.

15. **Xu, G. et al. Novel Protein Interactions with Endoglin and Activin Receptor-Like Kinase 1: Potential Role in Vascular Networks.** *Mol. Cell. Proteomics.* 2014. 13(2): 489-502. doi: 10.1074.mcp.m113.033464

    Senior Investigator: M. Letarte
    Molecular Structure and Function Program, Hospital for Sick Children, Toronto, ON, Canada.

    Using LUMIER interactome mapping approaches, authors identify 181 novel protein-protein interactions for endoglin, Alk1, TGFBR2, including 13 interactions that involve all three receptors. In particular, authors present data suggesting that all three receptors interact with PPP2R2B – a regulatory subunit of the PP2A protein phosphatase complex that influences eNOS phosphorylation status and function – and that endoglin negatively regulates this process. Finally, authors note that PPP2R2B is a possible candidate for the currently unknown HHT3 gene.

16. **Zucco, L. et al. Circulating Angiogenic Cell Dysfunction in Patients with Hereditary Hemorrhagic Telangiectasia.** *PLoS One*. 2014. 9(2): e89927. doi: 10.1371/journal.pone.0089927

    Senior Investigator: M.J. Kutryk
    Division of Cardiology, Keenan Research Center for Biomedical Science at the Li Ka Shing Knowledge Institute, St. Michael's Hospital, University of Toronto, Toronto, ON, Canada.

    Authors report that the percentage of circulating CD34+ angiogenic cells is increased in HHT patients, but that when these cells are maintained in culture, a higher percentage are apoptotic and fewer develop mature endothelial cell characteristics. These data suggest that circulating angiogenic cells in HHT patients may be less capable of repairing vascular lesions compared to that of healthy patients.

2015

1. **Alaa el Din, F. et al. Functional and Splicing Defect Analysis of 23 *ACVRL1* Mutations in a Cohort of Patients Affected by Hereditary Hemorrhagic Telangiectasia.** *PLoS One*. 2015. 10(7): e0132111. doi: 10.1371/journal.pone.0132111

    Senior Investigator: A. Kitzis
    Genetics of Rare Diseases, University of Poitiers, Poitiers, France; Department of Genetics, University Hospital of Poitiers, Poitiers, France.

    Authors compare 23 missense mutations in the *ACVRL1* (Alk1) gene identified in a screen of 400 HHT2 patients, and find that most mutations result in defective Alk1 protein folding, membrane localization, or loss of BM9 responsiveness, while two mutations affect intron-exon splicing resulting in a premature stop codon.

2. **Blanco, F.J. et al. Genome-Wide Transcriptional and Functional Analysis of Endoglin Isoforms in the Human Promonocytic Cell Line U937.** *J. Cell. Physiol.* 2014. 230(4): 947-958. doi: 10.1002/jcp.24827

   Senior Investigator: C. Bernabéu
   Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas (CSIC), 28040 Madrid, Spain; and Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), 28040 Madrid, Spain.

   Authors perform whole transcriptome analysis of cultured cells expressing either alternately spliced variants of endoglin, L-endoglin or S-endoglin, and find that signaling pathways associated with cell adhesion and migration are particularly affected. Specifically, forced expression of either endoglin variant reduces expression of several members of the integrin family of cell adhesion and signaling molecules while reduction of endoglin expression enhances integrin expression. Authors suggest that endoglin may influence integrin expression via a signaling pathway intermediary, Activin A.

3. **Donaldson, J.W. et al. Complications and Mortality in Hereditary Hemorrhagic Telangiectasia: A Population-Based Study.** *Neurology.* 2015. 84: 1886-1893. doi: 10.1212/WML.0000000000001538

   Senior Investigator: A.W. Fogarty
   Divisions of Epidemiology and Public Health, University of Nottingham, United Kingdom.

   In a large population-based study, authors find greater incidence of hemorrhage (including anemia, epistaxis, gastrointestinal bleeding, and hemoptysis), neurological complications (including migraine, cerebral abscess, and stroke), and cardiac failure among HHT patients compared to controls. They further find that HHT is associated with a two-fold increase in patient mortality.

4. **Mallet, C. et al. Functional Analysis of Endoglin Mutations from Hereditary Hemorrhagic Telangiectasia Type I Patients Reveals Different Mechanisms for Endoglin Loss of Function.** *Human Mol. Genet.* 2015. 24(4): 1142-1154. doi: 10.1093/hmg/ddu531

   Senior Investigator: E. Tillet
   Inserm, U1036, Grenoble F-38000, France, CEA, DSV, iRTSV, Laboratoire Biologie du Cancer et de L'Infection, Grenoble F-38000, France, University Grenoble-Alpes, Grenoble F-38000, France.

   In a comparative study of 31 endoglin missense mutations that were previously reported in HHT1 patients, authors find that missense mechanisms exert diverse effects on endoglin function. 16 of the studied mutations appear to be benign and alone may produce few symptoms of HHT, while the remaining mutations affect protein trafficking or BMP9 ligand binding. Furthermore, authors identify two dominant-negative mutations that lead to internalization of co-expressed wild-type endoglin.

5. **Massa, M. et al. Increase of Circulating Endothelial Cells in Patients with Hereditary Hemorrhagic Telangiectasia.** *Int. J. Hematol.* 2015. 101(1): 23-31. doi: 10.1007/s12185-014-1698-4

   Senior Investigator: C. Olivieri
   Laboratori Sperimentali di Ricerca Area Biotecnologie, IRCCS Fondazione "Policlinico S. Matteo", Viale Golgi 19, 27100, Pavia, Italy.

   Authors report that the percentage of circulating progenitor endothelial cells – which are implicated in vessel repair – is increased in HHT patients, while the percentage of hematopoietic progenitor cells is reduced. For HHT1 patients (but not HHT2 patients), increases in circulating endothelial cells are inversely correlated with patient age.

6. **Moon, E-H. et al. Essential Role for TMEM100 in Vascular Integrity but Limited Contributions to the Pathogenesis of Hereditary Haemorrhagic Telangiectasia.** *Cardiovasc. Res.* 2015. 105(3): 353-360. doi: 10.1093/cvr/cvu260

   Senior Investigator: S.P. Oh
   Department of Physiology and Functional Genomics, College of Medicine, University of Florida, Gainesville, FL, USA; and Lee Gil Ya Cancer and Diabetes Institute, Gachon University of Medicine and Science, Incheon, Republic of Korea.

   Authors show that TMEM100 expression (which has previously been found to be regulated by BMP9-Alk1 signaling) matches the spatiotemporal expression of Alk1 in developing blood vessels, and that endothelial-specific deletion of TMEM100 expression replicates the effect of Alk1-deficiency on retinal vascular sprouting. However, unlike with Alk1 deficiency, loss of TMEM100 expression does not induce arteriovenous malformation in the neonatal retinal vasculature or following skin wounding in adult animals, and produces only mild, non-tortuous arteriovenous malformations in adult lung and liver.

7. **Rozenberg, D. et al. Prevalence and Nature of Dyspnea in Patients with Hereditary Hemorrhagic Telangictasia (HHT).** *Resp. Med.* 2015. 109(6): 768-777. doi: 10.1016/j.rmed.2015.04.003

   Senior Investigator: M.E. Faughnan
   Toronto HHT Centre, Division of Respirology, Department of Medicine, University of Toronto, St. Michael's Hospital, Toronto, ON, Canada; and Li Ka Shing Knowledge Institute of St. Michael's Hospital, Toronto, ON, Canada.

   Authors conduct a retrospective review of 790 patients including 506 patients with a definite diagnosis of HHT, and find that 35% of HHT patients have dyspnea, including 44% of HHT patients with detectible pulmonary arteriovenous malformations (PAVMs) as well as 29% of HHT patients without detectible PAVMs. Authors find that several other factors are associated

with dyspnea among HHT patients including older age, higher body mass index, as well as HHT clinical manifestations such as anemia and symptomatic liver vascular malformations.

8. **Tørring, P.M. et al. Global Gene Expression Profiling of Telangiectasial Tissue from Patients with Hereditary Hemorrhagic Telangiectasia.** *Microvasc. Res.* 2015. 99: 118-126. doi: 10.1016/j.mvr.2015.04.002

   Senior Investigator: K. Brusgaard
   Department of Clinical Genetics, Odense University Hospital, Denmark; Human Genetics, Institute of Clinical Research, University of Southern Denmark, Denmark.

   Authors perform global gene expression analysis on telangiectasial and non-telangiectasial samples obtained from HHT1 and HHT2 patients. In HHT1 patients, 67 genes are differentially expressed in telangiectasial tissue, and gene ontology analysis identifies an enrichment of terms associated with Wnt signaling.

9. **Young, K. et al. BMP9 Crosstalk with the Hippo Pathway Regulates Endothelial Cell Matricellular and Chemokine Responses.** *PLoS One.* 2015. 10(4): e0122892. doi: 10.1371/journal.pone.0122892

   Senior Investigator: C.P. Vary
   Center for Molecular Medicine, Maine Medical Center Research Institute, Scarborough, ME, USA; and Graduate School of Biomedical Sciences and Engineering, University of Maine, Orono, ME, USA.

   Authors present evidence showing crosstalk between BMP9-endoglin and Hippo signaling pathways to regulate endothelial cell-extracellular matrix adhesion, as well as endothelial cell migration and inflammatory chemokine expression. Specifically, BMP-endoglin signaling promotes zyxin localization to endothelial cell focal adhesions, and the nuclear translocation of Hippo signaling pathway effector Yap1 to enable binding with nuclear Smad1/5 and transactivation of downstream genes, CTGF and CYR61.

10. **Zemankova, L. et al. Atorvastatin-Induced Endothelial Nitric Oxide Synthase Expression in Endothelial Cells is Mediated by Endoglin.** *J. Physiol. Pharmacol.* 2015. 66(3): 403-413.

    Senior Investigator: P. Nachtigal
    Charles University in Prague, Faculty of Pharmacy in Hradec Kralove, Department of Biological and Medical Sciences, Hradec Kralove, Czech Republic.

    Authors report that treatment of cultured endothelial cells with atorvastatin prevents TNF-α-induced decreases in eNOS and endoglin. They further show using siRNA gene silencing approaches that atorvastatin regulation of eNOS requires endoglin expression, suggesting that this statin may be less effective in HHT1 patients.

2016

1. **Brinjikji, W. et al. High Rates of Bleeding Complications among Hospitalized Patients with Hereditary Hemorrhagic Telangiectasia in the United States.** *Ann. A. Thorac. Soc.* 2016. 13(9): 1505-1511.
doi: 10.1513/annalsats.201603-200oc

   Senior Investigator: V.N. Iyer
   Division of Pulmonary and Critical Care Medicine, Mayo Clinic, Rochester, MN, USA.

   In a study of HHT patients hospitalized in the United States between 2000 and 2012 and their outcomes, authors find that most patients are hospitalized for bleeding-related complications, including anemia, epistaxis and gastrointestinal bleeding. In addition, HHT patients frequently present with cardiopulmonary complications, including congestive heart failure.

2. **Gkatzis, K. et al. Interaction Between ALK1 Signaling and Connexin40 in the Development of Arteriovenous Malformation.** *Arterioscler. Thromb. Vasc. Biol*. 2016 36(4): 707-717.
doi: 10.1161/atvbaha.115.306719

   Senior Investigator: F. Lebrin
   CNRS Unité mixte de recherche 7241/INSERM U1050, Center for Interdisciplinary Research in Biology, Collège de France, Paris, France; and MEMOLIFE Laboratory of Excellence, Paris Sciences et Lettres Research University, Paris, France.

   Authors identify *Gja5* as a transcriptional target of BMP9/Alk1 signaling, and show that the protein encoded by *Gja5*, Connexin40, is reduced in HHT2 patients. They further demonstrate that combined haploinsufficiency of both Alk1 and Connexin40 in mice increases the appearance of HHT2-like vascular lesions compared to mice haploinsufficient for Alk1 alone, associated with increased production of reactive oxygen species.

3. **Hunter, B.N. et al. An Evaluation of the Severity and Progression of Epistaxis in Hereditary Hemorrhagic Telangiectasia (HHT) 1 vs. HHT 2.** *Laryngoscope*. 2016. 126(4): 786-790.
doi: 10.1002/lary.25604

   Senior Investigator: K.F. Wilson
   Division of Otolaryngology-Head & Neck Surgery, University of Utah, Salt Lake City, UT, USA.

   In retrospective review of HHT1 and HHT2 patients, authors find that HHT2 patients have a later age of onset for epistaxis compared to HHT1 patients, but that epistaxis severity scores are more severe over time in HHT2 compared to HHT1. In addition, HHT2 patients are more likely to seek intranasal cautery or laser photocoagulation therapy to control epistaxis compared to HHT1 patients.

4. **Ojeda-Fernández, L. et al. Mice Lacking Endoglin in Macrophages Show an Impaired Immune Response.** *PLoS One*. 2016. 12(3): e1005935.
doi: 10.1371/journal.pgen.1005935

   Senior Investigator: L.M. Botella
   Centro de Investigación en Red de Enfermedades Raras (CIBERER), Valencia, Spain; and Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Biológicas (CSIC), Madrid, Spain.

   Authors show that endoglin is expressed upon differentiation of circulating monocytes into tissue-resident macrophages, and that deletion of endoglin expression in macrophages increases susceptibility to opportunistic infection in mice. Loss of macrophage endoglin is associated with decreased leukocyte transmigration, reduced macrophage phagocytotic efficiency, and altered TGF-β target gene expression, which likely contributes to the impaired innate immune response observed in these mice.

5. **Ola, R. et al. PI3 Kinase Inhibition Improves Vascular Malformations in Mouse Models of Hereditary Haemorrhagic Telangiectasia.** *Nat. Comm.* 2016. 7: 13650.
doi: 10.1038/ncomms13650

   Senior Investigator: A. Eichmann
   Cardiovascular Research Center, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT, USA; Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT, USA; and Inserm U970, Paris Cardiovascular Research Center, Paris 75015, France.

   Authors find that VEGF and PI3K/Akt signaling is increased in endothelial cells deficient in BMP9/Alk1 signaling, and that pharmacological PI3K inhibition (via administration of Wortmannin or Pictilisib) both prevents and reverts arteriovenous malformations and other hypervascularization phenotypes in the neonatal mouse retina of endothelial Alk1-deficient mice, as well as in the gastrointestinal tract. By contrast, endothelial VEGFR2 deletion prevents vascular hyperdensity but only partially protects against arteriovenous malformation in the neonatal retinas of these mice.

6. **Rochon, E.R. et al. Alk1 Controls Arterial Endothelial Cell Migration in Lumenized Vessels.** *Development*. 2016. 143: 2593-2602.
doi: 10.1242/dev.135392

   Senior Investigator: B.L. Roman
   Department of Human Genetics, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, USA.

   Using the zebrafish model, authors present evidence showing that loss of Alk1 expression does not affect arterial endothelial cell proliferation or apoptosis, but Alk1 is required for arterial endothelial cell migration against the direction of bloodflow.

7. **Rossi, E. et al. Endoglin Regulates Mural Cell Adhesion in the Circulatory System.** *Cell. Mol. Life Sci.* 2016. 73(8): 1715-1739
doi: 10.1007/s00018-015-2099-4

Senior Investigator: C. Bernabéu
Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas (CSIC), 28040 Madrid, Spain Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), 28040 Madrid, Spain; and Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), 28040, Madrid, Spain.

Authors present evidence for a novel role for endoglin in mediating CXCR12-induced, integrin-mediated adhesion between endothelial cells and mural cells to stabilize vessels and reduce vascular permeability. Authors further show that soluble endoglin inhibits this signaling pathway.

8. **Ruiz, S. et al. A Mouse Model of Hereditary Hemorrhagic Telangiectasia Generated by Transmammary-Delivered Immunoblocking of BMP9 and BMP10.** *Sci. Rep.* 2016. 5: 37366.
doi: 10.1038/srep37366.

Senior Investigator: P. Marambaud
Litwin-Zucker Research Center for the Study of Alzheimer's Disease, The Feinstein Institute for Medical Research, Manhasset, NY, USA; and Hofstra Northwell School of Medicine, Hempstead, NY, USA.

Authors report that transmammary delivery of BMP9 and BMP10 blocking antibodies is an effective mouse model of HHT. Specifically, blocking antibodies are efficiently delivered from lactating dams to pups resulting in reliable dysregulation of neonatal retinal vascular morphology. In whole transcriptome analysis, authors further find that BMP9/10 signaling strongly upregulates gene expression of angiopoietin-2 in neonatal retinas as well as in cultured endothelial cells.

9. **Young, K. et al. Endoglin is Required in *Pax3*-Derived Cells for Embryonic Blood Vessel Formation.** *Dev. Biol.* 2016. 409(1): 95-105.
doi: 10.1016/j.ydbio.2015.10.019

Senior Investigator: C.P. Vary
Maine Medical Center Research Institute, Scarborough, ME, USA; and Graduate School of Biomedical Sciences and Engineering, University of Maine, Orono, ME, USA.

Authors deleted endoglin expression in Pax3-positive vascular precursor cells, which contribute primarily to smooth muscle cells of the dorsal aorta as well as a subpopulation of aortic and intersomitic endothelial cells. Loss of endoglin in this vascular precursor cell population results in dilated cranial, aortic, and intersomitic vessels with disorganized smooth muscle cell layers in mice.

2017

1. **Albiñana, V. et al. Mutation Affecting the Proximal Promoter of *Endoglin* as the Origin of Hereditary Hemorrhagic Telangiectasia Type 1.** *BMC Med. Genet.* 2017. 18(1): 20. doi: 10.1186/s12881-017-0380-0

   Senior Investigator: L.M. Botella
   Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas (CSIC), and Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Ramiro de Maeztu 9, Madrid, 28040, Spain.

   In genomic sequencing of individual members of a family with HHT, authors identify for the first time a pathogenic single nucleotide mutation located in the promoter region of endoglin that likely disrupts transcription factor complex binding necessary for endoglin gene transcription.

2. **Baeyens, N. et al. Defective Fluid Shear Stress Mechanotransduction Mediates Hereditary Hemorrhagic Telangiectasia.** *J. Cell Biol.* 2017. 214(7): 807-816. doi: 10.1083/jcb.201603106

   Senior Investigator: M.A. Schwartz
   Department of Medicine (Cardiology), Yale University School of Medicine, New Haven, CT, USA; Department of Cell Biology, Yale University, New Haven, CT, USA; and Department of Biomedical Engineering, Yale University, New Haven, CT, USA.

   Using a combination of in vivo and in vitro models, authors show that fluid shear stress potentiates BMP9/10 activation of Alk1 signaling likely by promoting association between Alk1 and endoglin, and that this synergistic relationship between flow and BMP9/Alk1/endoglin signaling functions to stabilize the vasculature by restraining endothelial cell proliferation and promoting mural cell recruitment.

3. **Brinjikji, W. et al. Prevalence and Characteristics of Brain Arteriovenous Malformations in Hereditary Hemorrhagic Telangiectasia: A Systematic Review and Meta-Analysis.** *J. Neurosurg.* 2017. 127: 302-310. doi: 10.3171/2016.7.jns16847

   Senior Investigator: G. Lanzino
   Neurosurgery, Mayo Clinic, Rochester, MN, USA.

   In meta-analysis of 39 previously published studies, authors find that the prevalence of brain arteriovenous malformations among HHT patients Is 10%, with over 40% of these patients exhibiting multiple arteriovenous malformations. The prevalence of brain arteriovenous malformations is higher in HHT1 patients compared to HHT2 patients and does not appear to change with patient age, suggesting that brain arteriovenous malformations may form early in life and are present among pediatric HHT patients. Finally, authors find that 50% of HHT

patients with brain arteriovenous malformations report symptoms, and 20% will experience hemorrhage of brain arteriovenous lesions.

4. **Jin, Y. et al. Endoglin Prevents Vascular Malformation by Regulating Flow-Induced Cell Migration and Specification Through VEGFR2 Signaling.** *Nat. Cell Biol.* 2017. 19(6): 639-652. doi: 10.1038/ncb3534

   Senior Investigator: L. Jakobsson
   Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Scheeles väg 2, 171 77 Stockholm, Sweden.

   Using mouse models of endothelial endoglin deletion and overexpression, authors show that endoglin controls venous-to-arterial migration of endothelial cells against the direction of bloodflow, and that loss of endoglin expression leads to venous and arteriolar retention of endothelial cells and subsequent arteriovenous malformation with secondary increases in endothelial cell proliferation. In vitro experiments further show that endoglin regulates VEGFA/VEGFR2 signaling kinetics to suppress Akt signaling. PI3K/Akt inhibition partially rescues flow-directed migration in endoglin-deficient endothelial cells in vitro, and reduces arteriovenous malformation in endothelial endoglin-deficient mutant mice.

5. **Palagallo, G.J. et al. The Prevalence of Malformations of Cortical Development in a Pediatric Hereditary Hemorrhagic Telangiectasia Population.** *AJNR Am. J. Neuroradiol.* 2017. 38: 383-386.
   doi: 10.3174/ajnr.A4980

   Senior Investigator: A.J. White
   Department of Pediatrics, Washington University in St. Louis School of Medicine, St. Louis, MO, USA.

   Authors find that malformations of cortical development – particularly, unilateral subclinical polymicrogyria – are relatively more prevalent among pediatric HHT patients, and appear to correlate with the presence of pulmonary or brain arteriovenous malformations in these patients.

6. **Ruiz, S. et al. Tacrolimus Rescues the Signaling and Gene Expression Signature of Endothelial ALK1 Loss-of-Function and Improves HHT Vascular Pathology.** *Hum. Mom. Genet.* 2017. (in press)
   doi: 10.1093/hmg/ddx358.

   Senior Investigator: P. Marambaud
   Litwin-Zucker Research Center for the Study of Alzheimer's Disease, The Feinstein Institute for Medical Research, Manhasset, NY, USA; and Hofstra Northwell School of Medicine, Hempstead, NY, USA.

   Authors identify FDA-approved drug tacrolimus as a potent Alk1 signaling mimetic that reverses many of the signaling changes observed with Alk1 signaling blockade in cultured

endothelial cells, including loss of Smad1/5/8 signaling and upregulation of Dll4 expression. Tacrolimus increases Smad1/5/8 activation in cells expressing mutant Alk1, and treatment of mice wherein BMP9/10 signaling has been blocked – a mouse model of HHT – with tacrolimus partially rescues vascular development in the neonatal retina.

7. **Saito, T. et al. Structural Basis of the Human Endoglin-BMP9 Interaction: Insights into BMP Signaling and HHT1.** *Cell Reports.* 19(9): 1917-1928.
doi: 10.1016/j.celrep.2017.05.011.

   Senior Investigator: L. Jovine
   Department of Biosciences and Nutrition and Center for Innovative Medicine, Karolinska Institutet, Huddinge 14183, Sweden.

   Authors present the crystal structure of the N-terminal ectodomain of human endoglin complexed with the ligand, BMP9. The crystal structure shows that a highly hydrophobic region of the endoglin ectodomin interacts with BMP9 at a different site compared to the region of BMP9 bound by Alk1, and that several endoglin mutations associated with HHT affect hydrophobic packing of the protein likely disrupting protein stability and trafficking.

8. **Sugden, W. et al. Endoglin Controls Blood Vessel Diameter Through Endothelial Cell Shape Changes in Response to Haemodynamic Cues.** *Nat. Cell Biol.* 2017. 19(6): 653-665.
doi: 10.1038/ncb3528

   Senior Investigator: A.F. Siekmann
   Max Planck Institute for Molecular Biomedicine, Roentgenstrasse 20, D-48149 Muenster, Germany; and Cells-in-Motion Cluster of Excellence (EXC 1003 - CiM), University of Muenster, D-48149 Muenster, Germany.

   Authors identify the zebrafish homolog of endoglin (*eng*) and report that homozygous deletion of the *eng* gene results in fish that survive to adulthood but with cranial arteriovenous malformations. In zebrafish embryos, *eng* deletion results in excessively large dorsal aortas and posterior cardinal veins that fail to reduce in caliber in response to flow. Finally, authors report that the remodeling defect in the dorsal aorta is due to failure of *eng* mutant endothelial cells to decrease endothelial cell surface area in response to shear stress.

9. **Tachida, Y. et al. Mutual Interaction Between Endothelial Cells and Mural Cells Enhances BMP9 Signaling in Endothelial Cells.** *Biol. Open.* 2017. 6(3): 370-380.
doi: 10.1242/bio.020503

   Senior Investigator: H. Kobayashi
   Pain and Neuroscience Laboratories, R&D Division, Daiichi Sankyo Co., Ltd., Tokyo, Japan.

   Using cell culture models, authors present evidence that direct cell-cell contact between endothelial cells and mural cell types potentiates BMP9-induced gene expression changes.

10. **Vorselaars, V.M.M. et al. *SMAD4* Gene Mutation Increases the Risk of Aortic Dilation in Patients with Hereditary Haemorrhagic Telangiectasia.** *Int. J. Cardiol.* 2017. 245: 114-118. doi: 10.1016/j.ijcard.2017.06.059

    Senior Investigator: M.C. Post
    Department of Cardiology, St. Antonius Hospital, Nieuwegein, The Netherlands.

    Authors report that the incidence of aortic root dilation is significantly increased in HHT patients with mutations in Smad4 compared to either non-HHT patients or HHT patients bearing mutations in Alk1 or endoglin.

11. **Vorselaars, V. et al. Pulmonary Hypertension in a Large Cohort with Hereditary Hemorrhagic Telangiectasia.** *Respiration.* 2017. 94(3): 242-250.
    doi: 10.1159/000458447

    Senior Investigator: M.C. Post
    Department of Cardiology, St. Antonius Hospital Nieuwegein, Nieuwegein, The Netherlands.

    In a large study of HHT patients, authors find that incidence of pulmonary hypertension is significantly increased in HHT patients. Furthermore, incidence was higher among HHT2 patients compared to HHT1 patients, and presence of hepatic arteriovenous malformations was among the strongest predictors of pulmonary hypertension in HHT patients.

SELECTED REVIEWS

1. **Ardelean, D.S. and Letarte, M. Anti-Angiogenic Therapeutic Strategies in Hereditary Hemorrhagic Telangiectasia.** *Front. Genet.* 6: 35.
   doi: 10.3389/fgene.2015.00035

   Senior Investigator: M. Letarte
   Molecular Structure and Function Program, Hospital for Sick Children, Toronto, ON, Canada.

   Authors review current findings regarding dysregulated angiogenesis in HHT and discuss the evidence supporting the use of anti-angiogenic strategies and treatments as an HHT therapeutic approach.

2. **Botella, L.-M. et al. Research on Potential Biomarkers in Hereditary Hemorrhagic Telangiectasia.** *Front. Genet.*2015. 6: 115.
   doi: 10.3389/fgene.2015.00115

   Senior Investigator: C. Bernabéu
   Department of Cellular and Molecular Medicine, Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Cientificas , Madrid, Spain ; Centro de Investigación Biomédica en Red de Enfermedades Raras , Madrid, Spain.

   Authors review current findings on several possible HHT plasma biomarkers, as well as other novel strategies and techniques for diagnosing HHT in the patient population.

3. **Brinjikji, W. et al. Cerebrovascular Manifestations of Hereditary Hemorrhagic Telangiectasia.** *Stroke.* 2015. 46: 3329-3337.
   doi: 10.1161/strokeaha.115.010984

   Senior Investigator: G. Lanzino
   Department of Neurosurgery, Mayo Clinic, Rochester, MN, USA.

   Authors review the incidence and characteristics of cerebrovascular malformations and other nervous and non-nervous system manifestations of HHT.

4. **Dingenouts, C.K.E., et al. Mononuclear Cells and Vascular Repair in HHT.** *Front. Genet.* 2015. 6: 114.
   doi: 10.3389/fgene.2015.00114.

   Senior Investigator: W. Bakker
   Department of Molecular Cell Biology, Leiden University Medical Center Leiden, Netherlands.

   Authors review current findings regarding the contribution of disrupted inflammatory responses – and specifically, dysfunction of mononuclear cells – in the pathogenesis of HHT.

5. **Jerkic, M. and Letarte, M. Contribution of Oxidative Stress to Endothelial Dysfunction in Hereditary Hemorrhagic Telangiectasia.** *Front. Genet.* 2015. 6: 34.
doi: 10.3389/fgene.2015.00034

   Senior Investigator: M. Letarte
   Molecular Structure and Function Program, Hospital for Sick Children, Toronto, ON, Canada.

   Authors review studies demonstrating that production of reactive oxygen species is elevated in mouse models of HHT leading to endothelial damage and impaired nitric oxide-mediated vasodilation.

6. **McDonald, J. et al. Hereditary Hemorrhagic Telangiectasia: Genetics and Molecular Diagnostics in a New Era.** *Front. Genet.* 2015. 6:1.
doi: 10.3389/fgene.2015.00001

   Senior Investigator: P. Bayrak-Toydemir
   Department of Pathology, University of Utah Salt Lake City, UT, USA ; ARUP Institute for Clinical and Experimental Pathology Salt Lake City, UT, USA.

   Authors review the current literature regarding identified mutations in known genes associated with HHT, and they further propose integration of next-generation sequencing approaches to enhance molecular diagnosis strategies for the disease.

7. **Peacock, H.M. et al. Arteriovenous Malformations in Hereditary Haemorrhagic Telangiectasia: Looking Beyond ALK1-NOTCH Interactions.** *Cardiovasc. Res.* 2016. 109(2): 196-203.
doi: 1093/cvr/cvv264

   Senior Investigator: E.A. Jones
   Department of Cardiovascular Science, Centre for Molecular and Vascular Biology, KU Leuven, UZ Herestraat 49-Box 911, 3000 Leuven, Belgium

   Authors discuss current perspectives regarding involvement of Notch signaling in HHT-related arteriovenous malformation and review additional targets of BMP9/Alk1 signaling that may contribute to the etiology of HHT.

8. **Roman, B.L. and Hinck, A.P. ALK1 Signaling in Development and Disease: New Paradigms.** *Cell. Mol. Life Sci.* 2017. (in press)
doi: 10.1007/s00018-017-2636-4

   Senior Investigators: B.L. Roman and A.P. Hinck
   Department of Human Genetics, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, USA (B.L.R.); and Department of Structural Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA (A.P.H.).

Authors provide a comprehensive overview of BMP9/10-Alk1 signaling during vascular development and in HHT.

9. **Rossi, E. et al. Endoglin Involvement in Integrin-Mediated Cell Adhesion as a Putative Pathogenic Mechanism in Hereditary Hemorrhagic Telangiectasia Type 1 (HHT1).** *Front. Genet.* 2015. 5: 457.
doi: 10.3389/fgene.2014.00457

   Senior Investigator: C. Bernabéu
   Department of Cellular and Molecular Medicine, Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Cientificas , Madrid, Spain ; Centro de Investigación Biomédica en Red de Enfermedades Raras , Madrid, Spain.

   Authors review and discuss the emerging evidence for a novel role for endoglin in regulating integrin expression and integrin-mediated cell adhesion and signaling that may contribute to the pathogenesis of HHT1.

10. **Thalgott, J. et al. Pericytes as Targets in Hereditary Hemorrhagic Telangiectasia.** *Front. Genet.* 2015. 6: 37.
doi: 10.3389/fgene.2015.00037

    Senior Investigator: F. Lebrin
    CNRS Unité Mixte de Recherche 7241/INSERM U1050, Center for Interdisciplinary Research in Biology, Collège de France, Paris, France; and MEMOLIFE Laboratory of Excellence, Paris Sciences et Lettres Research University, Paris, France.

    Authors review recent advances regarding TGF-β signaling on endothelial cell-mural cell interactions that stabilize developing vessels, and authors further discuss the implications of these findings in the pathogenesis and treatment of hereditary hemorrhagic telangiectasia.

11. **Tual-Chalot, S. et al. Mouse Models of Hereditary Hemorrhagic Telangiectasia: Recent Advances and Future Challenges.** *Front. Genet.* 2015. 6: 25.
doi: 10.3389/fgene.2015.00025

    Senior Investigator: H.M. Arthur
    Institute of Genetic Medicine, Newcastle University, Newcastle, United Kingdom.

    Authors review and synthesize key findings from studies conducted on multiple mouse models of HHT.