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**9th International
Hereditary Hemorrhagic Telangiectasia
Scientific Conference**

20-24 May 2011

Kemer, Antalya – Turkey

Guest Editor: Kevin Whitehead, USA

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20-24 May 2011
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Guest Editor: Kevin Whitehead, USA

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9th International Hereditary Hemorrhagic Telangiectasia Scientific Conference

20-24 May 2011
Kemer, Antalya – Turkey
PROGRAM

20 May 2011, Friday

17:30-19:00 WELCOME RECEPTION

21 May 2011, Saturday

08:30-10:00 SESSION I: WELCOME SESSION (*Session chairs: Kevin Whitehead and Pinar Bayrak-Toydemir*)

Rosemary Ackhurst, *Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, CA, USA*. **“Genetic Modifiers of HHT: A Mouse to Human Approach”**

Scott Grosse, *Division of Blood Disorders, National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, GA, USA*. **“Using Administrative Data Sources for Population Surveillance for HHT”**

A17 – Scott Grosse, *Division of Blood Disorders, National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, GA, USA*. **“Prevalence of Hereditary Hemorrhagic Telangiectasia in a Privately Insured U.S. Population”**

A14 – Pinar Bayrak-Toydemir, *ARUP Institute for Clinical and Experimental Pathology and Department of Pathology, University of Utah, Salt Lake City, UT, USA*. **“New Home for Hereditary Hemorrhagic Telangiectasia (Including SMAD4) Mutation Database”**

10:15-12:15 SESSION II: HARNESSING STEM CELLS IN HHT (*Session chairs: Helen Arthur and Paul Oh*)

Bruno Péault, *Centre for Cardiovascular Science, The University of Edinburgh, Edinburgh, UK and Orthopedic Hospital Research Center, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA*. **“Multipotent Stem Cells in Adult Human Organs: Identification, Purification and Potential Therapeutic Use”**

Joyce Bischoff, *Vascular Biology Program, Department of Surgery, Harvard Medical School, Boston, MA, USA*. **“Building Vascular Networks with Human Endothelial and Mesenchymal Progenitor Cells”**

Christine Mummery, *Department of Anatomy and Embryology, Leiden University Medical Center, Leiden, The Netherlands*. **“Induced Pluripotent Stem Cells as Novel Models for HHT”**

D13 – Christian Freund, *Department of Anatomy & Embryology, Leiden University Medical Center, Leiden, the Netherlands*. **“Induced Pluripotent (iPS) Cell Lines from Patients as a Disease Model to Study Hereditary Hemorrhagic Telangiectasia (HHT)”**

D07 – Cesare Danesino, *Medical Genetics – University of Pavia, Pavia, Italy*. **“Rendu-Osler-Weber Patients have an Increased Frequency of Mature Endothelial Cells in Peripheral Blood”**

13:30-15:15 SESSION III: HEPATIC ARTERIOVENOUS MALFORMATIONS (*Session chairs: Guadalupe Garcia-Tsao and Sophie Dupuis-Girod*)

Sukru Emre, *Department of Surgery, Yale University, New Haven, CT, USA*. **“Challenges in Orthotopic Liver Transplant for Patients with Hereditary Hemorrhagic Telangiectasia (HHT)”**

C22 – Elisabetta Buscarini, *Gastroenterology Department, HHT Center, Maggiore Hospital, Crema, Italy*. **“Natural History and Outcome of Hepatic Vascular Malformations in a Large Cohort of Patients with Hereditary Hemorrhagic Telangiectasia”**

A02 – Elsa Guillot, *Centre Hospitalier Lyon Sud, Service de Radiologie, Pierre Bénite, France*. **“Comparison of CT-scan and Contrast-enhanced Ultrasonography to Screen Liver Involvement in HHT”**

A28 – Alexander Massmann, *Saarland University Hospital, Saar, Germany and Clinics of City Cologne, Holweide, Germany*. **“Magnetic Resonance Imaging of the Liver in Patients with Hepatic Involvement of HHT”**

A03 – Sophie Dupuis-Girod, *Hospices Civils de Lyon, Hôpital Louis Pradel et Service de Génétique et Centre de Référence sur la Maladie de Rendu-Osler, Faculté de Médecine, Université Lyon, Lyon, France*. **“Efficacy and Safety of Bevacizumab in the Treatment of Hereditary Hemorrhagic Telangiectasia (HHT) Associated with Severe Hepatic Vascular Malformations”**

D24 – Kevin Whitehead, *Utah HHT Center of Excellence and Division of Cardiology, University of Utah, Salt Lake City, UT, USA*. **“Use of Bevacizumab in Complicated HHT: North American HHT Center Experience”**

15:30-17:30 SESSION IV: MEDICAL THERAPY FOR HHT (*Session chairs: Elisabetta Buscarini and Karen Swanson*)

Franck Lebrin, *Early Development and Pathologies, Center for Interdisciplinary Research in Biology, INSERM U1050, Collège de France, Paris, France*. **“From Mechanisms of Disease to Therapeutic Assays: New Approaches to Treatment of Bleeding in HHT Patients”**

C03 – Ségolène Gaillard, *INSERM, CIC 201, Hospices Civils de Lyon, Lyon, France*. **“Atero: Tranexamic Acid for the Treatment of Epistaxis in Hereditary Haemorrhagic Telangiectasia Patients”**

A24 – Luisa-Maria Botella, *Centro de Investigaciones Biológicas, and Centro de Investigación Biomédica en Red de Enfermedades Raras, Instituto de Salud Carlos III, Madrid, Spain and Hospital de Sierrallana, Torrelavega, Santander, Spain*. **“Raloxifene Hydrochloride as the First Orphan Drug for HHT”**

B14 – Carlo Sabbà, *Geriatric Unit and Rare Disease Center, University of Bari “A. Moro”, Italy*. **“Efficacy of Low-Dose Bevacizumab in Hereditary Hemorrhagic Telangiectasia”**

C16 – Gregor Bachmann-Harildstad, *Department of Otorhinolaryngology, Akershus University Hospital and Oslo University, Lørenskog, Norway*. **“Bevacizumab with 3-Weeks Intervals is Effective on Anemia in a Patient with Severe Hereditary Hemorrhagic Telangiectasia”**

A05 – Tuvia Hadar, *Nose and Sinus Institute, Department of Otorhinolaryngology, Rabin Medical Center, Beilinson Campus, Petah Tiqwa and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel*. **“Anti-Estrogen Therapy for Hereditary Hemorrhagic Telangiectasia – A Long Term Clinical Trial”**

A04 – Geraldine Samson, *Hospices Civils de Lyon, Pôle Information Médicale Evaluation Recherche, Université Lyon, Lyon, France*. **“Ex vivo Study of Bevacizumab Transport Through Porcine Nasal Mucosa”**

22 May 2011, Sunday

08:00-09:45 SESSION V: BRAIN ARTERIOVENOUS MALFORMATIONS (*Session chairs: Karel terBrugge and William Young*)

William Young, *Department of Anesthesia and Perioperative Care, University of California, San Francisco, CA, USA.*
“Lumpers versus Splitters: Is Hereditary Hemorrhagic Telangiectasia (HHT) a Biological Model of Sporadic Brain AVM?”

B08 – Takeo Nishida, *Yale HHT Center, Yale University School of Medicine, New Haven, CT, USA.* **“Correlations Between Genotypes and Clinical Manifestations in Brain AVMs with HHT”**

D22 – Neil Woodall, *Georgia Health Sciences University, Augusta, GA, USA.* **“Neurologic Sequelae of Hereditary Hemorrhagic Telangiectasia; the Georgia Health Sciences University Experience”**

C25 – Karel terBrugge, *Division of Neuroradiology, Department of Medical Imaging, University of Toronto, Toronto, Ontario, Canada.* **“Brain Arteriovenous Malformation Multiplicity is Strongly Associated with Hereditary Hemorrhagic Telangiectasia: Quantitative Assessment”**

B01 – Tsugio Seki, *Georgia Health Sciences University, Augusta, GA, USA.* **“A Novel Mouse AVM Model with Partial Lethality”**

Z01 – Augustine Ozanne, *Consultation pluridisciplinaire Rendu-Osler, APHP, Boulogne, France.* **“Cerebral Arteriovenous Fistula in Children: Genetic and Angiographic Analysis of 26 Cases (HHT versus RASA1)”**

10:00-12:00 SESSION VI: SCREENING AND IMAGING IN HHT (*Session chairs: Justin McWilliams and Robert White*)

Felix Ratjen, *Division of Respiratory Medicine, Hospital for Sick Children, University of Toronto, Toronto, Canada.*
“Screening for AVMs in Children: Who, When and How?”

B02 – Faisal Budhani, *Department of Medical Imaging, St. Michael's Hospital, University of Toronto, Toronto, Ontario, Canada.* **“Sensitivity of Low Dose CT Thorax for PAVM Detection in HHT”**

A29 – Alexander Massmann, *Saarland University Hospital, Saar, Germany and Clinics of City Cologne, Holweide, Germany.* **“Whole-body Magnetic Resonance Imaging as a Screening Procedure for Patients with HHT”**

C09 – John A. Livesey, *Imperial College School of Medicine and NHLI Respiratory Sciences, Imperial College London, London, UK.* **“Smoking – A New Risk Factor for Cerebral Abscess in Hereditary Haemorrhagic Telangiectasia?”**

D26 – Marco W. F. van Gent, *Department of Cardiology, St. Antonius Hospital, Nieuwegein, the Netherlands.* **“Activin Receptor-like Kinase-1 and Endoglin are not Related with Pulmonary Hypertension in Hereditary Hemorrhagic Telangiectasia”**

B15 – Carlo Sabbà, *Geriatric Unit and Rare Disease Center, University of Bari “A. Moro”, Italy.* **“Formation of Renal Cysts in Patients with Hereditary Haemorrhagic Telangiectasia”**

C08 – Helen Fennimore, *Nutrition and Dietetics, Kings College London, London, UK and University of Liverpool School of Medical Education, Liverpool, UK.* **“Diet and Hereditary Haemorrhagic Telangiectasia”**

13:00-15:00 SESSION VIIA: PULMONARY ARTERIOVENOUS MALFORMATIONS (*Session chairs: Hans-Jurgen Mager and Meir Mei-Zahav*)

- D27 – Marco W. F. van Gent, *Department of Cardiology, St. Antonius Hospital, Nieuwegein, the Netherlands*. **“Diagnostic Accuracy of Transthoracic Contrast Echocardiography as a Screening Method for Pulmonary Arteriovenous Malformations”**
- B21 – Pascal Lacombe, *Consultation pluridisciplinaire Rendu-Osler, APHP, Boulogne, France*. **“Thrombosis of Pulmonary Arteriovenous Malformations: Clinical Presentation and Treatment”**
- C07 – Vatshalan Santhirapala, *Imperial College School of Medicine and NHLI Respiratory Sciences, Imperial College London, London, UK*. **“Which Patients with Pulmonary Arteriovenous Malformations are Dyspnoeic? Retrospective Analysis of a Single-Centre 2005-2010 Cohort”**
- E01 – S. Veltuis, *Department of Cardiology, St. Antonius Hospital, Nieuwegein, the Netherlands*. **“Grade of Pulmonary Right-to-Left Shunt Using Contrast Transthoracic Echocardiography Associated with Neurological Complications”**
- C11 – Graham Robinson, *Department of Radiology, Hull and East Yorkshire NHS Trust, Hull, UK*. **“Long Term Results of Types 1 and 2 Amplatzer Vascular Plugs in Treating Pulmonary Arteriovenous Malformations”**
- B19 – Chantale Bélanger, *Centre Hospitalier Universitaire de Montréal, Montréal, Québec, Canada*. **“Pulmonary Arteriovenous Malformations Reperfusion after Percutaneous Embolization: Sensitivity and Specificity of Non-Enhanced CT Assessed with Pulmonary Angiography”**
- D09 – Urban W. Geisthoff, *Departments of Otorhinolaryngology, Internal Medicine and Radiology, Hospital of the City of Cologne, Cologne, Germany*. **“Does Transesophageal Echocardiography have a Place in the Screening Cascade for Pulmonary Arteriovenous Malformations?”**
- D28 – Marco W. F. van Gent, *Department of Cardiology, St. Antonius Hospital, Nieuwegein, the Netherlands*. **“Contrast Transthoracic Echocardiography in Hereditary Hemorrhagic Telangiectasia: a 5-Years Follow Up Study”**

13:00-15:00 SESSION VIIB: MOLECULAR AND CELLULAR BASES OF HHT (*Session chairs: Carmelo Bernabeu and Chris Hughes*)

- B29 – Paul D. Upton, *University of Cambridge School of Clinical Medicine, Addenbrookes and Papworth Hospitals, Cambridge, UK*. **“BMP9 Suppresses MCP1 Production by Human Pulmonary Artery Endothelial Cells”**
- D06 – Cesare Danesino, *Medical Genetics, University of Pavia, Pavia, Italy*. **“Distribution of TGF-Beta1 SNPs at Codon 10 and 25 in 169 Italian Patients Affected with Hereditary Hemorrhagic Telangiectasia”**
- B25 – Mirjana Jerkic, *Molecular Structure and Function Program, Hospital for Sick Children, Toronto, Ontario, Canada*. **“Increased Oxidative Stress Leads to Endothelial Dysfunction and Pulmonary Hypertension in Eng and Alk1 Heterozygous Mice”**
- C18 – Andreas Lux, *Q-bios GmbH and Faculty of Medicine at Mannheim, University of Heidelberg, Mannheim, Germany*. **“Analysis of Endoglin Homo- and Heteromers by Bimolecular Fluorescence Complementation and Further Fluorescence Microscopy Methods”**
- D18 – S. Paul Oh, *Department of Physiology and Functional Genomics, University of Florida, Gainesville, FL, USA*. **“Enhanced Migratory Property in Alk1-Deficient Endothelial Cells upon Angiogenic Stimulation”**
- C21 – Michael Kutryk, *Terrence Donnelley Heart Centre and Division of Cardiology, St. Michael's Hospital, Toronto, Ontario, Canada*. **“Angiogenesis Related MicroRNA in Patients with Hereditary Hemorrhagic Telangiectasia”**
- D08 – Cesare Danesino, *Medical Genetics, University of Pavia, Pavia, Italy*. **“Structural Modelling of ALK1 Ectodomain”**

15:15-17:00 SESSION VIII: EPISTAXIS MANAGEMENT AND RELATED TOPICS (*Session chairs: Donald Mintz and Urban Geischoff*)

- D02 – Christian A. Merlo, *Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD, USA*. **“Longitudinal Variation of Epistaxis in Individuals with Hereditary Hemorrhagic Telangiectasia”**
- A07 – Dario Morais, *ORL Service, Clinic University Hospital of Valladolid, Valladolid, Spain*. **“Local Sclerotherapy with Polidocanol (Etoxisclerol®) in Treatment of Epistaxis in HHT or Rendu-Osler-Weber Disease. 10 Years of Experience”**
- B06 – Sara Richer, *St. Vincent’s Medical Center, Bridgeport, CT, USA*. **“The modified Youngs Procedure in Patients with Transfusion Dependent Severe Epistaxis from Hereditary Hemorrhagic Telangiectasia”**
- C12 – Claus-Günther Konnerth, *Department of Otolaryngology, Head and Neck Surgery, Karl-Hansen-Medical-Center, Bad Lippspringe, Germany*. **“Coagulation of Telangiectasia of the Oral Cavity and Lips in HHT Patients Using a 980 nm Diode Laser”**
- Z02 – Johannes J. Mager, *St. Antonius Hospital, Nieuwegein, the Netherlands*. **“Bevacizumab Nasal Spray for Frequent Epistaxis in HHT”**
- A08 – Anette Kjeldsen, *Department of Otorhinolaryngology, Odense University Hospital, Odense, Denmark*. **“Efficiency of Laser Treatment in Patients with Hereditary Haemorrhagic Telangiectasia”**
- A20 – Giuseppe A. Latino, *Toronto HHT Program, Department of Medicine, Division of Respiratory, St. Michael’s Hospital, Toronto, Ontario, Canada*. **“Opportunities for Improving Diagnosis Rates of Hereditary Hemorrhagic Telangiectasia”**

23 May 2011, Monday

08:00-10:00 SESSION IX: ANIMAL MODELS (*Session chairs: Beth Roman and Tsugio Seki*)

- Kevin Whitehead, *Division of Cardiology, University of Utah, Salt Lake City, UT, USA*. **“Mechanisms and Models in Hereditary Vascular Diseases: Cavernous Malformations”**
- D17 – S. Paul Oh, *Department of Physiology and Functional Genomics, University of Florida, Gainesville, FL, USA*. **“Comparison of Conditional Knockout Mouse Models Suggests Divergent Pathogenetic Mechanisms Underlying Hereditary Hemorrhagic Telangiectasia Types 1 and 2”**
- D04 – Marwa Mahmoud, *Institute of Human Genetics, Newcastle University, Newcastle, UK*. **“Characterization of Arteriovenous Malformation Development in Two Inducible Mouse Models of HHT1 and HHT2”**
- C23 – Michelle Letarte, *Molecular Structure and Function Program, Hospital for Sick Children, Toronto, Ontario, Canada*. **“Beneficial Effects of Anti-Angiogenic Therapy in Endoglin Heterozygous Mice”**
- D16 – S. Paul Oh, *Department of Physiology and Functional Genomics, University of Florida, Gainesville, FL, USA*. **“Preclinical Assessment of VEGF Blockades for Inhibition of Arteriovenous Malformation in a Mouse Model for HHT”**
- C24 – Madonna Peter, *Molecular Structure and Function Program, Hospital for Sick Children, Toronto, Ontario, Canada*. **“The Potential Role of Endoglin in Modulating Immune and Angiogenic Mediators of Inflammatory Bowel Disease”**
- B18 – Wineke Bakker, *Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, the Netherlands*. **“Regenerative Capacity of Macrophages is Impaired in Hereditary Hemorrhagic Telangiectasia-1”**

10:15-12:10 SESSION X: GENOTYPES, PHENOTYPES AND CORRELATIONS (*Session chairs: Cesare Danesino and Tom Letteboer*)

- C10 – Whitney Wooderchak-Donahue, *ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, USA*. **“Determination of Causative Genes in Hereditary Hemorrhagic Telangiectasia (HHT) Using Exome Sequencing”**
- B12 – Frank Schwenter, *Samuel Lunenfeld Research Institute, Mount Sinai Hospital Zane Cohen Familial Gastro-Intestinal Cancer Registry, Department of Surgery, Mount Sinai Hospital, Toronto, Ontario, Canada*. **“Juvenile Polyposis, Hereditary Hemorrhagic Telangiectasia and Early Onset Colorectal Cancer in Patients with SMAD4 Mutation”**
- D15 – David A. Stevenson, *Department of Pediatrics, University of Utah, Salt Lake City, UT, USA*. **“Mutations in the 5’ UTR of ENG Gene Cause Hereditary Hemorrhagic Telangiectasia (HHT)”**
- B13 – Carlo Sabbà, *Geriatric Unit and Rare Disease Center, University of Bari “A. Moro”, Italy*. **“Patients with Hereditary Haemorrhagic Telangiectasia wait >30 Years on Average to Receive a Correct Diagnosis!”**
- D14 – Kristy Damjanovich, *ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, USA*. **“ACVRL1 and ENG Mosaicism in Hereditary Hemorrhagic Telangiectasia”**
- B27 – Els de Gussem, *Department of Internal Medicine, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada*. **“Phenotype-Genotype Correlations in Patients with Hereditary Hemorrhagic Telangiectasia and Pulmonary Arteriovenous Malformations”**
- D21 – Christine Edwards, *Division of Respiratory, Department of Medicine, St. Michael’s Hospital, Toronto, Ontario, Canada*. **“Genotype-Phenotype Correlations of Canadians with Hereditary Hemorrhagic Telangiectasia”**

24 May 2011, Tuesday

08:00-10:00 SESSION XI: TGF- β AND BMP PATHWAYS IN DISEASE (*Session chairs: Sabine Bailly and Michelle LeTarte*)

- Mark deCaestecker, *Division of Nephrology, Vanderbilt University Medical Center, Nashville, TN, USA*. **“BMP Signaling and Pulmonary Hypertension”**
- Navin Kapur, *Division of Cardiology, Tufts Medical Center, Boston, MA, USA*. **“Osler, Weber, and Rendu: Providing Insights into Cardiac Remodeling a Century Later”**
- B16 – Francisco J. Blanco, *Centro de Investigaciones Biológicas and Centro de Investigación Biomédica en Red de Enfermedades Raras, Madrid, Spain*. **“Analysis of the Molecular Mechanisms Involved in the Regulation of the Intron Retention of the Endoglin Gene During Endothelial Senescence”**
- A23 – Masato Morikawa, *Department of Molecular Pathology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan*. **“Genome-wide Analysis of SMAD1/5 Binding Regions in Endothelial Cells”**
- B07 – Beth Roman, *Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA, USA*. **“Alk1 Regulation of Flow-Responsive Gene Expression”**
- B17 – Carmelo Bernabeu, *Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas and Centro de Investigación Biomédica en Red de Enfermedades Raras, Madrid, Spain*. **“Vascular Injury Triggers KLF6 Mobilization and Cooperation with SP1 to Promote Endothelial Activation Through Upregulation of the ALK1 Gene”**

10:15-12:15 SESSION XII: CLINICAL MANAGEMENT OF HHT PATIENTS (*Session chairs: Carlo Sabba and James Gossage*)

Claire Shovlin, *National Heart & Lung Institute Cardiovascular Sciences, Imperial College, London, UK*. **“Pregnancy and Other Stressors of the HHT Phenotype”**

C05 – John A. Livesey, *Imperial College School of Medicine and NHLI Respiratory Sciences, Imperial College London, London, UK*. **“Associations of Iron Deficiency and Iron Treatment with Venous Thromboembolism and Biomarkers in Replicate HHT Cohorts”**

B26 – Els de Gussem, *Department of Internal Medicine, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada*. **“Brain Abscesses in Patients with Hereditary Hemorrhagic Telangiectasia”**

A18 – Walter Li, *Yale University School of Medicine, New Haven, CT, USA*. **“Reproducibility of Oxygen Saturation Monitoring During Six-Minute Walk and Exercise Testing in Patients with Pulmonary Arteriovenous Malformations.”**

C06 – Christopher G. Mason, *Respiratory Medicine, Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, UK*. **“Is Flying Safe for Individuals with Hereditary Haemorrhagic Telangiectasia?”**

C01 – Amy Østertun Geirdal, *Oslo University College, Faculty of Social Science and Department of Medical Genetics, Oslo University Hospital, Rikshospitalet, Oslo, Norway*. **“Living with Hereditary Haemorrhagic Telangiectasia. Coping and Psychological Distress”**

D10 – Urban Geithoff, *Department of Otorhinolaryngology, Hospitals of the City of Cologne, Cologne, Germany*. **“Trauma can Induce Telangiectases in Patients with Hereditary Hemorrhagic Telangiectasia”**

12:15-13:30 SESSION XIII: SUMMARY SESSION (*Session chairs: Jamie McDonald and Marie Faughnan*)

Henri Plauchu, *National Reference Center for Rendu-Osler disease - Clinical genetics department - French Centers of Excellence RO-Network coordinator and Hospices Civils de Lyon, Hôpital Louis Pradel, Bron, France*. **“Hereditary Hemorrhagic Telangiectasia Honorary Lecture: A Career Devoted to HHT”**

Session I Welcome Session

GENETIC MODIFIERS OF HHT: A MOUSE TO HUMAN APPROACH

T Letteboer^{1,2}, F Clermont¹, M Benzinou¹, S Espejel¹, MT Luu¹, KA Harradine¹, R Roy³, BE Aouizerat^{4,5}, JK Ploos van Amstel², S Giraud⁶, S Dupuis-Girod⁶, G Lesca⁶, H Plauchu⁶, CJJ Westermann⁷, **RJ Akhurst**^{1,5}

¹UCSF Helen Diller Family Comprehensive Cancer Center, Department of Medical Genetics, University Medical Centre, Utrecht, The Netherlands; ²UCSF HDFCCC Biostatistical Core Facility, ³UCSF Department of Physiological Nursing; ⁴UCSF Institute of Human Genetics, ⁵Service de Génétique Moléculaire et Clinique, Hôpital Edouard Herriot, Université de Lyon, Lyon, France; ⁶Hospices Civils de Lyon, Hôpital Louis Pradel, Service de Génétique et centre de référence sur la maladie de Rendu-Osler, Université de Lyon, Lyon, France; ⁷St Antonius Hospital, Nieuwegein, The Netherlands

HHT shows considerable clinical variability in severity of phenotypes even within families harboring the same pathogenic mutation, suggestive of environmental and/or genetic factors that modify the penetrance and expressivity of the clinical manifestations. We took a mouse to human genetics approach to screen for variant genetic loci that influence clinical severity of HHT, as scored by the presence of PAVM. We focused on genomic regions syntenic to murine modifier loci, Tgfbm2 and Tgfbm3, which are known to influence TGFβ1-dependent vascular development. We screened SNPs covering human syntenic loci TGFBM2 and TGFBM3, together with SNPs within >70 additional candidate genes encoding TGFβ and BMP signaling or response components, including ENG and ACVRL1. Genetic variation within the wild type ENG allele showed association with elevated risk of PAVM in Dutch HHT1 patients. Of all 760 SNPs screened, SNPs within TGFBM2 demonstrated strongest genetic association with PAVM in Dutch HHT1 and HHT2. This genetic association was validated for PAVM in French HHT patients. eQTL analysis of human lymphoblastoid and human lung tissues showed genetic interaction between ENG and TGFBM2. *In vitro* functional studies on candidate genes from TGFBM2 and TGFBM3 suggest possible mechanisms of action in predisposition to PAVM in HHT.

Acknowledgements: this work was funded by the HHT Foundation, NIH grants HL078564 (RJA) and GM60514 (RJA), the Ter Meulen Fund (TGWL) and American Heart Association (FC). We thank the patients for their consent and contributions.

USING ADMINISTRATIVE DATA SOURCES FOR POPULATION SURVEILLANCE FOR HHT

S Grosse

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Population-based public health surveillance involves the ongoing, systematic collection, analysis, and interpretation of health data to assess emerging or under-recognized health threats and to inform health policy-making. Monitoring populations requires statistical analysis of data from sources such as birth and death records, health surveys based on scientifically chosen samples, and/or administrative data (e.g., hospital discharges or health insurance claims). Surveillance for rare disorders using such sources is challenging because surveys are unlikely to provide sufficient numbers of cases, survey self-reports of diagnoses may not be reliable, diagnostic codes in death records or administrative data may not be sensitive or specific in identifying cases, and health insurance plans may not be representative. Codes for symptoms, co-morbidities, and procedures can also be used in administrative data to improve case ascertainment. Clinical registries, although not population-based, are invaluable sources of information for rare diseases and are used to conduct surveillance for diseases in populations where most affected persons are seen at specialized treatment centers. A relatively new and promising approach for rare disease surveillance involves linking registry and administrative data by matching individually identifiable information. This presentation discusses methods and experiences in using administrative data for surveillance for rare diseases and for HHT in particular.

(A17) PREVALENCE OF HEREDITARY HEMORRHAGIC TELANGIECTASIA IN A PRIVATELY INSURED U.S. POPULATION

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Currently, there are no population-based estimates of the prevalence of hereditary hemorrhagic telangiectasia (HHT) for the U.S.; however, it is estimated that 1 in 5,000 are affected. We used data from the 2003-2008 MarketScan® Commercial Claims and Encounters and Medicare Supplemental Insurance databases to identify individuals with an HHT diagnosis (ICD-9-CM code 448.0) noted on at least one inpatient claim or ≥2 outpatient claims ≥30 days apart. The administrative prevalence of HHT was calculated by age, sex and region. The frequency of reported symptoms consistent with HHT was assessed to evaluate the potential misclassification of HHT in claims data. Of the 28 million individuals included in the analysis, 821 had HHT reported (0.3 per 10,000). Prevalence was highest for individuals over 70 years of age (1.1 per 10,000) and those

living in the Western U.S. (0.44 per 10,000). The most frequently reported symptoms among individuals with HHT were anemia (41%), epistaxis (23%), and unspecified hemorrhage of the gastrointestinal (GI) tract (17%). The symptoms which were most specific to HHT, measured by the relative frequencies among people with HHT and in the general population were arteriovenous malformation (AVM), particularly of the lung, and angiodysplasia of the stomach and duodenum. Lung AVM in combination with epistaxis or other AVM was highly specific to HHT, although only a minority had an HHT diagnosis recorded. Although HHT is underascertained in administrative data, the findings regarding the prevalence of reported HHT can inform future efforts for population surveillance of HHT.

(A14) NEW HOME FOR HEREDITARY HEMORRHAGIC TELANGIECTASIA (INCLUDING SMAD4) MUTATION DATABASE

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The Hereditary Hemorrhagic Telangiectasia (HHT) Mutation database is a freely available mutation repository in which all ENG and ACVRL1 sequence based mutations and polymorphisms have been stored. It has been developed and hosted by Heriot Watt University in Edinburgh since 2004. Database has 1244 entries so far. In 2011, the HHT Mutation database will be moving to the University of Utah-ARUP Laboratories (www.arup.utah.edu). The ARUP education web site has been hosting several disease specific mutation databases including SMAD4 for several years. The new database will keep the mutation information from previous entries. However, there will be some format changes with several improvements. The frequency of mutations (independent observations for each mutation) with phenotype information will be added. As seen in the figure, independent observations for each mutation with clinical features and references will be available on the main screen. Independent observations will be clickable to provide more information for each observation, including family concordance studies, if available. In addition, the database will also provide large deletion/duplication mutation information for both genes. Individual or diagnostic laboratory submission will be available, and the database will be updated quarterly.

Patient	Clinical Findings	Family Concordance	Reference
1	E, F	unknown	McAllister (1994)
2	E, T		Yamaguchi (1997)
3	T, P	yes	Bourdieu (2000)
4	E, H, P	yes	Kjeldson (2001)

Session II Harnessing Stem Cells in HHT

MULTIPOTENT STEM CELLS IN ADULT HUMAN ORGANS: IDENTIFICATION, PURIFICATION AND POTENTIAL THERAPEUTIC USE

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Multipotent progenitor cells can be recovered from cultured adult tissues, the archetype of which remains the mesenchymal stem cell. In spite of its recognized usability for cell therapy, the MSC has been of unknown native identity, tissue distribution and frequency, and its extraction has relied entirely on extended culture. Hypothesizing that blood vessels naturally harbor primary MSC, we have identified, then sorted to homogeneity by flow cytometry endothelial and perivascular cells in multiple human organs. The latter – that include pericytes in microvessels and adventitial cells around larger ones – express MSC markers and are natively multipotent. Sorted pericytes, regardless of tissue origin, are identical to MSC in terms of growth in vitro, developmental potential and ability to migrate, inhibit T-cell proliferation and support hematopoiesis. These results indicate a perivascular origin for mesenchymal stem cells. Accordingly, human pericytes regenerate bone and skeletal muscle and promote functional recovery in the diseased heart, lung and kidney.

BUILDING VASCULAR NETWORKS WITH HUMAN ENDOTHELIAL AND MESENCHYMAL PROGENITOR CELLS

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For many diseases and injuries, there is a pressing need to rebuild the vasculature in order to quickly resupply organs and tissues with oxygen and nutrients. Towards this goal, we have built vascular networks in vivo using human endothelial and mesenchymal progenitor cells (EPCs and MPCs respectively). The EPCs can be isolated from umbilical cord blood or from adult peripheral blood (~100mls). MPCs can be isolated from umbilical cord blood or adult bone marrow (~25mls). The two cell populations are resuspended together in an extracellular matrix and injected subcutaneously in immune-deficient mice. Within 7 days, the EPCs and MPCs form microvascular networks with connections to the host vasculature (1). The nascent human vessels are perfused and organized with MPCs closely surrounding EPC-lined lumens. We recently expanded this model to show that human EPCs

and MPCs form perfused blood vessels when injected in a variety of extracellular matrices— type I collagen, fibrin and PuraMatrix, a hydrogel-forming peptide (2), which underscores the versatility of EPCs and MPCs. Our ongoing studies are focused on 1) the earliest onset of perfusion, 2) direct visualization of anastomoses between the nascent vessels built from human EPCs and MPCs and host vessels, and 3) building vessels with human EPCs and MPCs in ischemic hind limbs. The presence of EPCs in human peripheral blood, albeit at very low levels, raises the question of how EPCs may function in the vasculature over a lifetime, and how this might be altered in the context of HHT.

1. Melero-Martin, J.M., De Obaldia, M.E., Kang, S.Y., Khan, Z.A., Yuan, L., Oettgen, P., and Bischoff, J. 2008. Engineering robust and functional vascular networks in vivo with human adult and cord blood-derived progenitor cells. *Circ Res* 103:194-202.
2. Allen, P., Melero-Martin, J., and Bischoff, J. 2011. Type I collagen, fibrin and PuraMatrix matrices provide permissive environments for human endothelial and mesenchymal progenitor cells to form neovascular networks. *J Tissue Eng Regen Med*.

INDUCED PLURIPOTENT STEM CELLS AS NOVEL MODELS FOR HHT

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One of the most significant breakthroughs in the last decade of biomedical research has been the discovery that normal somatic skin cells can reprogrammed to a pluripotent state similar to that of embryonic stem cells. This means that cell lines can be derived from any individual that have the capacity to form all cell types of the body. We have now derived these induced pluripotent stem cells (iPS cells) from patients with various cardiovascular diseases. These range from patients with inherited cardiac diseases to, more recently, patients with ROW or HHT. Mutations in Endoglin, a co-receptor for transforming growth factor β (TGF β), or ALK1, a type 1 TGF β receptor in endothelial cells cause human hereditary telangiectasia type 1 and 2 respectively (HHT1 and HHT2). Through endoglin and ALK1, TGF β signals to the BMP-Smads 1/5/8 instead of the usual Smad2/3, specifically in endothelial cells. We show that absence of Endoglin severely disrupts vascular smooth muscle cell (VSMC) / endothelial cell (EC) association in an in vitro model based on mutant mouse embryonic stem cells and in some strains of mutant mice. The disease in humans is characterized by weak vessel walls and hemorrhage, resulting in severe nosebleeds and mucutaneous telangiectases. These vascular lesions cause arteriovenous malformations (AVMs) forming a direct connection between arterioles

and venules without intervening capillaries. Using nasal epithelium derived from patients undergoing the Saunders operation to replace it with skin from the arm of the same patient, we have derived fibroblasts in culture that we have reprogrammed to human iPS cells. We now have two fully characterized lines from HHT1 patients and one line from HHT with several more in the pipeline. Control human iPSC cell lines are able to generate mesenchymal cells/VSMC fairly efficiently but ECs with much lower efficiencies. Preliminary results indicate that sprouting angiogenesis in some of the patient lines is defective. We will describe these results in the context of the growing importance of human iPS cells from patients in understanding the molecular mechanisms of disease. These cells could provide a platform for new drug discovery to enhance vessel stability, much as Thalidomide has been shown to do recently (Lebrin et al, 2010, Nature Medicine) but preferably with fewer side effects. Human models are of importance not only to elucidate molecular mechanisms, but also to develop improved future therapies.

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(D13) INDUCED PLURIPOTENT CELL LINES FROM PATIENTS AS A DISEASE MODEL TO STUDY HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant vascular disorder caused by mutations in genes of the TGF- signaling pathway, notably Endoglin (HHT type 1) or ALK-1 (HHT2). HHT is characterized by telangiectasias in multiple organs. The observed phenotype results from severe disruption of vessel wall maturation, as a consequence of loss of pericyte and smooth muscle cell (SMC) coverage. Despite that mouse models of HHT are available, the human model system has not been developed yet. We have recently derived induced pluripotent stem (iPS) cells from patients with HHT1 and HHT2 by retroviral overexpression of the Yamanaka factors. iPS cells closely resemble embryonic stem cells (ESC) and can differentiate into derivatives of the three germ layers in vitro and in vivo. Vascular differentiation of iPS cells can be enhanced in the presence of serum and VEGF. Notably, differentiated EBs give rise to the two major vascular cell types, namely vascular smooth muscle cells and to a lesser extent endothelial cells (~3%). Endothelial cells express typical markers such as CD31, CD34, CD144 and von Willebrand factor. The development of methods for enrichment of both cell types, functional assays for vascular differentiation, heterotypic endothelial-smooth muscle cell interaction is cur-

rently ongoing. This will allow us to study the mechanism of HHT disease and to develop novel drugs by utilizing vascular cells derived from iPS cells from HHT patient or healthy individuals.

(D07) RENDU-OSLER-WEBER PATIENTS HAVE AN INCREASED FREQUENCY OF MATURE ENDOTHELIAL CELLS IN PERIPHERAL BLOOD

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Vascular diseases are associated with an increase in circulating progenitor cells, specially endothelial cells. We evaluated the frequency of these circulating progenitor cells in HHT patients and correlated it with parameters such as mutation frequencies and age of patients. The characterization of these cells is still debated today; we evaluated the CD34+ (hematopoietic and circulating endothelial progenitors and mature endothelial cells); the subpopulation CD34+ VEGFR-2+ (progenitor and mature endothelial cells) and subpopulation CD34+ CD133+ VEGFR-2+ CD45- (endothelial progenitors). The hematopoietic progenitors were identified as CD34+ CD133+ VEGFR2-. We studied peripheral blood samples from 150 patients and 30 healthy subjects (CTRLs). We noted that both the frequency of total CD34+ cells and the frequency of CD34+VEGFR-2+ cells is significantly increased in patients compared to CTRLs. Instead, the frequency of CD34 + CD133 + VEGFR2- cells is significantly decreased. These data indicate that in HHT patients there is an increase of circulating endothelial progenitors (CD34+ CD133+ VEGFR-2+ CD45- cells). Therefore, the increase in CD34 + cells is due to an increment of mature endothelial cells. These changes do not depend on gene involved or mutation type.

Session III Hepatic Arteriovenous Malformations

CHALLENGES IN ORTHOTOPIC LIVER TRANSPLANT FOR PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Over the past 2 decades, there have been numerous reports detailing the comparative success of orthotopic liver transplantation (OLT) for treatment of hereditary hemorrhagic telangiectasia (HHT). Based on the accumulated experience, the current consensus favors OLT rather than temporary surgical measures when patient develops severe liver and cardiac complications of HHT. Outcomes of OLT have been mostly positive in the literature with more than 82% 10 year patient survival. Decision making for OLT is well established in patients presenting with biliary tract complications including biliary strictures and cholangitis episodes. The rationale for early OLT in patients with mildly symptomatic heart failure would be to prevent irreversible cardiopulmonary pathology that cannot be alleviated by OLT. However, the predictors of poor outcome in these patients are not well understood and are critical to define in order to optimize the strategic use of selected patients with high output heart failure for OLT. Performing liver transplantation for patients with HHT has unique problems. Bleeding from the extrahepatic AVMs, especially retroperitoneal area, is always challenging. Appropriate evaluation of the pulmonary artery pressure and vascular resistance is paramount important. In patients with HHT, secondary to hepatic AVMs, the size of the hepatic artery can reach almost the size of the aorta.. Therefore, surgeons must be prepared to vary the operative approach based on the patient's vasculature. In conclusion, liver transplantation should be the procedure of choice for patients with HHT whom developed serious complications of the disease process. Operative interventions other than OLT are detrimental to the long-term care of these patients.

(C22) NATURAL HISTORY AND OUTCOME OF HEPATIC VASCULAR MALFORMATIONS IN A LARGE COHORT OF PATIENTS WITH HEREDITARY HEMORRHAGIC TELEANGIECTASIA

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Background. Hereditary hemorrhagic telangiectasia (HHT) is a genetic disease characterized by teleangiectasias involving virtually every organ. There are limited data in the literature regarding the natural history of liver vascular malformations (VMs) in HHT and their associated morbidity and mortality. **Aim.** This prospective cohort study sought to assess the outcome of liver involvement in HHT patients. **Methods.** We analyzed 16 years of surveillance data from a tertiary HHT referral center in Italy. We considered for inclusion in this study 502 consecutive Italian patients at risk of HHT who presented at the HHT referral center and underwent a multidisciplinary screening protocol for the diagnosis of HHT 154 of the 502 individuals assessed in the center had hepatic VMs and were the subject of the study, 198 patients with HHT and without hepatic VMs were the controls. Additionally, we report the response to treatment of patients with complicated hepatic VMs. **Results.** The 154 patients were included and followed for a median period of 44 months (range 12-181); of these, eight (5.2%) died from VM-related complications, and 39 (25.3%) experienced complications. The average incidence rates of death and complications were 1.1 and 3.6 per 100 person-years, respectively. The median overall survival and event-free survival after diagnosis were 175 and 90 months, respectively. The rate of complete response to therapy was 63%. **Conclusions.** This study shows that substantial morbidity and mortality are associated with liver VMs in HHT patients.

(A02) COMPARISON OF CT-SCAN AND CONTRAST-ENHANCED ULTRASONOGRAPHY TO SCREEN LIVER INVOLVEMENT IN HHT

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Introduction: Liver vascular malformations are present in 50-78% of HHT patients and Doppler ultrasound is currently recommended for liver VM screening. Computed tomography (CT scan), however, is more sensitive and allows an indirect visualization of shunts. Contrast-enhanced ultrasound (CEUS) provides continuous real time vascular study of the liver parenchyma during the different phases of contrast enhancement. Our objective was to evaluate the effectiveness of CEUS in the diagnosis of hepatic liver lesions in HHT versus CT scan. **Methods:** Prospective single-center study. CEUS was proposed to patients with a definite HHT diagnosis and normal hepatic Doppler US. Then CEUS was compared to hepatic CT scan. CEUS used a low-MI technique (Toshiba AplioXG[®]) with i.v boluses of SonoVue[®] (Bracco, Italy). After injection, the ROW data were recorded to determine perfusion parameters using CHIQ software. Multiphasic CT scans were performed for all patients with multiplanar and angiographic reconstructions. After CEUS and CT scan, data were classified in three groups: no, possible or definite liver involvement. **Results:** 29 patients were included and 25 underwent CEUS and CT scan (4 patients were excluded after doppler US). Sensitivity of CEUS compared to CT scan was 89%. 84% (n= 21) of patients with normal doppler US had hepatic involvement on CT scan and/or CEUS. **Conclusion:** CEUS is a promising approach in the non-invasive characterization of HHT liver lesions and can be useful as a first-line imaging technique, as sensitive as CT scan. Further studies are needed in HHT.

(A28) MAGNETIC RESONANCE IMAGING OF THE LIVER IN PATIENTS WITH HEPATIC INVOLVEMENT OF HHT

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Purpose. To evaluate magnetic resonance tomography and magnetic resonance angiography findings of hepatic involvement in patients suffering from HHT. **Methods and Materials.** 228 patients (mean age 46.5) with confirmed HHT according to Curacao criteria or their first degree relatives underwent a screening examination of the liver. Patients with signs of liver involvement underwent an additional non-enhanced and dynamic contrast-enhanced MRI including a hepatobiliary excretion phase and MRA after gadolinium-BOPTA (Multihance, Bracco) 0.05 mmol/kg bodyweight i.v. **Results:** MRI revealed 42 patients (mean age 55.3 years; male 8; female 34) with hepatic and vascular pathologies related to HHT. 12 of 42 patients were associated also with PAVM. Hepatomegalia was found in 30 patients. Right-heart-insufficiency (RHI), due to HAVM, was present in 11 patients, who did not suffer from hemodynamically relevant pulmonary AVM (PAVM). Patients with an enlarged diameter of the hepatic artery (HA) showed increased nodular hyperplastic changes of the liver (14 patients), a lower RHI-rate, and a rather normal diameter of the portal vein. **Conclusion.** HHT-patients with hepatomegaly and nodular hyperplastic changes of the liver most

likely have shunts at the sinusoidal level. Direct arteriovenous and arterio-portal shunts are associated with an almost normal liver size without hyperplastic changes. The increased arterial blood supply to the liver could explain the nodular hyperplastic changes similar to the mechanism of focal overgrowth of liver tissue discussed in the development of FNH. HAVM causing hyperplastic nodules do not result in direct hemodynamical left-to-right-shunts, while HAVM without hyperplastic nodules is likely to cause RHI.

(A03) EFFICACY AND SAFETY OF BEVACIZUMAB IN THE TREATMENT OF HEMORRHAGIC HEREDITARY TELANGIECTASIA (HHT) ASSOCIATED WITH SEVERE HEPATIC VASCULAR MALFORMATIONS. A PHASE II STUDY

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Background. Hereditary hemorrhagic telangiectasia is a dominantly inherited genetic vascular disorder in which hepatic shunts can be associated with high-output cardiac failure. The possible use of anti-VEGF treatments such as Bevacizumab in cases of HHT with liver vascular malformations is an interesting new therapeutic approach. The objective was to test the efficacy of Bevacizumab in the severe hepatic forms of HHT. Efficacy is defined as a decrease in cardiac index (CI), 3 months after the first injection, in relation to the initial value at the start of the trial. **Methods.** Single-center phase II trial. A two-step Gehan design was used. Inclusion criteria: age between

18 and 70 years, confirmed HHT, severe liver involvement and high cardiac index (CI). **Treatment.** Bevacizumab, 6x5 mg / kg every 14 days. **Results.** A total of 25 patients were included. In January 2011, 17 patients achieved a 6 months follow up. Mean CI measured at 3 months was significantly improved in 12/17 patients with 2 complete responses (CR) (mean at baseline 4.9 vs 4.2) ($p=0.004$). At 6 months, CI decreased in 15/17 patients with 4 CR ($p < 0.001$), epistaxis improved in duration (mean 158 vs 28 min, $p < 0.001$) and frequency ($p=0.007$) and quality of life was significantly improved for physical and mental component ($p=0.042$ and 0.025 respectively). Mean liver volume was significantly decreased ($p=0.026$). Skin telangiectasia were not improved as well as pulmonary AVM. **Conclusion.** Bevacizumab efficiently reduced CI in 15/17 treated HHT patients with severe liver shunting.

(D24) USE OF BEVACIZUMAB IN COMPLICATED HHT: NORTH AMERICAN HHT CENTER EXPERIENCE

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Introduction. Anecdotal reports of bevacizumab in HHT have been published. HHT patients may develop complicated disease for which therapy is suboptimal. Adverse effects have been observed during therapy for cancer, yet complications have not been reported for systemic use in HHT. We report the collective experience of the North American HHT centers, reporting both efficacy and safety outcomes. **Methods.** Center directors for the 13 North American HHT centers were contacted to retrospectively identify patients treated with systemic bevacizumab. Indications, dosages, outcomes and adverse events were reported. **Results.** Twelve patients were identified that had received systemic bevacizumab primarily for complicated HHT. The average age was 57 years (range 25 to 77). Indications included hepatic arteriovenous malformations with heart failure (5 – benefit in 4) or biliary ischemia (1 – partial response), gastrointestinal bleeding (4 – response in all 4), and diffuse pulmonary arteriovenous malformations (1 – no response). One further patient received a single infusion of bevacizumab as an attempt at rescue of severe tracheal bleeding. Hemorrhage did not respond and ultimately led to death within days of the infusion. One patient with heart failure relapsed after 1 year, received a second course with benefit for another year, and is now receiving a third course. One patient reported nausea and fatigue during infusion. One patient (with GI bleeding) developed peripheral neuropathy. Worsening of epistaxis during therapy was seen in a single patient. **Conclusion.** Beneficial responses to bevacizumab have been seen for heart failure and gastrointestinal bleeding. Adverse effects cannot be excluded.

Session IV Medical Therapy for HHT

FROM MECHANISMS OF DISEASE TO THERAPEUTIC ASSAYS: NEW APPROACHES TO TREATMENT OF BLEEDING IN HHT PATIENTS

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Development of vascular malformations in HHT patients has been attributed to deregulation of key angiogenic factors leading to abnormal endothelial cell proliferation and migration, extracellular matrix production and degradation, as a result of which endothelium and surrounding mural cells disengage. This causes vessels to weaken and become prone to bleeding, a major feature of HHT. Inhibitors of angiogenesis could therefore be beneficial in treating symptoms of these vascular anomalies in HHT patients. However, anti-angiogenic drugs may also affect normal physiological processes and therefore requires cautious clinical implementation and preferably a well-described mechanism of action. We have recently reported that thalidomide, a drug with anti-angiogenic and anti-inflammatory properties, reduced both severity and frequency of nosebleeds in the majority of a small group of HHT patients as a result of enhanced vessel stabilization providing an new and exciting therapeutic strategy for the treatment of vascular malformations in HHT. We will describe the mechanisms underlying vessel anomalies in HHT and how thalidomide stimulates vessel maturation to prevent bleeding. Because thalidomide is not without risk since it has poor specificity affecting a range of physiological processes and has side effects, we aim to develop molecules with the same biological responses in HHT but with fewer side effects than thalidomide.

(C03) ATERO: TRANEXAMIC ACID FOR THE TREATMENT OF EPISTAXIS IN HEREDITARY HAEMORRHAGIC TELANGIECTASIA PATIENTS

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Background. HHT is characterized by recurrent epistaxis, cutaneous telangiectasia and visceral arteriovenous malformations that affect many organs. The objective of our study was to demonstrate the efficacy and explore the safety of tranexamic acid (TA) for the treatment of epistaxis in HHT. **Methods.** In this randomized, placebo-controlled, cross-over trial, HHT patients suffering from recurrent epistaxis were randomized to receive either TA then placebo or the alternative sequence, each period lasting over three months. The primary efficacy outcome was the mean duration of epistaxis per month. The monthly number of epistaxis, the haemoglobin level and the safety were secondary endpoints. Thrombophlebitis were systematically searched by Doppler ultrasound at each visit. The primary endpoint was analysed on a log scale using a hierarchical linear regression model. **Results.** A total of 118 patients (mean age 51.7 years) were evaluated. The mean duration of epistaxis per month was significantly lower under TA (median=105 minutes) than in the placebo group (median=121 minutes), ($p < 0.05$). A mean of 26 episodes of epistaxis per month was observed under TA versus 27 under placebo ($p < 0.05$). The haemoglobin level did not change throughout all the study, in each group; 10 transfusions were required under placebo versus 6 under TA. We did not record any thrombophlebitis. No particular treatment-related complications were observed. **Conclusions.** ATERO demonstrated a statistically significant 19% reduction in the duration of epistaxis per month under TA versus placebo. The cumulative exposure to 354 months of treatment did not raise serious concern in the safety of use of TA.

(A24) RALOXIFENE HYDROCHLORIDE AS THE FIRST ORPHAN DRUG FOR HHT

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Raloxifene has been recently designated by EMA and FDA, as the first orphan drug for the treatment of HHT bleeding. This designation was obtained by the Spanish HHT unit in 2010. Epistaxis increases with age and decreases HHT patients' quality of life. Pharmacological treatments assayed for HHT bleeding belong to one of these groups: 1. Antifibrinolytic agents: é-aminocaproic acid, tranexamic acid. 2. Hormonal therapy: estrogens and SERMs. 3. Anti-angiogenic drugs: thalidomide, bevacizumab. 4. Immunosuppressors: sirolimus, tacrolimus. These drugs were only used in case reports. 5. Anti-inflammatory/antioxidant compounds: N-acetylcysteine (de Gussem et al., 2009). A clinical survey was carried on in a series of 19 HHT women, daily treated with 60 mg of

raloxifene. Prescription was advised according to the criteria of suffering moderate or severe epistaxis in postmenopausal ages, and with osteoporosis. After 18 months of treatment, women reported a decrease in the frequency and amount of nose bleedings. In parallel, the studies carried out in the laboratory of Madrid (CSIC), showed that raloxifene increases expression of Endoglin and ALK1, thus counteracting the haploinsufficiency of these HHT genes (Albiñana et al., 2010). The explanation for the increase in Endoglin and ALK1 is that for the first time we have shown that the estrogen receptor binds to Endoglin promoter (promoter of ALK1 is also very likely bound), stimulating the transcription of both genes, Endoglin and ALK1. The conclusion is that raloxifene may be useful in the therapy of epistaxis and a clinical trial will be carried out to develop the orphan designation.

(B14) EFFICACY OF LOW-DOSE BEVACIZUMAB IN HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Bevacizumab has been effective in anecdotal cases of HHT. An increased VEGF serum level in HHT patients and its influence on the severity of epistaxis episodes has already been demonstrated. We initiated an independent prospective pilot study with a three-arm experimental design to evaluate different dosages of bevacizumab: 6 patients (1 mg/kg), 6 patients (2.5 mg/kg), and 6 patients (5 mg/kg), with one i.v. injection every 3-weeks for 3 months. All presented severe bleeding episodes, epistaxis and/or GI hemorrhaging provoking hemoglobin values ≤ 10.0 gr/dL. The patient database included: frequency and severity of epistaxis, gastrointestinal bleeding, hemoglobin, hematocrit, serum iron, VEGF and data from echocardiography, rhinoscopy, videocapsule endoscopy and total body CT. Patients were monitored with clinical-chemistry tests for 5 months pretreatment, every month during treatment and for 1 year after suspension. The first-arm 3-month follow-up (lowest dosage) resulted in an epistaxis reduction returning to pretreatment levels after 3-month suspension. Transfusions dramatically decreased and the mean cell hemoglobin increased at end of treatment. Moreover, a distinct regression of facial telangiectases was noted in 3/6 patients with this sign. Quality of life improved during therapy and all patients resumed their normal activities. No adverse side effects were observed, possibly due to low dosage. The second study arm is now in progress. These preliminary results establish both the efficacy of bevacizumab in HHT and its effective use at the lowest dosage. The return to pretreatment conditions after drug suspension indicates that chronic treatment might be necessary, hence the importance of drug dosage.

(C16) BEVACIZUMAB WITH 3-WEEKS INTERVALS IS EFFECTIVE ON ANEMIA IN A PATIENT WITH SEVERE HEREDITARY HEMORRHAGIC TELANGIECTASIA

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The medical treatment of hereditary hemorrhagic telangiectasia (HHT) is still experimental. Only tamoxifen has shown a significant effect on epistaxis in a double-blind, placebo-controlled study. Cases, which were treated with anti-VEGF (bevacizumab), showed a remarkable effect on hepatic AV-malformations and on anemia. Side effects of bevacizumab as a long-term treatment for HHT are not known. We present a patient with severe anemia due to HHT. An endoglin splice mutation c.817-1G>T in exon 6 was found. Anemia was due to intestinal bleedings and epistaxis. The hemoglobin levels were around 7 g/L and between 4 and 6 units packed red cells were given weekly since 2003. The patient was treated with three complete courses of bevacizumab 5 mg/kg i.v. The first course was given with two-week intervals in autumn 2009; the second and third courses were given with three-week intervals in 2010. A forth course was started in 2011. Before treatment with bevacizumab, thalidomide was given for a 21-months period. Thalidomide showed some effect, but had to be stopped because of peripheral neuropathy. The effect of bevacizumab was impressive and the hemoglobin level rose up to 13 g/l at the end of the first course and transfusions with packed red cells were not necessary. Also, when given with three-week intervals during the second and third course, bevacizumab had a substantial effect on anemia. Side effects were not observed. The effect of bevacizumab on anemia was remarkable. Over a period of 16 months, side effects of bevacizumab did not occur in this case.

(A05) ANTI-ESTROGEN THERAPY FOR HEREDITARY HEMORRHAGIC TELANGIECTASIA – A LONG TERM CLINICAL TRIAL

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Background. Hereditary hemorrhagic telangiectasia (HHT) is associated with recurrent epistaxis in 90% of cases. Good response to hormone treatment has been documented, although its use remains controversial. The aim of this study was to examine the efficacy of an anti-estrogenic agent, tamoxifen, in the treatment of HHT-asso-

ciated epistaxis. *Methods.* From February 2005 to February 2009, 46 patients at our center started treatment with tamoxifen 20 mg/day for severe epistaxis due to HHT. All met at least 3 of the 4 established criteria for diagnosis. 38 patients (20 men, 18 women; mean age, 52.0 years) completed 3-50 months (mean 23.4±16 months) of treatment. Patients were examined every 3 months in the first year of treatment and every 6 months thereafter. At onset of treatment and at every follow-up visit, blood was collected to determine blood count, clotting time, and kidney and coagulation function. In addition all patients filled out a self-assessment questionnaire of rhinologic quality of life and epistaxis grading scale. *Results.* Bleeding score improved from 5.7±1.8 to 3.8±1.6 ($p=0.0001$). Quality of life score improved from 78.8±30.9 to 63.5±27.6 ($p=0.0001$). Hemoglobin concentration improved from 10.6±2.9 to 11.9±2.5 gr/dL ($p=0.0001$). None of the patients needed blood transfusions during the treatment period. Only one patient had minor side effects of the drug. *Conclusions.* The present long-term clinical trial indicates that tamoxifen 20 mg/d is effective for the treatment of epistaxis due to HHT, with almost no adverse effects.

(A04) EX VIVO STUDY OF BEVACIZUMAB TRANSPORT THROUGH PORCINE NASAL MUCOSA

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Introduction. Hereditary hemorrhagic telangiectasia (HHT) is responsible for disabling epistaxis, for which bevacizumab is reported as new therapeutic option. To limit the systemic side effects of bevacizumab, local administration seems most suitable. The aim of this study is to characterize bevacizumab transport into and through porcine nasal mucosa ex vivo. *Materials and Methods.* Cavity mucosa samples were mounted on static vertical diffusion cells with a donor compartment in contact with the anterior surface of the mucosa and a receptor compartment in contact with the posterior surface of the mucosa. Bevacizumab solution (25 mg/ml, 500 µg) was applied on the anterior surface of the nasal mucosa for 2.5 h. Bevacizumab concentrations were measured by enzyme-linked immunosorbent assays. Mucosa integrity was estimated by biophysical measurements (transmucosal water loss and mucosal relief parameters) and histological examination. *Results.* Transmucosal transport of bevacizumab followed a Fickian diffusion process (permeability coefficient: $[63\pm22] \times 10^{-8}$ cm/s; and steady-state flux: 56.4 ± 19.6 µg/cm²/h). Total recovery of bevacizumab throughout 2.5 hour experiment was 83% of the initial dose distributed as following : 95 ± 14 µg (~19%) in the mucosa, 56 ± 26 µg (~11%) in the receptor compartment, and 263 ± 73 µg (~53%) on the surface of the mucosa. Biophysical measurements and histological results before and after topical treatment were not significantly different, confirming the harmlessness of nasal bevacizumab delivery. *Conclusion.* This characterisation study of bevacizumab transport allows us to consider its future use by local administration in humans.

Session V Brain Arteriovenous Malformations

LUMPERS VERSUS SPLITTERS: IS HEREDITARY HEMORRHAGIC TELANGIECTASIA A BIOLOGICAL MODEL OF SPORADIC BRAIN ARTERIOVENOUS MALFORMATION?

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Brain arteriovenous malformations (bAVM) are a relatively rare but important cause of hemorrhagic stroke, especially in children and young adults. Progress in the development of novel therapeutic approaches has been challenged by poor understanding of their etiology and pathogenesis [1]. There is a widely-held belief that the disease is caused by some kind of developmental error in utero that remains silent for a variable period of time until clinical presentation later in life. Although there are surprisingly few data to support this hypothesis, such a view provides further inertia to devising innovative “post-natal” medical therapies. When a sporadic disease has a phenotypic similarity to even a very rare familial disorder, identification of a gene or pathway that is central to the disease pathogenesis can be a powerful point of departure for mechanistic study. Over 95% of bAVMs are sporadic, the most notable exception being the association of bAVM with HHT. Interestingly, bAVM associated with HHT has not generally been viewed as a familial instance of the sporadic disease. The relatively few reports on the subject have tended to stress the differences between familial and sporadic syndromes rather commonalities [2]. Improving synergy between investigations of sporadic bAVM and HHT has substantial practical consequences. First, establishing the similarities between sporadic and familial syndromes has immediate clinical impact in terms of extrapolating clinical management data from the more prevalent sporadic cases to the rarer instances of HHT-related bAVM. Second, if we assume that there is a common pathway or pathways for both syndromes, study of mechanisms should be more productive proceeding from a known genetic locus. A conceptually similar example relevant to bAVM may be cerebral cavernous malformations, where considerable progress has been made in understanding the disease from insights into the familial syndromes. Coordinated research on both familial and sporadic syndromes is likely to provide maximal utility, both for clinical [3] and basic science [4] investigations. A promising concept for further study is that bAVM represents a “response-to-injury,” requiring both an environmental (i.e., pro-angiogenic) stimulus and a genetic pre-disposition. HHT represents the most severe instance, in that bAVM is roughly 100-1000-fold more likely in HHT patients than in the general population, based on inferences from published series.

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(B08) CORRELATIONS BETWEEN GENOTYPES AND CLINICAL MANIFESTATIONS IN BRAIN AVMS WITH HHT

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Background and Objective. Five-20% of HHT patients harbor brain AVMs (BAVMs), which can cause intracranial hemorrhage (ICH), disability and death. Genotype-phenotype studies have demonstrated an association between endoglin (ENG) mutation and presence of BAVMs. However, there have been no studies correlating genotypes with clinical manifestations of BAVMs amongst HHT patients. Our objective was to study associations between HHT genotype and BAVM phenotype. **Methods.** 100, 50 and 11 HHT patients with BAVM were registered from Yale HHT Center, Toronto HHT Center and NIH Brain Vascular Malformation Consortium (BVMC), respectively. Age at diagnosis, history of ICH, age at ICH, number of BAVMs and sex, were collected from records and compared amongst genotypes. **Results.** Of 161 patients (M:F=39%:61%), 101(62.7%) patients had had genetic testing. Demographic information is shown in the Table.

	ENG	ACVRL1	SMAD4	No mutation found	Not tested	Total
#(%) of pts	73 (72.3%)	15(14.9%)	2 (2.0%)	11 (10.9%)	60	161
Age of Dx (mean yrs±SD)	27.7±16.6	24.3±17.5	11.0±7.1	22.0±17.0		
Hx of ICH	16 (24.2%)	4 (33.3%)	0 (0%)	7 (63.6%)	13 (24.5%)	40 (27.8%)
Age at ICH (mean yrs±SD, range)	25.8±15.6 2-50.4	18.0±21.4 0-48	N/A	15.7±10.6 1-29	30.7±18.9 1-58.2	24.9±17.0 0-58.2
Multiple BAVMs	20 (27.8%)	3 (20.0%)	0 (0%)	2 (18.2%)	12 (21.0%)	37 (23.6%)
# of BAVM per patient (mean±SD)	1.44 ± 0.80	1.27 ± 0.59	1.00±0.00	1.45±1.21	1.72±1.90	1.52±1.32

There was no significant difference in age at diagnosis, frequency of ICH, age at ICH, multiplicity of BAVMs or gender amongst genotypes. *Conclusion:* In this first large series of BAVMs in HHT, we have demonstrated BAVMs presence in all genotypes and that ICH can occur with all genotypes even in childhood. Screening and treatment for BAVMs should be considered in all HHT patients.

(D22) NEUROLOGIC SEQUELAE OF HEREDITARY HEMORRHAGIC TELANGECTASIA; THE GEORGIA HEALTH SCIENCES UNIVERSITY EXPERIENCE

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Hereditary hemorrhagic telangiectasia (HHT), or Osler-Weber-Rendu Disease, is a hereditary disorder characterized by mucocutaneous telangiectasias, frequent nosebleeds, and visceral arteriovenous malformations. The association between HHT and cerebral vascular malformations has been described. The prevalence of CVM in patients with HHT has been estimated at 12-23% in this patient population. Few reports have outlined the prevalence of the various cerebral vascular malformations found in HHT patients. A retrospective review of prospectively collected data was carried out using a database of 368 patients seen and examined at the Medical College of Georgia HHT Center. Of 280 patients with definite or probable HHT, 275 were screened with MRI examinations of the brain and 5 by CT Angiogram. Forty-seven CVMs were identified in 41 patients. Nineteen cerebral arteriovenous malformations, seven intracranial aneurysms, eight telangiectasias, three cavernous malformations, nine developmental venous anomalies, and one arteriovenous fistula were identified. Six patients had multiple lesions. Of the 230 patients with definite HHT in our series, 17.8% (41) had a brain vascular malformation. Neurologic presentations included headache in 121 cases, seizure in 19 cases, intracranial hemorrhage in five cases, and cerebral abscess in five cases. These findings highlight the importance of screening for brain vascular malformations in this patient population and serve as a reminder of the varied neurologic sequelae of HHT. We are currently compiling data from an age-matched control group that received MRI scans at our institution for comparison to the HHT group.

(C25) BRAIN ARTERIOVENOUS MALFORMATION MULTIPLICITY IS STRONGLY ASSOCIATED WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA: QUANTITATIVE ASSESSMENT.

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Background: The prevalence of brain arteriovenous malformations (BAVMs) is known to be elevated in patients

with hereditary hemorrhagic telangiectasia (HHT), estimated at 5-15%. The prevalence of multiple BAVMs is greater in patients with HHT than in those with sporadic BAVMs, however the magnitude of this difference remains unclear. *Purpose:* The purpose of this study is to quantitatively evaluate the relationship between multiplicity of BAVMs and the diagnosis of HHT. *Methods:* Retrospective review of clinical and imaging records in all patients with BAVMs referred to two large North American tertiary referral centers over the past 25 years, including demographics, clinical data and angiographic characteristics. Statistical comparisons of BAVM multiplicity in patients with HHT and those with sporadic BAVMs were performed. *Results:* A total of 1999 patients were included. Out of all BAVM patients, 2.8% had diagnosed HHT and 1.9% had multiple BAVMs. Out of patients with multiple BAVMs, 59% had HHT. Of all BAVM patients with HHT, 39% had multiple BAVMs, while in BAVM patients without HHT, 0.7% had multiple BAVMs. Multiplicity of BAVMs was highly associated with the diagnosis of HHT in univariate ($p < 0.0001$) and multivariable ($p < 0.001$) models, with an OR of 86 (95% CI: 38 -196). The positive likelihood ratio of bAVM multiplicity was 51 (95% CI: 28-92). *Conclusion:* Multiplicity of BAVMs is highly associated with HHT. The presence of BAVM multiplicity should alert the clinician to the possibility of HHT and lead to comprehensive investigation for this diagnosis.

(B01) A NOVEL MOUSE AVM MODEL WITH PARTIAL LETHALITY

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A novel mouse model of arteriovenous malformation (AVM) was developed by inter-crossing activin receptor-like kinase 1 (Alk1; Acvrl1) conditional knockout mice, Alk1 knockout mice, and tissue specific Cre-deleter mice. A majority of Alk1 mutant mice were paralyzed or died by 10-15w of age due to hemorrhages in intracranial, spinal, and/or abdominal cavities. Intriguingly, the mice surviving this early lethal period (LP) did so in spite of carrying multiple AVMs and experiencing minor bleedings in their neurological tissues. A majority of these surviving mice lived for more than 1 year of age. In order to quantify the severity of AVMs, a novel mouse AVM grading system for the neurological tissues was developed. The grading system revealed that almost all Alk1 mutant mice in stable period ($\geq 16w$ of age) had high AVM scores. Furthermore, preliminary longitudinal magnetic resonance (MR) angiography observation revealed that gross morphologies, relative sizes, and numbers of cerebral AVMs were largely unchanged after 3w of age, indicating that most AVMs ceased growth by 3w of age. This novel AVM mouse model will be an invaluable tool for elucidating AVM and HHT pathogenesis as well as for developing AVM drug treatments.

(Z01) CEREBRAL ARTERIOVENOUS FISTULA IN CHILDREN: GENETIC AND ANGIOGRAPHIC ANALYSIS OF 26 CASES (HHT VERSUS RASA1)

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Background and purpose. Curaçao criteria may not be all present in the pediatric population, and genetic analysis is recommended in case of central nervous system (CNS) high flow arteriovenous fistula (single or multiple). **Material and methods.** We selected 26 cases of high flow cerebral arteriovenous fistula (CAVF) presenting at the pediatric age among patients referred for endovascular treatment of CNS arteriovenous shunt in Bicêtre. We excluded single nidus or micro arteriovenous malformation (AVM), and spinal arteriovenous shunt. Angiograms were analysed, and specific attention was paid to the possible multiplicity of AV shunts. Genetic variation of HHT1 (Endoglin), HHT2 (ACVRL1) and RASA genes was assayed by PCR and sequencing of the entire coding sequence and intronic junctions of the 3 genes. Multiplex ligation-dependent probe amplification (MLPA) was used to screen for large rearrangements of ACVRL1 and Endoglin genes. **Results.** Mutations in HHT1, HHT2 and RASA1 genes were found both in single and multiple CAVF. The rate of single versus multiple CAVF seems predominantly associated to RASA1 mutations than HHT1 or HHT2 mutations. The rate of positive genetic analysis in this cohort of pediatric arteriovenous high-flow fistula is 61.5%. **Conclusions.** In contrast to HHT1 and HHT2 mutations, RASA1 mutations seem to predominate in single CAVF. This study emphasizes the importance of the RASA1 gene in pediatric CAVF.

Session V Screening and Imaging in HHT

FROM MECHANISMS OF DISEASE TO THERAPEUTIC ASSAYS: NEW APPROACHES TO TREATMENT OF BLEEDING IN HHT PATIENTS

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Development of vascular malformations in HHT patients has been attributed to deregulation of key angiogenic factors leading to abnormal endothelial cell proliferation and migration, extracellular matrix production and degradation, as a result of which endothelium and surrounding mural cells disengage. This causes vessels to weaken and become prone to bleeding, a major feature of HHT. Inhibitors of angiogenesis could therefore be beneficial in treating symptoms of these vascular anomalies in HHT patients. However, anti-angiogenic drugs may also affect normal physiological processes and therefore requires cautious clinical implementation and preferably a well-described mechanism of action. We have recently reported that thalidomide, a drug with anti-angiogenic and anti-inflammatory properties, reduced both severity and frequency of nosebleeds in the majority of a small group of HHT patients as a result of enhanced vessel stabilization providing an new and exciting therapeutic strategy for the treatment of vascular malformations in HHT. We will describe the mechanisms underlying vessel anomalies in HHT and how thalidomide stimulates vessel maturation to prevent bleeding. Because thalidomide is not without risk since it has poor specificity affecting a range of physiological processes and has side effects, we aim to develop molecules with the same biological responses in HHT but with fewer side effects than thalidomide.

(B02) SENSITIVITY OF LOW DOSE CT THORAX FOR PAVM DETECTION IN HHT

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Purpose. The purpose of this study is to evaluate the sensitivity of low dose computed tomography (CT) for detection of pulmonary arteriovenous malformations (PAVMs) in hereditary hemorrhagic telangiectasia (HHT) patients compared to standard dose CT. **Materials and methods.** All patients who had both an initial standard dose CT thorax for PAVM detection as well as a follow-up low dose CT since January 2008 were identified. These scans were reviewed to assess whether all PAVMs initially detected on the standard dose scan were detected on the subsequent low dose CT. **Results.** In total, 271 patients were scanned in this 27 month period. One hundred and six patients had a low dose CT. Of these, 64 patients had a low dose scan following a standard dose CT at our institution. One hundred and seven PAVMs were detected in 23/64 (35.9%) patients on standard dose CT, with feeding artery diameter as small as 1 mm. In addition to the embolized PAVMs, 86/89 (Sensitivity = 96.6%; 95% CI, 90.4-98.9%) of untreated PAVMs were detected on follow-up low dose CT. **Conclusions:** Our results indicate that the sensitivity of low dose CT for detection of PAVMs in HHT patients is comparable to that of standard dose non-contrast CT. **Clinical relevance/Application.** Since the completion of this study, all patients at our HHT center now receive an initial low dose CT scan due to its excellent sensitivity for detection of PAVMs.

(A29) WHOLE-BODY MAGNETIC RESONANCE IMAGING AS A SCREENING PROCEDURE FOR PATIENTS WITH HHT

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Purpose. To evaluate magnetic resonance imaging (MRI) as a screening procedure for the detection of cerebral (CAVM), pulmonary (PAVM) and hepatic arterio-venous malformations (HAVM) in patients suffering from HHT. **Methods and Materials.** Overall, 264 patients (mean age 46.3; male 109; female 155) with confirmed HHT according to Curacao criteria or their first degree relatives underwent screening MRI including non-contrast magnetic resonance tomography and contrast-enhanced magnetic resonance angiography (CE-MRA). AVM presence was scored as "0" (=none present), "1" (=definitely present) or "2" (=uncertain) and was evaluated by patient gender, age and size. **Results.** MRI revealed 6 of 218 patients (2.8%, mean age 43.8; male 3, female 3) with definite CAVM (size: 8-20 mm). 5 of 6 patients with a definitive CAVM showed coincidence of PAVM. Pulmonary CE-MRA detected in 87 of 259 patients (33.6%) 265 PAVM (120 in 39 men, 145 in 48 women, 87 in 30 women of childbearing age ≤ 50 years). One PAVM was detected in 38 patients, two PAVMs in 16 patients, three in 8 patients and ≥ 4 in 25 patients. Most PAVMs detected on CE-MRA were small (< 10 mm). 67 of 87 patients with 218 evaluable PAVMs detected on CE-MRA underwent global or selective DSA. Significantly ($p < 0.001$) fewer PAVMs (163/218 [74.8%]) were demon-

strated on DSA of which 152 were embolized. HAVM were detected in 42 of 228 patients (18.4%; mean age 55.3 years; male 8; female 34). 12 of 42 patients were associated with PAVM. **Conclusion:** MRI and CE-MRA is a suitable screening procedure for patients with HHT, permitting accurate detection and staging of AVMs.

(C09) SMOKING - A NEW RISK FACTOR FOR CEREBRAL ABSCESS IN HEREDITARY HAEMORRHAGIC TELANGIECTASIA?

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Background. Cerebral (brain) abscesses are one of the most feared complications of pulmonary arteriovenous malformations (PAVMs). Our previous studies have demonstrated associations with male gender, venous thromboemboli, and dental microorganisms [1]. **Methods.** A further series of individuals with CT-proven PAVMs and hereditary haemorrhagic telangiectasia (HHT) was recruited prospectively between 2007-2010. Associations between cerebral abscess (defined by requirement for neurosurgical drainage and long term antibiotics), and patient-specific variables were assessed in univariate and stepwise multiple regression analyses (STATA XP). **Results.** The series comprised 165 consecutive PAVM/HHT patients who had not been part of any previous study. Six (3.70%) had developed a cerebral abscess (3 men; 3 women). In this series, the only variable that was significantly associated with cerebral abscess was a current or previous history of smoking (5/6 abscess patients [83%], compared to 53 [32.3%] non-abscess patients. The odds ratio for cerebral abscess in smokers was 10.5 [95% confidence intervals 1.20, 92.3], $p = 0.034$). Pooling with our earlier series to enhance power was not statistically valid due a positive series indicator variable, meaning that the series differed substantially in characteristics, most obviously overall smoking rates (47% compared to 35%). **Conclusions.** While the numbers involved are small, the findings if replicated, would not be surprising since smoking avoidance is known to minimise gingival inflammation [2]. This provides a further incentive for smoking cessation advice for individuals with HHT.

1. Shovlin et al, Thorax 2008; Shovlin et al Thromb Haem 2007
2. Sakki et al. Community Dent Oral Epidemiol. 1995;23:155-8

(D26) ACTIVIN RECEPTOR-LIKE KINASE-1 AND ENDOGLIN ARE NOT RELATED WITH PULMONARY HYPERTENSION IN HEREDITARY HAEMORRHAGIC TELANGIECTASIA

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Purpose. According to guidelines activin receptor-like kinase-1 (Alk-1) and endoglin mutations seem to be related to pulmonary hypertension (PH). However, the data supporting this relation are limited. Therefore we evaluated the presence of PH in patients with these mutations in hereditary hemorrhagic telangiectasia (HHT). **Methods:** All consecutive persons screened for HHT between May 2004 and December 2010 underwent transthoracic echocardiography (TTCE). They were included if an appropriate tricuspid insufficiency signal was present to measure the right-ventricular systolic pressure (RVSP) and if a definite mutation analysis was available. PH was suspected if the RVSP was ≥ 30 mmHg. **Results:** In 411 of the 662 screened persons (62%) the RVSP could be reliably measured, and in 298 (73%) a definite mutation analysis was available. An Alk-1 mutation was found in 91 patients (52% women, mean age 47 ± 13 years), an endoglin mutation in 99 patients (60% women, mean age 42 ± 15 years), and no mutation was found in 108 controls (70% women, mean age 42 ± 16 years). The mean RVSP was 21 ± 8 mmHg, 23 ± 10 mmHg, and 21 ± 8 mmHg in these groups, respectively ($p > 0.05$). A RVSP ≥ 30 mmHg was found in 35 patients, of whom 17 (49%) Alk-1 mutation, 10 (29%) endoglin mutation carriers, and 8 (23%) controls ($p = 0.35$). HHT was not a predictor for an increased RVSP. **Conclusion.** In contrast to previous small studies, the presence of an Alk-1 or endoglin mutation does not appear to be a predictor for PH in HHT.

(B15) FORMATION OF RENAL CYSTS IN PATIENTS WITH HEREDITARY HAEMORRHAGIC TELANGIECTASIA

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There is experimental evidence that VEGF released by renal tubules is involved in cyst formation. Since, VEGF plays a role in the aberrant angiogenesis in HHT we have screened 275 HHT patients admitted to our outpatients Centre for detection of cysts. As shown in Table 1 we have detected, via echography, 13 renal cysts, 5 renal and hepatic cysts, 1 renal and ovarian cyst and 6 hepatic cysts. No major alterations of renal functions were found. Only in this group of 25 cystic patients out of 275 we have observed 3 cases of paraproteinemia (IgM K, IgG-lambda and IgA K). One can speculate that VEGF released by endothelial cells and/or mesenchymal cells may account for cystic formation. Finally, VEGF released by renal endothelial cells may lead to expansion of B cell clones, thus generating monoclonal gammopathies.

Table 1: Characteristics of cysts.

Renal cysts: 8 cortical left kidney; 1 cortical right kidney; 4 bilateral.
 Renal and hepatic cysts: Kidney (4 cortical left, 1 bilateral); Liver (5 left lobe).
 Hepatic cysts: 4 left hepatic lobe; 2 right lobe.
 Renal and ovarian cysts: Kidney (1 cortical left); ovary (bilateral).

(C08) DIET AND HEREDITARY HAEMORRHAGIC TELANGIECTASIA

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Introduction: Iron deficiency and iron deficiency anaemia, are frequently present in individuals with hereditary haemorrhagic telangiectasia (HHT), provoked by recurrent nosebleeds and gastrointestinal bleeding¹. Ferrous sulphate tablets are recommended as the first line treatment for iron-deficiency anaemia in the UK², and it is recognised that aggressive iron therapies may be required in HHT³. **Methods:** Since requirements for iron supplementation should reflect dietary intake, in addition to iron losses (through bleeding), we performed relevant literature searches using PubMed, and reviewed the information available to patients. **Results:** PubMed searches in January 2011 using the search terms "diet" and "HHT" identified 8 hits, none of which were relevant. More detailed searches also revealed no studies into dietary iron and iron status in HHT. The recently published HHT Guidelines³ do not refer to dietary issues such as foods that modify iron absorption. The website of the HHT Foundation International provides a useful link to the National Anaemia Action Council website, but this does not emphasise the need to enhance iron intake when nosebleeds or other specific bleeds are more frequent. **Conclusions:** The potential for dietary manoeuvres to reduce the severity of iron deficiency for individuals with HHT and significant blood loss appears to be poorly appreciated. We have therefore designed a prospective study for the Spring of 2011, the findings of which will be presented.

1. Shovlin. Blood Reviews 2010; 24:203-19
2. http://eng.mapofmedicine.com/evidence/map/iron_deficiency_anaemia1.html.
3. Faughnan et al, J Med Genet 2011;48:73-87.

Session VIIa: Pulmonary Arteriovenous Malformations

(D27) DIAGNOSTIC ACCURACY OF TRANSTHORACIC CONTRAST ECHOCARDIOGRAPHY AS A SCREENING METHOD FOR PULMONARY ARTERIOVENOUS MALFORMATIONS

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Background: Patients with hereditary hemorrhagic telangiectasias (HHT) are routinely screened for pulmonary arteriovenous complications (PAVMs) because of the associated neurological complications. We compared the detection of intrapulmonary shunting on transthoracic contrast echocardiography (TTCE) with the presence of PAVMs on high-resolution chest computed tomography (HRCT) in a large series of screened persons. **Methods:** In the period from May 2004 till December 2010, 626 persons with possible HHT were screened with both a TTCE and chest HRCT. TTCE was judged positive or negative for a pulmonary shunt, or indeterminate when intracardiac and intrapulmonary shunting could not be discriminated. Chest HRCT was interpreted as positive, negative or indeterminate for the presence of PAVMs. **Results:** TTCE was positive for a pulmonary shunt in 252 patients (40.3%), negative in 369 patients, and in 5 patients the origin of the shunt could not be discerned. Chest HRCT showed PAVMs in 122 patients (19.5%), and in 18 patients the presence of a PAVM was uncertain. The negative predictive value of TTCE for the absence of PAVMs on chest HRCT was 99.4%, and sensitivity was 98.3%. In two patients with PAVMs on chest HRCT but no shunt on TTCE, PAVMs were very small and far beyond possibility for treatment. The positive predictive value of TTCE for the presence of PAVMs was 48.4%, and specificity 73.9%. **Conclusion:** In this large series directly comparing TTCE with chest HRCT, TTCE appears to have an excellent negative predictive value. When used as a first-line screening tool, no PAVMs amenable for embolisation were missed. Intrapulmonary shunting on TTCE is frequently present in patients without macroscopic PAVMs on chest HRCT.

(B21) THROMBOSIS OF PULMONARY ARTERIOVENOUS MALFORMATIONS: CLINICAL PRESENTATION AND TREATMENT

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Purpose. To describe clinical presentation, imaging findings and therapeutic options in patients with thrombosis of pulmonary arteriovenous malformations (PAVMs).

Materials and methods. All consecutive patients with thrombosis of PAVMs during a 5-year period were recorded from our database. The following criteria were analyzed: age at presentation, clinical presentation, imaging findings using enhanced multidetector computed tomography (MDCT). The technique of embolization and its results were also analyzed. **Results.** From a database of 157 patients with PAVMs, 7 (4 women and 3 males) were identified to have a thrombosis within a PAVM, one immediately after embolization and 6 spontaneous before embolization. Five of them were adults (mean age 46 years) and two were children (17 years). Six patients were already diagnosed with HHT. Four patients presented neurological complications, stroke in three and cerebral abscess in one. Two patients presented with diffuse PAVMs, 4 with multiple PAVMs, and one with a single large PAVM. Calcifications were present in two cases. In one case, thrombosis was detected within the feeding artery of the PAVM. Two patients had a pulmonary hypertension, and two presented a polycythemia. Treatments were embolization in six patients and surgery in one patient. All but one patient made a full recovery. **Conclusion.** Spontaneous thrombosis within PAVMs can lead to severe neurological complications. Early diagnosis with enhanced MDCT facilitates the therapeutic options and the technique of embolization. Thrombosis can also occur at the time of embolization, presumably related to pulmonary venous hypertension.

(C07) WHICH PATIENTS WITH PULMONARY ARTERIOVENOUS MALFORMATIONS ARE DYSPNOEIC? RETROSPECTIVE ANALYSIS OF A SINGLE-CENTRE 2005-2010 COHORT

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Background. Pulmonary arteriovenous malformations (PAVMs) affect ~50% of HHT patients, often causing hypoxaemia (low blood oxygen levels). Most PAVM patients however, do not present with breathlessness. **Methods.** To identify factors contributing to breathlessness in the PAVM population, a retrospective analysis was performed in 165 new patients presenting with CT-proven PAVMs between 06/05 and 07/10. Based on self-reported exercise tolerance at presentation, and blinded to physiological measurements, two investigators assigned patients to the Medical Research Council dyspnoea scale, with individuals undertaking regular/intense sport reclassified as Grade "0". Four separate blood oxygen saturation (SpO2) measurements after standing for 7, 8, 9 and 10 minutes were used for statistical calculations using GraphPad software. **Results.** 44 (26.7%) of the population were dyspnoeic (Figure 1A), reducing to 24% when 5 patients with severe co-existent respiratory pathologies were excluded (sole Grade 4 patient; 4/7 Grade 3 patients). In univariate analyses, SpO2, which varied widely, displayed no clear relationship with dyspnoea

grade (Figure 1B). Patient ages ranged from 17-87 years, and older patients were more dyspnoeic (Figure 1C). To assess whether differing ages had masked an SpO₂-dyspnoea relationship, multiple regression analyses were performed. Excluding the patients with dual pathology, once adjusted for age, SpO₂ was inversely associated with dyspnoea grade ($p=0.0019$), although age and SpO₂ did not account for most of the population variation (overall model: $r^2 = 18.9\%$, $p < 0.0001$). **Conclusions.** These data imply that it is unusual for PAVMs alone to account for moderate to severe dyspnoea.

Figure 1: A) MRC dyspnoea grades. B) Distribution of SpO₂ by dyspnoea grade. C) Distribution of age by dyspnoea grade. All graphs indicate full cohort of 165 patients. Error bars represent mean and standard deviation.

(E01) GRADE OF PULMONARY RIGHT-TO-LEFT SHUNT USING CONTRAST TRANSTHORACIC ECHOCARDIOGRAPHY ASSOCIATED WITH NEUROLOGICAL COMPLICATIONS

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Objective. The presence of a pulmonary arteriovenous malformations in patients with hereditary hemorrhagic teleangiectasia is associated with neurological complications. Pulmonary right-to-left shunt (RLS) can be diagnosed and graded by contrast transthoracic echocardiography (TTE). We evaluate whether the grade of pulmonary RLS measured by contrast TTE is associated with neurological complications in patients screened for hereditary hemorrhagic teleangiectasia (HHT). **Methods:** All persons, screened for HHT between May 2004 and December 2010 and who underwent a contrast TTE, were included. The shunt using contrast TTE was graded as none, minimal, moderate, and large. All neurological events were diagnosed by neurologists and confirmed by appropriate imaging techniques. **Results.** In total 703 persons were screened for HHT during the study period. A sufficient contrast TTE was performed in 642 persons (mean age 43.7 ± 15.4 years, 61.4% female). A pulmonary RLS was present in 45.3% (minimal 18.4%, moderate 9.7%, and large 17.3%). Within all screened persons, 38.2% suffered headaches, 21.4% overall migraine, and 11.5% migraine with aura (MA). The life-time prevalence of CVA was 2.5%, and TIA 1.4%. Only in patients with a large pulmonary RLS on contrast TTE the prevalence of MA and CVA were increased compared to those without a shunt (28.7% versus 8.6% for MA, and 10.1% versus 1.2% for CVA, $p < 0.001$ for both). There was no difference in the prevalence of headaches, migraine without aura, and TIA. **Conclusion.** The prevalence of a CVA and migraine with aura is increased only in the presence of a large shunt on contrast transthoracic echocardiography.

(C11) LONG TERM RESULTS OF TYPES 1 AND 2 AMPLATZER VASCULAR PLUGS IN TREATING PULMONARY ARTERIOVENOUS MALFORMATIONS

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Purpose. PAVMs treated by embolotherapy may recur as a result of recanalisation or the development of new feeding arteries. The Amplatzer Vascular Plugs (AVP), both types 1 and 2, are recoverable, screw detachable nitinol embolic devices that offer lower risks of paradoxical embolisation than using regular pushable coils. This study presents our medium term results of their use in PAVMs. **Materials and Methods.** 32 patients with PAVMs (age range 19-67 years, M:F=10:22), 25 of whom were asymptomatic and detected on screening, and 7 of whom were symptomatic, were treated with AVPs between December 2004 to January 2011. Our initial experience was with type 1 devices, but since the release of the type 2 device this has been preferred as it produces more rapid occlusion. **Results.** A total of 39 PAVMs were deemed suitable for the AVP, and all were successfully occluded. 15 type 1 and 24 type 2 AVPs were deployed. There were no device or procedural complications. One type 1 AVP has recanalised and required further occlusion. All patients remain well and symptom-free with respect to their PAVMs at a mean follow-up of 56 months (range 22-82) for type 1 and a mean of 21 months (range 1-40) for type 2. **Conclusions.** Embolotherapy is the preferred treatment modality for occlusion of PAVMs, and the AVP has added a new option to our range of embolic devices. We present our long term outcome of using these devices.

(B19) PULMONARY ARTERIOVENOUS MALFORMATIONS REPERFUSION AFTER PERCUTANEOUS EMBOLIZATION: SENSITIVITY AND SPECIFICITY OF NON-ENHANCED CT ASSESSED WITH PULMONARY ANGIOGRAPHY

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Purpose. To evaluate the sensitivity and specificity of non-enhanced CT to detect PAVMs reperfusion after embolization. **Materials and Methods.** The imaging files of all patients who had pulmonary angiography follow-up after PAVM embolization in our center from 2000 to 2010 were reviewed. PAVM feeding artery, draining vein and aneurismal sac diameters were measured on non-enhanced CT before and after embolization. Follow-up pulmonary angiography was the gold standard to assess reperfusion. ROC curves were used to assess sensitivity and specificity according to the percentage of diameter reduction. **Results.** From the 67 patients who had PAVM embolization, 39 (58%) had no follow-up angiography, 9 (13%) no longer had pre or post embolization CT available and 19

(28%) patients had 52 PAVMs assessed by pulmonary angiography, performed during additional PAVMs embolizations (5 patients, 26%) or because of suspicion of PAVM reperfusion on CT (14 patients, 74%). Twenty-seven (51%) PAVMs were occluded and 25 (49%) were reperfused, 18 (72%) of these were re-embolized. Mean diameter reduction in occluded versus reperfused PAVMs were respectively $51,6 \pm 22,6\%$ versus $26,5 \pm 20,7\%$ for the vein and $60,1 \pm 19,5\%$ versus $25,9 \pm 23,8\%$ for the aneurismal sac. A 50% diameter reduction of the PAVM feeding artery, vein and aneurismal sac respectively showed a sensitivity/specificity of 76/78%, 88/62%, and 72/78% for reperfusion. *Conclusion.* There is a wide overlap in diameter reduction between occluded and reperfused PAVMs after embolization. A 50% diameter reduction cutoff for the aneurismal sac on CT would still miss 25% of reperfused PAVMs.

(D09) DOES TRANSESOPHAGEAL ECHOCARDIOGRAPHY HAVE A PLACE IN THE SCREENING CASCADE FOR PULMONARY ARTERIOVENOUS MALFORMATIONS?

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Background. Transthoracic echocardiography (TTE) is often used as a screening procedure for PAVMs, and CT scan of the thorax with contrast material can be used as a subsequent imaging technique. However, the value of discrepant findings is still unknown. *Methods.* Patients were offered transesophageal echocardiography (TEE) if their TTE results did not depict a clear distinction between a pulmonary or cardiac shunting. *Results.* Sufficient data was available for 115 patients, with 94 showing signs of central shunting with the TTE. CT scan (66) or MRI (12) performed on 73 cases revealed visible AVMs in 26 patients, a questionable result in 1 patient, and no hints of pulmonary shunting in 47 patients. Furthermore, 22 patients decided to have TEE performed. 19 showed pulmonary shunting and 3 had atrial septal defects. *Conclusion.* The significance of combined TTE-positive and imaging-negative findings is still not clear. 43 to 64

out of 115 patients of our cohort (37% - 56%) may have had so-called microscopic PAVMs. 3 out of 22 patients benefited from TEE by avoidance of long-term antibiotic prophylaxis. It has yet to be determined if this justifies the possible lethal risks of TEE.

(D28) CONTRAST TRANSTHORACIC ECHOCARDIOGRAPHY IN HEREDITARY HEMORRHAGIC TELEANGIECTASIA: A 5-YEARS FOLLOW UP STUDY

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Background. Because of the potential growth of small pulmonary arteriovenous malformations (PAVMs) to a size amenable for embolisation, follow-up of hereditary hemorrhagic teleangiectasia (HHT) patients with PAVMs on chest CT is recommended. However, little is known about the long-term follow-up of patients with intrapulmonary shunts on transthoracic contrast echocardiography (TTCE) in the absence of treatable PAVMs. *Methods.* A repeat TTCE 5 years after initial screening was performed in 27 patients with HHT who did not display treatable PAVMs on their first chest HRCT. Shunt size was divided in small, moderate and large. *Results.* In 24 patients HHT was genetically confirmed, of whom 8 with a mutation of Endoglin and 15 with a mutation of ALK-1. Three patients did not give permission for DNA mutation analysis and were clinically diagnosed with HHT. At baseline, TTCE showed no shunt in 12 patients (44.4%), and a small, moderate, and large shunt in 7 (25.9%), 3 (11.1%), and 5 (18.5%) patients, respectively. A small PAVM on chest CT scan was present in 3 patients (11.1%), of whom 2 patients with a large shunt and 1 with a moderate shunt on TTCE. After 5 years, an overall shunt on TTCE was present in 13 (48.1%) patients. There was a change in shunt size in 7 patients: in 1 patient there was an increase in shunt size from absent to small, and in 6 patients there was a decrease in shunt size (small to absent in 3 patients, moderate to small in 2 patients, and large to moderate in 1 patient). The distribution of shunt size was significantly altered after 5 years ($p < 0.001$). *Conclusion.* There was no increase in pulmonary shunt size on contrast echocardiography after five-year follow-up in HHT patients without treatable PAVMs on chest CT.

Session VIIb Molecular and Cellular Bases of HHT

BMP9 SUPPRESSES MCP1 PRODUCTION BY HUMAN PULMONARY ARTERY ENDOTHELIAL CELLS

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BMP9 is the major ligand for the ALK1/BMPR-II receptor complex in endothelial cells. BMPR-II mutations underlie pulmonary arterial hypertension (PAH) and ALK1 mutations cause hereditary hemorrhagic telangiectasia (HHT) or combined HHT+PAH. Both diseases are characterised by an unstable vasculature susceptible to remodelling events. We reported that BMP9 induces interleukin-8 (IL8) and E-selectin, factors that can positively regulate vessel stability, in human pulmonary artery endothelial cells (HPAECs). Here, we examine the regulation of monocyte chemoattractant protein-1 (MCP1), a negative regulator of vessel stability, by BMP9 in HPAECs. HPAECs were treated with M199/0.1% FBS ± BMP9 (1 ng/ml). Protein lysates were analysed for Smad phosphorylation. MCP1 expression was determined using quantitative PCR and release measured using MCP1 ELISA. In some experiments, cells were transfected with siRNAs for ALK1, BMPR-II, Smad1, Smad2, Smad4, Smad5 or Smad8 to examine their contribution to the BMP9 response. MCP-1 release by HPAECs under cyclic stretch was also assessed. BMP9 induced phosphorylation of Smad1/5 and Smad2 in HPAECs. BMP9 potentially repressed MCP1 expression at 8 and 12 hours and MCP1 release over 24 hours. ALK1 and Smad4 siRNAs abolished this effect. Smad1/5 siRNA co-transfection caused a partial reversal, whereas BMPR-II siRNA had no effect. BMP9 also inhibited cyclic stretch induced MCP-1 release by HPAECs. BMP9 represses basal and stretch-induced MCP1 release by HPAECs. The repression of MCP1 is dependent on ALK1 and Smad4, but not BMPR-II. Therefore, attenuated MCP1 repression by BMP9 may be relevant to the pathogenesis of HHT, but not PAH.

(D06) DISTRIBUTION OF TGF-BETA1 SNPS AT CODON 10 AND 25 IN 169 ITALIAN PATIENTS AFFECTED WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA

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TGFβ1 is secreted by several cell types, including peripheral blood mononuclear cells, endothelial and vascular smooth muscle cells. TGFβ1 plasma and serum concentration can be measured but the interpretation of the results is hampered as a wide range of variation in response to physiological and pathological processes is known. Although the mechanisms controlling plasmatic TGFβ1 concentration are poorly understood, a significant genetic contribution to the production of active and latent TGFβ1 in plasma has been demonstrated. In humans, TGFβ1 gene shows several polymorphisms and some of them correlate with different TGFβ1 plasma levels. Studies of the association of these polymorphisms with different disorders have been published and most of them reported a significant differences in distribution between patients and controls of the cod 10 Pro/Leu and cod 15 Pro/Arg, in the signal peptide of TGFβ1 gene. Plasma levels of TGFβ1 in HHT patients have been studied by two different groups with opposite results. In order to obtain information on the genotype-related TGFβ1 production, irrespective of phisio/pathological/environmental responses, we studied the distribution of codon 10 and 25 polymorphisms in 169 HHT patients (100 index cases), with known causing mutation in ENG or ACVRL1, and compared results to a control group. A statistically significant difference in the distribution of codon 25 polymorphisms was observed. Moreover, correspondence analysis showed a possible association between the disease and the heterozygous genotypes in TGFβ1 both at codon 10 and 25 and therefore to low/intermediate producers of the cytokine.

(B25) INCREASED OXIDATIVE STRESS LEADS TO ENDOTHELIAL DYSFUNCTION AND PULMONARY HYPERTENSION IN ENG AND ALK1 HETEROZYGOUS MICE

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We demonstrated previously that endoglin and Alk1 interact with endothelial NO synthase (eNOS) and play a role in its activation. Reduced endoglin/eNOS complex in endothelial cells deficient in endoglin leads to eNOS uncoupling and generation of reactive O₂⁻ species (ROS) rather than NO. We have now shown that lungs of adult Eng and Alk1 heterozygous mice produce less NO and more ROS than lungs of control mice. The increase in ROS was partially inhibited by L-NAME and eNOS-dependent. These mice developed signs of pulmonary

hypertension (PH) with age, showing increased vascular remodeling, pulmonary resistance and right ventricular hypertrophy, the latter occurring earlier in Alk1 than Eng heterozygous mice. Prevention of PH onset by 6 week oral treatment with the antioxidant Tempol (1mM) confirmed a role for ROS in disease initiation. We also measured NO and H₂O₂ levels in other vascularized tissues such as heart and liver to determine the extent of eNOS uncoupling in the mouse models of HHT. We found decreased NO production in both tissues and increased H₂O₂ levels in hepatic but not cardiac tissues of heterozygous mice. Mitochondria-derived ROS production in liver was higher in heterozygous than control mice. This is in agreement with recent findings that reduction in NO may inhibit mitochondrial potassium ATP channel opening, and further increase oxidative stress caused by mitochondrial ROS release. Our results strongly suggest that oxidative stress plays an important role in endothelial dysfunction, which can lead to PH and/or HHT, and that anti-oxidant treatment will benefit the patients.

(C18) ANALYSIS OF ENDOGLIN HOMO- AND HETEROMERS BY BIMOLECULAR FLUORESCENCE COMPLEMENTATION AND FURTHER FLUORESCENCE MICROSCOPY METHODS

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Most of our current knowledge regarding endoglin's ability to form homodimers or heterodimers as well as oligomers with other proteins is coming from biochemical assays by i.e. using immunoprecipitation. Although, these are valuable experimental data they do not have the charm of in vivo experiments that can be done by fluorescence microscopy. Here we present data on endoglin homodimerization and heterodimerization obtained by different fluorescence microscopy techniques. In particular, we will show the potential value of bimolecular fluorescence complementation (BiFC) assays in protein interaction studies. In order to evaluate this method, the interaction of wild type and mutant endoglin was exemplarily investigated. These studies were complemented by immunofluorescence microscopy and fluorescence microscopy with GFP-tagged endoglin proteins. The study of six different missense mutants showed that depending on the location of the mutation these mutant proteins remain either intracellular or are regular processed to the cell surface. Intracellular mutants are localized to the ER confirmed by immunofluorescence based colocalization for the ER protein calnexin. Furthermore, BiFC analyses unambiguously showed that all of the investigated missense mutations dimerize with wild-type endoglin and that depending on the position of the mutant's affected amino acid these heterodimers either remain in the ER or are transported to the cell membrane. In addition, the conformation of these wild-type/mutant heterodimers

obstructs binding of the endoglin-specific monoclonal antibody SN6. We also analyzed the possible cellular function of the mutants in cell proliferation and signalling. All these studies were done with transient and/or stable transfected endothelial and non-endothelial cells.

(D18) ENHANCED MIGRATORY PROPERTY IN ALK1-DEFICIENT ENDOTHELIAL CELLS UPON ANGIOGENIC STIMULATION

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For induction of Alk1 deletion in adult mice, we employed the ROSA26-CreER deleter strain in which Cre recombinase is ubiquitously expressed and can be activated upon tamoxifen (TM) treatment. By single TM injection, the adult mice carrying homozygous Alk1-conditional alleles died in 2-3 weeks with hemorrhages in the lungs and gastrointestinal tract. While subdermal vessels were unaffected by the Alk1-deletion, wounding or angiogenic stimulation to skin could induce AVM formation in this model. Interestingly, any pathological features or wound-induced AVMs were found in Alk1-heterozygous mice, indicating that homozygous Alk1 deletion might be required for development of such vascular lesions. We hypothesized that the ALK1 signaling in endothelial cells (ECs) controls cellular responses to angiogenic stimulations. To test this hypothesis and to compare the differences in responses to angiogenic stimulations between Alk1-heterozygous and -homozygous ECs, we have established pulmonary EC lines (pECs) from ROSA26-CreER; Alk1(1f/2f) mice in which the conditional "2f" allele can be converted to the null "1f" allele by hydroxy-TM treatment. We found that migratory and sprouting properties in response to bFGF stimulation was significantly enhanced in Alk1-deleted pECs(1f/1f). In vivo Matrigel plug assay and ex vivo aortic ring assay also support this finding. Anti-angiogenic effects of BMP-9 was blunted in Alk1-deleted pECs(1f/1f), but SMAD1 phosphorylation by BMP9 was unaffected in pECs(1f/1f). Taken together these data suggest that enhanced sensitivity of pECs(1f/1f) to angiogenic stimulations may be an important mechanism of AVM formations in Alk1-deficient mice.

(C21) ANGIOGENESIS RELATED MICRORNA IN PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Background. MicroRNAs are tissue and cell specific, single-stranded, non-coding small RNAs that regulate gene

expression at the post-transcriptional level. Endothelial progenitor cells (EPCs) possess the capacity to differentiate into endothelial lineage cells, and play an important role in angiogenesis, vascular repair, and maintenance of vascular homeostasis. We hypothesize that the dysregulated angiogenesis seen in HHT patients with ENG and ACVRL1 mutations may be partially explained by changes in microRNA regulation of key angiogenic and antiangiogenic factors. **Results:** EPCs were isolated from venous blood of healthy individuals and those with HHT1 and HHT2. MicroRNAs miR-574-3p and miR-484, which target activin A receptor type II-like 1 (ACVRL1) mRNA, and microRNAs miR-17 and miR-21, which target thrombospondin 1 (THBS1) mRNA, were seen to be upregulated in both HHT1 and HHT2. miR-548a-3p, which targets both activin receptor-like kinase 5 (ALK5) and vascular endothelial growth factor (VEGF), was downregulated. Using gain of function techniques, enhanced expression of miR-548a-3p in EPCs from those with HHT resulted in upregulation of PDGFB (a cytokine involved in vascular maturation) mRNA expression. **Conclusions:** The upregulation of proangiogenic microRNAs, and the downregulation of antiangiogenic microRNAs, favors blood vessel formation in HHT. Restoration of levels of downregulated microRNAs also results in the increased levels of cytokines implicated in the maintenance of vessel integrity. The observed changes in microRNA levels in patients with HHT may therefore account for dysregulated angiogenesis and angiodysplasia in those with HHT.

(D08) STRUCTURAL MODELLING OF ALK1 ECTO-DOMAIN

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Activin A receptor, type II-like kinase 1 (also called ALK1), is a serine-threonine kinase predominantly expressed on endothelial cells surface. While a preliminary structural model of Endoglin extracellular domain is already available, structure determination of the ALK1 ectodomain (ALK1EC) has been elusive so far. We here describe the building of a homology model for ALK1EC exploiting the most updated structural prediction methods based on threading. The potential structural effect of HHT2-associated mutations and ALK1EC potential interaction mode with its natural ligand BMP9 were also explored. The predicted interaction mode of wild-type ALK1EC with the structure of BMP9 shows an unexpected orientation of the type I receptor strongly supported by the surface localisation of some HHT2 associated mutations. This study gives a preliminary insight into the potential structure of ALK1EC and into the structural effects of HHT2 associated mutations. Though modelling results must always be taken with caution, the potential effect of each single mutation can be predicted, and used in the formulation of new biological experiments and in the interpretation of the biological significance of new mutations, private mutations, or non-synonymous polymorphisms.

Session VIII Epistaxis Management and Related Topics

(D02) LONGITUDINAL VARIATION OF EPISTAXIS IN INDIVIDUALS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Background. Epistaxis among individuals with hereditary hemorrhagic telangiectasia (HHT) is common. Although there is a wide spectrum of severity of disease, little is known about longitudinal changes in epistaxis over time. **Objectives.** The purpose of this study was to examine longitudinal trends in epistaxis severity over time and to determine factors associated with worsening epistaxis. **Design.** Prospective cohort study. **Methods.** Individuals with HHT were sent a monthly HHT-Related Epistaxis Questionnaire and an Epistaxis Severity Score (ESS) was calculated. Subject demographics, geographic location, and time of year were collected. Descriptive analyses were performed with calculations of means and medians for continuous variables and proportions for categorical variables. Multiple linear regression models using generalized estimating equations were developed to assess the effect of geography, season, and ambient air characteristics on epistaxis severity over time. **Results.** One hundred thirty nine subjects over a nine-month period were included in the study. The mean (SD) age was 55.7 (11.4) years, and the mean (SD) ESS was 4.7 (1.04). The majority (80.4%) of subjects at baseline had moderate epistaxis (ESS 4-7). Over time, the ESS remained constant within individuals with a change of -0.007 (p=0.59) per month during the study. The ESS was significantly lower in the summer (-0.38; p=0.01), fall (-0.57; p=0.36), and winter (-0.76; p=0.42) months when compared to the spring months. **Conclusions.** Epistaxis severity tends to remain constant in individuals with HHT over time. However, seasonal variation significantly affects epistaxis severity. Further study investigating geographic location and ambient air characteristics is warranted.

(A07) LOCAL SCLEROTHERAPY WITH POLIDOCANOL (ETOXISCLEROL®) IN TREATMENT OF EPISTAXIS IN HHT OR RENDU-OSLER-WEBER DISEASE. TEN YEARS OF EXPERIENCE

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Introduction: None treatment has been completely effective to eliminate epistaxis in HHT. The polidocanol in submucous or subperichondrial injection was first published in 2000. This is the result of our experience after 10 years using the polidocanol in 41 patients and with more than 350 injections done. **Material and Methods.** 41 HHT Polidocanol (Etoxisclerol®) 0.5% in submucous/subperichondrial injection, under local anaesthesia. An average of 4-5 injections in each side of the nose and 350-400 injections were realized. **Results.** 32 patients (78%) responded very good (occasional and minimum bleeds); 6 patients (14.6%) responded quite good (significant improvement); 2 patients (4.87%) responded a bit (improved but they still had important epistaxis); 1 patient (2.43%) without response. One case suffered septal perforation (2.43%) and another one felt dizziness and blurred vision during few minutes. **Discussion.** Local sclerotherapy with different materials has been utilized in treatment of epistaxis in HHT. Polidocanol injured venous endothelium causing a secondary thrombosis without producing necrosis in the healthy tissue border. It is a stable product, it is not needed refrigeration, and his low viscosity allows an easier application. Until today, our serie is the biggest one published and we got 97.56% improvement in these epistaxis. We think that septal perforation, as collateral effect, is caused by simultaneous bilateral injections in a pathological mucosa. **Conclusions.** Local injections with Etoxisclerol® are effective in epistaxis control of HHT or Rendu-Osler-Weber disease. Its application is usually simple and side effects are not appreciable.

(B06) THE MODIFIED YOUNGS PROCEDURE IN PATIENTS WITH TRANSFUSION DEPENDENT SEVERE EPISTAXIS FROM HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Objective: Surgical treatment of epistaxis in Hereditary Hemorrhagic Telangiectasia (HHT) has historically been managed with laser procedures and the septodermoplasty procedure. On transfusion dependent patients with severe epistaxis we have been performing the modified Youngs procedure or surgical closure of the nostrils. Our objective is to report treatment of severe epistaxis related to HHT with a modified Young's procedure and assess patient outcome. **Methods:** Patients with severe, iron or blood transfusion dependent epistaxis who underwent a modified Young's procedure in two otolaryngology HHT centers were reviewed. Patients were evaluated for post-operative epistaxis. **Results:** 24 patients underwent a modified Young's procedure for severe epistaxis. The procedure was well tolerated by all patients and 22 of 24 patients (92%) experienced complete cessation of bleeding after the Young's procedure. Two patients (8%) experienced minor bleeding post-operatively with failure of complete nostril closure, but did not require blood transfusion post-operatively. No patients requested a reversal of the procedure. **Conclusions:** The modified Young's

procedure is a safe and efficacious procedure with complete cessation of epistaxis in most patients with severe epistaxis and HHT.

(C12) COAGULATION OF TELANGIECTASIA OF THE ORAL CAVITY AND LIPS IN HHT-PATIENTS USING A 980 NM DIODE LASER

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Introduction: Topical application of energetic laser light (e.g. Nd:YAG, KTP and diode laser) is one of the most effective therapies in the treatment of recurrent epistaxis due to nasal telangiectasia in patients suffering from Hereditary Hemorrhagic Telangiectasia. Telangiectasia can also occur in the oral cavity especially in the tongue, the lips and other organs and tend to spontaneous bleedings. **Material and methods:** We performed coagulation of telangiectatic vessels on the lips, the tongue and the oral mucosa with a fibercoupled GaAs-diode laser of 980 nm wavelength. 12 Patients (7male, 5 female, age 24-71 years, median age 58,4 years) were treated under general anaesthesia in a continuous wave noncontact mode with a 15W power setting. Ice cubes were used for intermittent tissue cooling. **Results:** The telangiectasia showed immediate blanching when irradiated with laser light. The tissue contracted but the epithelial layer stayed intact. The so called "Pop-Corn-Effect" was not seen in the oral cavity. During surgery no bleedings occurred. Postoperative we could not find mucosa swelling or wound healing disturbances. Patients were almost free of pain. Long term results showed disappearance of the respective telangiectasia. Hemorrhages did not occur any longer from treated telangiectasia. **Conclusion:** The 980 nm diode laser light seems to be an effective and save tool for surgical treatment of vascular lesions in the oral cavity in HHT-patients.

(Z02) BEVACIZUMAB NASAL SPRAY FOR FREQUENT EPISTAXIS IN HHT

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In a minority of the patientys with HHT epistaxis remains a significant problem despite the regular local treatments. Thalidomide often helps in these patients, but has side effects. Bevacizumab nasal spray has been reported in the recent literature as a promising treatment. We have treated 9 patients with continuing epistaxis or with side effects of Thalidomide with an intranasal Bevacizumab spray. An epistaxis diary, levels of haemoglobine and video of nasal telangiectasia are used to monitor the effect of the spray. The results seem indeed promising and will be discussed. Video imaging of the nasal mucosa before and after treatment will be shown.

(A08) EFFICIENCY OF LASER TREATMENT IN PATIENTS WITH HEREDITARY HAEMORRHAGIC TELANGIECTASIA

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Background: Earlier studies have shown the effect of laser treatment, on epistaxis, in patients with hereditary haemorrhagic telangiectasia (HHT). At present time only very few prospective trials, have been performed, and many studies are based on patients subjective assessment of the severity of epistaxis. This prospective study measures the objective effect of laser treatment, in HHT patients, with mild to moderate epistaxis. We introduce an objective measure to assess the severity of epistaxis, the bleeding time (BT). The patients Quality of life before and after treatment are assessed and compared to normative data. **Methods:** In 30 patients we measured the BT before laser treatment, 1,5 month- and 6,5 months after treatment. The Short form 36 (SF-36), a validated health questionnaire, was completed before and 6,5 months after treatment. **Results:** Compared to preoperative BT, the BT was significant reduced 1,5 and 6,5 month after laser treatment ($p < 0,05$) in both cases. No significant difference in quality of life, before and after treatment, was shown. The HHT patients quality of life before treatment was reduced in 5 out of 8 dimentions when compared to Danish background population. **Conclusion:** laser treatment reduces epistaxis in HHT patients with mild to moderate epistaxis for at least 6 months; this group of patients has reduced quality of life compared to background population.

(A20) OPPORTUNITIES FOR IMPROVING DIAGNOSIS RATES OF HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Background. Experts agree that HHT is under-diagnosed. To improve diagnosis rates, we need data about how and when HHT patients access health care professionals. As epistaxis is most common symptom, we focused on related health care utilization. We hypothesized that people with HHT consult ENT specialists and emergency room (ER) physicians, and that targeting these specialists may be an opportunity for improving diagnosis rates. **Objective:** To study consultation patterns and access to ENT and ER physicians, amongst people with HHT.

Methods: Consultation patterns were studied using a web-based survey of HHT Foundation International email contacts (N=2836), targeting diagnosed HHT patients and inquiring about consultation with ENT and ER physicians prior to diagnosis. Access to ENT physicians locally (province of Ontario) was studied in 556 definite HHT patients from Toronto HHT Centre, geocoded with postal codes. Driving network model was developed in ArcGIS to generate 30, 60 and 90 minute driving buffers around ENT clinics, and determine the proportion of Ontarians within ENT clinic service area. **Results:** See Table 1. In addition, most Ontarians have access to ENT consultation, with 93%, 98% and 99% of individuals being within a 30, 60 and 90 minute drive, respectively, of an ENT clinic.

Table 1. Web-based survey shows contact with ENT and ER physicians before HHT diagnosis

Respondents (%)	940/2836 (33%)
Mean age (SD)	54.1 (14.1) years
Mean age at diagnosis (SD)	37.1 (14.1) years
Sex (% female)	67%
Epistaxis (%)	807/940 (86%)
ENT consult pre-diagnosis (%)	349/940 (37%)
ER consult pre-diagnosis (%)	154/940 (16%)
Mean interval from ENT consult to diagnosis (SD)	13.9 (12.2) years
Mean interval from ER consult to diagnosis (SD)	11.3 (12.6) years
Mean age at first ENT consult (SD)	27.8 (16.8) years
Mean age at first ER consult (SD)	30.1 (17.4) years

Discussion and Conclusions: Nearly 40% of people with HHT consult ENT, a mean of 14 years pre-diagnosis. Most Ontarians have access to ENT consultation locally. We believe ENTs should be targeted to increase diagnosis rates of HHT.

Special thanks to HHT Foundation International.

Session IX Animal Models

MECHANISMS AND MODELS IN HEREDITARY VASCULAR DISEASES: CAVERNOUS MALFORMATIONS

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Cerebral Cavernous Malformation (CCM) is a common brain vascular malformation that causes hemorrhagic stroke. Familial CCM is linked to autosomal dominant loss-of-function mutations in KRIT1, CCM2, and PDCD10. Genetic diseases such as CCM and HHT open the possibility of developing animal models in genetically tractable organisms. However, often the genetic equivalent of human disease does not faithfully reproduce human disease pathology in mice and other organisms. We have used gene targeting in mice, supported by experiments in other organisms and in cell culture to explore the role of KRIT1, CCM2 and PDCD10 in development and disease, to identify potential mechanisms of disease, and to test potential therapies. In the process, we have determined the requirements in mice for the development of CCM pathology, and have identified candidate pathways for therapeutic intervention.

(D17) COMPARISON OF CONDITIONAL KNOCKOUT MOUSE MODELS SUGGESTS DIVERGENT PATHOGENETIC MECHANISMS UNDERLYING HEREDITARY HEMORRHAGIC TELANGIECTASIA TYPES 1 AND 2

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Mutations in ENG and ALK1 are associated with HHT types 1 and 2, respectively. As ENG and ALK1 are TGF- β receptors, it is generally believed that these receptors mediate a TGF- β superfamily ligand signal in a linear SMAD-dependent manner within endothelial cells (ECs), and that deficiency of such signal transduction may be the common mechanism for HHT1 and HHT2. It was previously shown that Alk1 conditional knockout (cKO) mice, in which Alk1 was either deleted within ECs during development or globally in adult mice, develop AVMs in multiple visceral organs. Interestingly, secondary insults (e.g. wounding) was necessary to induce Alk1-deficient subdermal vessels to form de novo AVMs. Thus, we generated Eng-cKO mice using the same Cre deleter lines and approaches, with the expectation that there would be overlapping or similar phenotypes between the Eng- and Alk1-cKO mice. We were surprised to find that L1cre(+);

Eng2f/2f mice were viable and their vasculature was largely unaffected by the loss of vascular endothelial Eng. Likewise, the onset, progression, and locations of vascular phenotypes seen in adult inducible Eng KO mice (Eng-iKO) were also distinct from Alk1-iKO mice. Strikingly, Eng-deficient subdermal vessels within the skin or ear rarely formed *de novo* AVMs around acute wounds. Instead, AVMs developed in tissue surrounding the ear identification tag, indicating chronic wounding may be more appropriate for inducing AVMs in Eng-iKO mice. Taken together, these data suggest that there may be differing pathogenetic mechanisms underlying HHT1 and HHT2.

(D04) CHARACTERISATION OF ARTERIOVENOUS MALFORMATION DEVELOPMENT IN TWO INDUCIBLE MOUSE MODELS OF HHT1 AND HHT2

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Background: Mutations in the TGF- β family receptors Endoglin and Alk1 are the underlying cause of the autosomal dominant vascular dysplasia hereditary haemorrhagic telangiectasia (HHT) Type 1 and Type 2, respectively. The development of arteriovenous malformations (AVMs) in various vascular beds is the most common feature of HHT. **Methods:** To identify the mechanisms underlying AVM development in HHT, we have utilised two inducible knockout mouse models of HHT1 and HHT2. These mouse models carry an endothelial specific inducible Cre (Cdh5-Cre-ERT2), which allows time and endothelial cell-specific inactivation of floxed Endoglin or floxed Alk1 genes following treatment with tamoxifen. The effect on angiogenesis following Endoglin or Alk1 gene depletion was examined in vivo using the well established neonatal mouse retinal angiogenesis model. **Results and Conclusion:** Endothelial specific loss of Endoglin or Alk1 in vivo leads to *de novo* development of AVMs in the neonatal mouse retina. The abnormal inter-connecting shunts exhibit increased cell proliferation, increased cellularity and show venous identity, as determined by expression of EphB4. A more severe phenotype was observed after Alk1 deficiency, as a deficiency of Alk1 in postnatal mice led to lethality within 48 hours whereas Endoglin deficient mice remain viable. Differences in vascular plexus remodelling, vascular branching and maintenance of arterial/venous identity were also identified between the two models, indicating that the pathogenetic mechanisms underlying the development of AVMs in HHT1 and HHT2 might be different. Changes in downstream molecular pathways implicates both Smad-dependent and independent pathways in HHT pathogenesis.

(C23) BENEFICIAL EFFECTS OF ANTI-ANGIOGENIC THERAPY IN ENDOGLIN HETEROZYGOUS MICE

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Endoglin is a major component of endothelial cells that plays a role in maintaining vascular integrity. Mutations in the Endoglin gene lead to Hereditary Hemorrhagic Telangiectasia (HHT), associated with dilated vessels and arteriovenous malformations (AVMs). Endoglin is a co-receptor for TGF- β 1, known to regulate angiogenesis, which is pathological in AVMs. Our hypothesis is that defective TGF- β signaling leads to increased VEGF levels, dysregulated angiogenesis and that anti-VEGF therapy will be beneficial. Anti-angiogenic therapy is being considered for the treatment of HHT patients; therefore we tested the use of monoclonal antibody B20-4.1.1 to VEGF in Endoglin heterozygous (Eng+/-) mice. We also treated Eng+/- mice challenged by a single course of 3% dextran sodium sulfate (DSS) for 5 days, which induces chronic colitis and pathological angiogenesis. Mice were injected i.p. with an optimal dose of 5 mg/kg. Mice received 4 injections, given weekly under basal conditions and every 5 days after DSS induction. Increased vascular permeability (VP) was observed in several tissues of Eng+/- mice relative to control mice and was restored to normal by anti-VEGF treatment. Antibody-bound VEGF levels were high in the plasma indicating that B20-4.1.1 was binding circulating VEGF. In DSS-induced colitis, anti-VEGF treatment significantly decreased the number of CD31+ vessels and the extent of inflammation and crypt damage, observed on distal colon sections from Eng+/- mice. We conclude that anti-VEGF treatment is safe in Eng+/- mice and leads to normal VP under basal conditions and to attenuated chronic inflammation and pathological angiogenesis in colitic mice.

(D16) PRECLINICAL ASSESSMENT OF VEGF BLOCKADES FOR INHIBITION OF ARTERIOVENOUS MALFORMATION IN A MOUSE MODEL FOR HHT

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We have shown that wounding induces the formation of *de novo* AVMs in Alk1-deficient subdermal vessels, indicating that "wound" can play as an essential secondary factor for AVM development in Alk1-deficient subdermal vessels. Since wound healing is a multi-step process involving a diverse range of biological events, we aimed to determine the specific pathway among "wound" responses relevant for AVM formation. We focused on two major wound responses, i.e. inflammation and angiogenesis. More specifically, we tested whether LPS (for

inflammation) or VEGF (for angiogenesis) can recapitulate the “wound” response in the skin AVM model by implanting LPS or VEGF into dorsal skin of Alk1-deficient mice instead of wounding. Both factors were sufficient to induce AVM formation without wounding, suggesting that inflammatory or angiogenic stimulus can work as a second hit to induce AVM formation with Alk1-deficiency. We found that LPS induced inflammation accompanied with angiogenesis. When VEGF neutralizing antibody (Ab) was treated, the LPS effect on AVM formation was significantly blunted, indicating that inflammation-induced AVM in Alk1-deficient vessels is associated with stimulation of angiogenesis. We then investigated whether angiogenesis inhibition using the VEGF neutralizing Ab can prevent wound-induced AVM formation and/or internal bleeding in Alk1-deficient mice. VEGF Ab-treatment has significantly inhibited skin AVM formation, loss of red blood cells, and internal bleeding in GI and lung compared to the control saline treatment. Taken together, these data strongly support the idea that angiogenesis blockades would have a great therapeutic potential for epistaxis in HHT patients.

(C24) THE POTENTIAL ROLE OF ENDOGLIN IN MODULATING IMMUNE AND ANGIOGENIC MEDIATORS OF INFLAMMATORY BOWEL DISEASE

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Endoglin, a co-receptor of the TGF- β superfamily, is expressed in endothelial cells and some hematopoietic subsets. Endoglin and TGF- β are known to regulate the functions of the vascular and immune systems. To amplify the effects of endoglin haploinsufficiency, we challenged Endoglin heterozygous (Eng^{+/-}) and control mice using a model of inflammatory bowel disease. Mice were given a 5-day course of 3% dextran sulfate sodium and both Eng^{+/-} and control mice developed acute colitis on day 9. However, Eng^{+/-} mice progressed to chronic inflammation and pathological angiogenesis (at days 19-22) while control mice recovered. We propose that development of chronic disease in Eng^{+/-} mice is due to both endothelial and immune defects. Under basal conditions, distal colons of Eng^{+/-} mice showed decreased TGF- β 1 levels, increased VEGF levels and vascular permeability. All 3 parameters were up-regulated under chronic inflammatory conditions. An angiogenesis PCR array revealed the down-regulation of the angiostatic factor Thrombospondin-1 in Eng^{+/-} mice, suggesting that an angiogenic imbalance is responsible for pathologic angiogenesis in chronically inflamed tissue. To assess for immune defects, gut lamina propria leukocytes were isolated and characterized by flow cytometry. Eng^{+/-} mice demonstrated a decrease in a CD11b⁺F480⁺Gr-1⁺ myeloid subset with potential immunosuppressive function. CXCL1, CCL11, IL-1 β and IL-6, which stimulate myeloid cells, and myeloperoxidase

activity, were also decreased in Eng^{+/-} mice. Future studies will involve additional markers to define myeloid subsets affected in this model and functional assays to assess the impact of myeloid dysfunction in chronic bowel inflammation.

(B18) REGENERATIVE CAPACITY OF MACROPHAGES IS IMPAIRED IN HEREDITARY HEMORRHAGIC TELANGIECTASIA-1

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Background. Previous work showed impaired homing of mononuclear cells (MNC) from Hereditary hemorrhagic telangiectasia-1 (HHT-1) patients towards ischemic tissue. MNC can differentiate towards inflammatory and regenerative macrophages after injury. The aim of this study was to investigate whether the ability of MNC to differentiate towards regenerative macrophages is impaired in HHT-1. **Methods:** Eng^{+/-} mice were used as a model for HHT-1. In these mice, hindlimb ischemia was induced by ligation of the femoral artery. After 1 week of recovery, bone marrow (BM)-derived MNC were isolated, characterized using flow-cytometry at basal level and after differentiation towards macrophages (8 days of culture with Granulocyte-macrophage colony-stimulating-factor (GM-CSF)). Classically activated and regenerative macrophages were distinguished by Ly6C and CD206 expression, respectively. **Results:** Eng^{+/-} mice showed skeletal muscle degradation after hindlimb ischemia compared to wild-type(WT) skeletal muscles. However, this could not be explained by a difference in influx of immune cells or by a difference in basal levels of bone marrow (BM)-derived inflammatory Ly6C⁺ cells. Interestingly, after culturing BM-derived MNC with GM-CSF, MNC from Eng^{+/-} mice showed a skew towards classically activated macrophages (Ly6C⁺) at the expense of regenerative macrophages, compared to MNC of WT mice. In addition, stimulation with GM-CSF and Transforming-Growth-Factor β (TGF β) resulted in a more regenerative phenotype (Ly6C-low/CD206-high) of the macrophages in WT MNC-cultures. **Conclusion:** In Eng^{+/-} mice, the ability of MNC to differentiate towards regenerative (Ly6C-low/CD206-high) macrophages is impaired, possible due to a defective TGF β response. The blunted differentiation towards regenerative macrophages might contribute to the vascular malformations in HHT-1.

Session X Genotypes, Phenotypes and Correlations

(C10) DETERMINATION OF CAUSATIVE GENES IN HEREDITARY HEMORRHAGIC TELANGIECTASIA (HHT) USING EXOME SEQUENCING

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All known genetic defects that cause Hereditary Hemorrhagic Telangiectasia (HHT) are found within the transforming growth factor-beta (TGF- β) signaling pathway. Endoglin (ENG, chromosome 9q34), activin A receptor type II-like 1 (ACVRL1, chromosome 12q13), and SMAD family member 4 (SMAD4, chromosome 18q21), are known to cause HHT1, HHT2, and Juvenile Polyposis/HHT syndrome, respectively. Mutations in two unidentified genes on chromosome 5q31 and chromosome 7p14 cause HHT3 and HHT4, respectively. Our goal is to determine the HHT4 causative gene using exome sequencing. We have identified six unrelated probands who have HHT but do not have ENG, ACVRL1, or SMAD4 coding region mutations or large gene deletions or duplications. The entire exome for these individuals was enriched for using a targeted exome kit (SureSelect, Agilent) and sequenced using an Illumina HiSeq2000 instrument. Variants from the affected individuals were filtered against eight HapMap individuals and dbSNP to eliminate non-disease causing and common variants, and then against each other to identify common genes with variants. Variants were also analyzed using VAAST, the Variant Annotation, Analysis, and Selection Tool. Top candidate genes were identified. The variants in these genes are being screened in the other affected family members to show the disease effect of the mutations. Determining new HHT causative genes may lead to the discovery of new pathways of this disease, improved diagnosis, and potentially new therapeutics for HHT.

(B12) JUVENILE POLYPOSIS, HEREDITARY HEMORRHAGIC TELANGIECTASIA AND EARLY ONSET COLORECTAL CANCER IN PATIENTS WITH SMAD4 MUTATION

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Background: Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disorder caused by mutation in the endoglin or ALK1 genes. A distinct syndrome combines the clinical features of HHT and juvenile polyposis (JP) and has been associated with SMAD4 mutation. The aim of this study was to identify patients with clinical JP-HHT and SMAD4 mutation and to compare their phenotype with HHT or JP patients with mutations other than SMAD4. **Methods:** Patients prospectively enrolled in the Toronto HHT and JP databases who underwent genotyping were included in the study. The phenotypic characteristics of JP-HHT patients with SMAD4 mutation and patients with mutations other than SMAD4 were analyzed and compared. **Results:** In total 358 patients underwent genetic testing (HHT n=332, JP n=26). Among fourteen patients identified with SMAD4 mutation, ten met the criteria for both JP and HHT syndromes (71%). Patients with SMAD4 mutation showed a 100% penetrance of the polyposis phenotype. All JP patients with SMAD4 mutation had features of HHT. Three JP-HHT patients developed early onset colorectal cancer (CRC) (mean age= 28). JP-HHT patients with SMAD4 mutation differed clinically from HHT patients with other mutations than SMAD4 by a significantly higher rate of anemia. **Conclusions:** Patients with HHT and SMAD4 mutation are at significant risk of JP and CRC, with a gastrointestinal phenotype similar to JP patients in general. As such, HHT patients should undergo genetic testing to determine if they have SMAD4 mutation and are therefore at risk for JP and candidates for routine gastrointestinal evaluation and surveillance.

(D15) MUTATIONS IN THE 5'UTR OF ENG GENE CAUSE HEREDITARY HEMORRHAGIC TELANGIECTASIA (HHT)

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The effects of noncoding region mutations of ENG are not well described and homozygous mutations are generally lethal. We report 3 families with novel mutations in the 5'UTR of ENG, one of the probands is homozygous. Sequence analyses of HHT genes including 5'UTR regions, and Western blot analyses were performed. An ENG heterozygous point mutation (c.1-9G>A), creating an upstream translation initiation site without affecting the reading frame, was identified in Family 1. The 4-year-old proband, had telangiectasias and mild epistaxis. The proband's mother had 4 subtle telangiectasias with infrequent mild epistaxis. The maternal grandmother had a history of mild epistaxis and a few punctate telangiectasias. None of the three had solid organ AVMs identified. The 74-year-old proband of Family 2 had >30 telangiect-

tasias, daily epistaxis, but no AVMs based on standard screening protocols, and was homozygous ENG c.1-9G>A. Family 3, which presented with classical HHT findings and previously linked to ENG locus, had c.1-127G>A of the ENG gene, creating a novel translation initiation site and results in an out-of-frame product. In vitro expression studies with mutant constructs showed downregulation of the endoglin protein for both mutations. Our data suggest that c.1-127G>A is the cause of HHT in family 3. Based on arguably milder than average manifestations in Family 1 and viability in the homozygous state, we hypothesized that c.1-9G>A results in reduced, but not absent, protein production or function due to leakiness of the initiation of translation. Expression analysis supports that c.1-9G>A is a hypomorphic mutation. Quantitative measurements are underway.

(B13) PATIENTS WITH HEREDITARY HAEMORRHAGIC TELANGIECTASIA WAIT >30 YEARS ON AVERAGE TO RECEIVE A CORRECT DIAGNOSIS!

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Rare diseases often require a longer time period to be diagnosed. Furthermore, HHT disease, often leads to misdiagnosis, also due to variable manifestations and to features overlapping with other disorders. Studies aiming to investigate the time delay before receiving a proper HHT diagnosis are lacking. Our purpose was to study the time period intervening between symptom onset and first correct HHT diagnosis. The study was conducted on all consecutive patients with definite clinical HHT (based on Curacao criteria) and/or carrying the familial mutation who have been recruited by our HHT Center (2001-2010). A questionnaire was administered upon telephonic interview. Information on the following data was collected: patient's demographics, age at onset of the first HHT manifestation, date and place of the first diagnosis received, specialty of the physician who provided the first diagnosis, correctness of first diagnosis, age at the first correct HHT diagnosis. A total of 234 patients were involved in the study and completed the questionnaire, 115 probands and 119 relatives. The time-lag for correct diagnosis was of 31.5 yrs (range from 0 to 73 yrs) in the probands' group, and 22 yrs (from -4 to 68 yrs) in the group of relatives. In both groups, a high grade of scholarization ($p < 0.05$, $p < 0.001$) and being carriers of ALK1 vs ENG mutation ($p < 0.001$, $p < 0.001$) were associated with a shorter time-lag. Most HHT patients suffer from an unacceptably lengthy time interval for correct diagnosis, so more incisive information programs are strongly needed.

(D14) ACVRL1 AND ENG MOSAICISM IN HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Genetic mosaicism is known to be associated with the clinical phenotype of many diseases. However, mosaicism has not previously been reported in hereditary hemorrhagic telangiectasia (HHT), or pulmonary arterial hypertension, an associated disorder which is reported to result from mutations in the ACVRL1 or ENG genes. Two cases were submitted to our laboratory for HHT clinical testing. Case 1 has a clinical diagnosis of pulmonary arterial hypertension and symptoms of HHT. Case 2 has a clinical diagnosis of HHT. Neither patient has a family history of HHT. The entire ACVRL1 and ENG genes were sequenced for both cases. In case 1, a c.1451G>A (p.Arg484Gln) mutation was identified in exon 10 in the ACVRL1 gene; a mutation previously reported in association with pulmonary arterial hypertension. In case 2, a c.591delG (p.Arg197fs) mutation was identified in exon 5 of the ENG gene. In each case, the mutant allele demonstrated low amplification, indicating possible mosaicism. A second primer set was used to confirm these findings and rule out any possible primer binding issue due to polymorphisms. We then used pyrosequencing to quantify the percentage of the mutant alleles. In peripheral blood, the percentage of mosaicism was approximately 20% in the c.1451G>A mutation found in case 1, and 60% in the c.591delG mutation found in case 2. These findings highlight the importance of identifying mosaic cases when performing clinical testing of hereditary hemorrhagic telangiectasia.

(B27) PHENOTYPE-GENOTYPE CORRELATIONS IN PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA AND PULMONARY ARTERIOVENOUS MALFORMATIONS

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Objective: to study phenotype-genotype correlations in patients with HHT and pulmonary arteriovenous malformations (PAVMs). **Methods:** Database review of patients with HHT from the Toronto HHT Database. Inclusion criteria: all patients with macro PAVMs. They are categori-

zed according to their personal genetic mutation or family mutation. Imaging was reviewed for number of PAVMs, size of PAVMs and characteristics. Student's T-test was performed to compare results of patients with Endoglin and ACVRL-1 mutation. The number of patients included in the SMAD4 group was too small to include in the statistical analysis. **Results:** 215 patients with HHT and PAVMs were included: 119/215 (55%) patients had an Endoglin mutation, 23/215 (11%) ACVRL-1 mutation, 5/9 (2%) SMAD4 mutation and in 68/215 (32%) patients mutation was not known. Patient characteristics are shown in Table 1. There was no significant difference between patients with Endoglin or ACVRL-1 mutation in clinical presentation. Imaging of PAVMs by angiography and/or CT chest was available in 63 cases. The results are shown in Table 2. There was a trend towards an increased diameter of maximal feeding artery in patients with an Endoglin mutation ($P=0.06$).

Table 1: Patient characteristics and symptoms and complications.
*At initial assessment.

	Endoglin	ACVRL-1	SMAD4
Number of patients with PAVM	119	23	5
Mean age at study (years) (SD)	50.7 (16.4)	49.3 (15.5)	38.4 (6.0)
Female (percentage)	67 (56%)	12 (52%)	2 (40%)
Mean age* (years) (SD)	44.7 (15.1)	41.7 (14.4)	28.5 (7.4)
Dyspnea * (N,%)	54 (45%)	6 (26%)	2 (40%)
Hypoxemia ($pO_2 < 80$ mmHg)*	49/104 (47%)	3/13 (23%)	2/3 (60%)
Stroke* (N,%)	17 (14%)	2 (9%)	1 (20%)
TIA* (N,%)	24 (20%)	3 (13%)	0
Hemoptysis* (N,%)	18 (15%)	2 (9%)	0
Hemothorax* (N,%)	6 (5%)	0	1 (20%)
Brain abscess* (N,%)	9 (8%)	0	0

Table 2: Results of Imaging review.

	Endoglin	ACVRL-1	SMAD4
Number of Patients (%)	63/119 (52%)	9/23 (39%)	4/5 (80%)
Diffuse PAVMs	1/63 (2%)	0	1/4 (25%)
Mean number of PAVMs (SD)	2.6 (2.1)	2.9 (4.0)	2.6 (2.9)
Largest feeding artery (mean mm) (SD)	4.2 (2.0)	2.6 (1.5)	3.0 (1.4)

Conclusions: There are no significant differences in clinical and angiographic presentation of PAVMs between HHT patients with Endoglin and ACVRL-1 mutations.

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(D21) GENOTYPE-PHENOTYPE CORRELATIONS OF CANADIANS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Background. Hereditary Hemorrhagic Telangiectasia (HHT) is an autosomal dominant disorder characterized by telangiectasia and arteriovenous malformations (AVMs) in the lungs, brain, liver, and gastrointestinal (GI) tract. A genetic diagnosis can be made if a mutation is found in the coding exon of the endoglin (ENG), activin A receptor type II-like 1 (ACVRL1), or SMAD4 gene. Previous studies have demonstrated an association between ENG mutation and presence of pulmonary AVMs (PAVMs) and cerebral AVMs (CAVMs). We sought to study these associations in a large Canadian HHT population. **Methods:** We collected data from four Canadian HHT centres (Edmonton, Montreal, Toronto, Vancouver), from initial assessment and follow up visits. This includes age, gender, HHT manifestations, and genetic mutations. **Results:** Genetic mutations were known in 437/1383 (31.6%) of patients assessed at three centres (Vancouver data collection ongoing). One hundred ninety-two/437 (43.9%) had ACVRL1 mutation, 232/437 (53.1%) had ENG mutation and 13/437 (3.0%) had SMAD4 mutation. Cerebral AVMs and PAVMs were significantly more prevalent in ENG patients, while anemia and symptomatic liver VMs were significantly more prevalent in ACVRL1 (see table). There was no significant association between genotype and epistaxis, telangiectasia, and GI bleeding.

Table 1. Genotype phenotype associations in Canadian HHT population.

	ACVRL1 N=192	ENG N=232
Age	52.0 (17-84, 15.5)	50.4 (18-89, 15.3)
Gender	58.3% (112/192)	59.5% (138/232)
HHT Clinical Diagnosis	96.2 (177/184)	95.1 % (212/223)
Epistaxis	94.6% (175/185)	90.4% (208/230)
Telangiectasia	97.3% (178/183)	94.3% (215/228)
PAVM	16.6% (30/181)	67.0% (148/221) **
CAVM	5.2% (8/153)	12.1% (23/190) **
GI Bleeding	29.4% (40/136)	23.0% (41/178)
Anemia (HB <100)	9.5% (14/148)	3.6% (6/168) **
Symptomatic Liver AVM	6.9% (11/160)	2.4% (5/207) **

** $P < 0.05$

Conclusion. The Canadian HHT population genotype is nearly evenly split between ENG and ACVRL1, with ENG mutation being associated with CAVM and PAVM and ACVRL1 mutation being associated with anemia and symptomatic liver VMs.

Session XI TGF- β and BMP Pathways in Disease

BMP SIGNALING AND PULMONARY HYPERTENSION

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Patients with hereditary pulmonary arterial hypertension (HPAH) inherit heterozygous mutations at the BMPR2 locus, but patients carrying BMPR2 mutations subject to non-sense mediated mRNA decay (NMD) have a better prognosis than those carrying NMD negative mutations in which the mutant products may exert dominant negative effects. To explore the mechanism by which these mutations promote PAH, PH responses have been evaluated in heterozygous germ line null and hypomorphic Bmpr2 mutant mice (Bmpr2DEx2, corresponding to a known NMD negative HPAH mutation). Both have increased susceptibility to PH, but PH responses are exacerbated in hypomorphic Bmpr2 mutants. Neither develops obliterative vascular disease seen in humans, but both mutants have increased pulmonary vasoconstriction, and hypomorphic Bmpr2 mutant mice have a marked defect in EC-dependent pulmonary vasodilatation associated with reduced eNOS expression and activity. To explore this further, we evaluated the Bmpr2DEx2 mutant products in pulmonary ECs from these mice and in patients carrying the corresponding NMD negative BMPR2 mutation. Both express BMPR2DEx2 mutant products, which are retained in the ER, interfere with BMP signaling and reduce BMP-dependent eNOS activation. These effects are reversed after treatment with chemical chaperones. This supports the hypothesis that NMD negative BMPR2 mutations exacerbate disease severity in HPAH patients by exerting dominant negative effects on BMP signaling, and suggest potential approaches to correct BMP signaling defects in HPAH. Parallel studies using inducible pluripotent stem cells are being developed to evaluate therapeutic approaches to correct BMP signaling defects associated with different NMD positive and negative BMPR2 mutations in HPAH patients.

OSLER, WEBER, AND RENDU: PROVIDING INSIGHTS INTO CARDIAC REMODELING A CENTURY LATER

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Cardiac fibrosis accompanies myocardial hypertrophy and contributes to adverse cardiac remodeling in heart failure. Endoglin (Eng), a Type III auxiliary receptor for transforming growth factor beta (TGF β 1), is expressed by cardiac fibroblasts, but its effect on cardiac fibrosis has not been explored. We studied the role of endoglin in car-

diac fibrosis using a mouse model of pressure-overload induced heart failure. Increased Eng expression was observed in the left ventricle (LV) after 2, 4, and 10 weeks of thoracic aortic constriction (TAC) in male, wild-type (WT) mice along with progressive cardiac fibrosis and LV hypertrophy. Eng haploinsufficient (Eng+/-) mice demonstrated preserved LV systolic function and improved survival compared to WT controls after 10 weeks of TAC. Reduced cardiac fibrosis and Type I collagen expression were observed in Eng+/- mice versus WT after TAC. Cardiomyocyte hypertrophy and changes in calcineurin, SerCA, and bMHC expression were comparable between groups, while capillary density was higher in Eng+/- mice after TAC compared to WT controls. The dependence of TGF β 1 induced collagen synthesis on Eng expression was tested in vitro using human cardiac fibroblasts (hCF). Neutralizing antibodies and siRNA against Eng independently attenuated TGF β 1 induced collagen synthesis. Both adenoviral-mediated overexpression of full-length endoglin (AdFL-Eng) and transfer of conditioned media from COS-7 cells over-expressing human soluble endoglin (AdhsEng) attenuated TGF β 1 induced collagen synthesis in hCF. To confirm the inhibitory role of sEng, hCF were treated with recombinant sEng, which attenuated TGF β 1 induced collagen synthesis in a dose-dependent manner. Taken together, these results suggest that Eng is required for cardiac fibrosis and may mediate an auto-inhibitory feedback loop to limit TGF β 1 induced collagen expression via increased expression of soluble endoglin. Reduced endoglin expression attenuates cardiac fibrosis and promotes angiogenesis, a combination that likely contributes to improved survival in heart failure.

(B16) ANALYSIS OF THE MOLECULAR MECHANISMS INVOLVED IN THE REGULATION OF THE INTRON RETENTION OF THE ENDOGLIN GENE DURING ENDOTHELIAL SENESENCE

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Alternative splicing involving intron retention plays a key role in the regulation of gene expression. We previously reported that the alternatively spliced short isoform of endoglin (S-endoglin) is induced during the aging or senescence of endothelial cells by a mechanism of intron retention. In this work, we find that the splicing factor ASF/SF2 is involved in the expression of S-endoglin. Overexpression of ASF/SF2 in endothelial cells switched the balance between the two endoglin isoforms, favoring the synthesis of S-endoglin. Using a minigene reporter vector and RNA immunoprecipitation experiments we demonstrated that ASF/SF2 was able to interact with the nucleotide sequence of the endoglin minigene, suggesting the direct involvement of ASF/SF2. Accordingly, the sequence recognized using bioinformatics by ASF/SF2 in the endoglin gene was identified inside the retained intron near the consensus branch point. Finally, the ASF/SF2 subcellular localization during endothelial senescence

showed a preferential scattered distribution throughout the cytoplasm, where it interferes with the activity of the minor spliceosome, leading to an increased expression of S-endoglin mRNA. In summary, we report for the first time the molecular mechanisms by which ASF/SF2 regulates the alternative splicing of endoglin in senescent endothelial cells, which may contribute to the severity of the HHT symptoms.

(A23) GENOME-WIDE ANALYSIS OF SMAD1/5 BINDING REGIONS IN ENDOTHELIAL CELLS

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Background: Hereditary hemorrhagic telangiectasia (HHT) has been mapped to ENG, ACVRL1 (also known as ALK-1) and SMAD4. They are components for bone morphogenetic protein (BMP) signaling, which is transduced through BMP-specific receptor-regulated Smads (BR-Smads; SMAD1/5/8). On the other hand, it has not been clarified whether BR-Smad signaling pathway activated by other BMP type I receptors is able to compensate the phenotypes. Moreover, downstream effectors of BR-Smad signaling have not been fully defined. Here, we generated genome-wide maps of SMAD1/5 binding sites in endothelial cells and characterized them in detail. **Methods:** We performed chromatin immunoprecipitation followed by sequencing (ChIP-seq) analyses using human umbilical vein endothelial cells (HUVECs) stimulated with BMP-9 or BMP-6. **Results:** The mapping data indicated that SMAD1/5 preferentially bound to the evolutionally conserved enhancer regions. These SMAD1/5 binding regions were shown to work as transcriptional enhancers. BMP-9 was more potent than BMP-6; SMAD1/5 recognized a greater number of target sites after BMP-9 treatment. De novo motif finding methods predicted a SMAD1/5 binding sequence, which was consistent with the previously reported GC-rich motif. Furthermore, combining the mapping data with gene expression profiles allowed us to identify seventy candidate target genes of SMAD1/5. Among them, a Notch ligand JAG1 was induced by SMAD1/5 signaling in vitro, transactivating Notch signaling in the neighboring cells. **Conclusion:** Endothelial-expressed JAG1 has been reported to induce differentiation and recruitment of vascular smooth muscle cells (vSMCs). Our results provide insights into the molecular mechanism of BMP signaling in ECs and pathogenesis of vascular lesions of HHT.

(B07) ALK1 REGULATION OF FLOW-RESPONSIVE GENE EXPRESSION

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To understand the molecular and cellular missteps underlying development of telangiectasias and arteriovenous malformations in Hereditary Hemorrhagic Telangiectasia (HHT) patients, we are studying Alk1 signaling in zebrafish embryos. Zebrafish express alk1 in a subset of cranial arteries proximal to the heart, and loss of function mutations result in arterial enlargement and, immediately downstream, arteriovenous shunts. We previously demonstrated that alk1 expression is fully dependent on blood flow, leading us to hypothesize that alk1 might lie in a flow-responsive signaling pathway necessary for vessel quiescence. Accordingly, we demonstrate that expression of the shear stress-repressed gene, cxcr4a, is strongly upregulated in arterial endothelial cells in zebrafish alk1 mutants. Measurement of shear stress within arteries exhibiting dysregulated cxcr4a expression demonstrates that this mechanical force is not altered in alk1 mutants, supporting the idea that alk1 might lie upstream of cxcr4a in a flow-responsive signaling pathway. Further support for this hypothesis comes from the observation that endothelial-specific overexpression of constitutively active alk1 obliterates cxcr4a expression, despite the fact that most arteries in these embryos experience no blood flow. Together, these studies suggest that increases in expression of genes encoding promigratory proteins might contribute to arterial enlargement and AVMs in the absence of Alk1 function. Current studies are focused on defining the mechanism by which Alk1 regulates cxcr4a expression and exploring the role of increased endothelial tip cell behavior in development of enlarged arteries and AVMs in alk1 mutants.

(B17) VASCULAR INJURY TRIGGERS KLF6 MOBILIZATION AND COOPERATION WITH SP1 TO PROMOTE ENDOTHELIAL ACTIVATION THROUGH UPREGULATION OF THE ALK1 GENE

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ALK1 expression in the vascular bed is critical for vasculogenesis in the embryo. In adults, ALK1 expression in the quiescent endothelium is low, but it is highly induced in newly forming vessels under stimuli such as wound healing. The molecular mechanisms underlying the

ALK1 regulation in response to vascular injury have not been characterized yet. We find that Alk1 becomes strongly upregulated in mouse femoral arteries during proliferation of the neointima after wire injury. In vitro, denudation of monolayers of Human Umbilical Vein Endothelial Cells (HUVEC) also leads to an increase in ALK1. Interestingly, a key factor in tissue remodeling, the Krüppel-like factor 6 (KLF6), translocates to the cell nucleus during wound healing, concomitantly with an increase in the ALK1 gene transcriptional rate. Following KLF6 knock down in HUVECs, ALK1 mRNA levels are downregulated. Moreover, *Klf6*^{+/-} mice have lower levels of Alk1 in their vasculature compared with their wild type siblings. Chromatin immunoprecipitation experiments show that KLF6 is interacting with ALK1 promoter in endothelial cells, and this interaction is enhanced during wound healing. As a result, KLF6 is transactivating ALK1 gene, and this transactivation occurs by a synergistic cooperative mechanism with Sp1, as demonstrated by luciferase reporters and wound healing assays. In summary, upon vascular injury, KLF6 activates the ALK1 gene transcription in order to activate the pro-angiogenic state of endothelial cells during vascular remodeling. These data suggest that ALK1 plays a critical role in activated endothelial cells during vascular remodeling.

Session XII Clinical Management of HHT Patients

PREGNANCY AND OTHER STRESSORS OF THE HHT PHENOTYPE

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Hereditary haemorrhagic telangiectasia (HHT) leads to the development of telangiectasia, and arteriovenous malformations (AVMs), particularly in the pulmonary, cerebral, and hepatic circulations. Haemorrhage, and complex pathophysiology, result from shunts that bypass specific capillary beds. HHT pathology develops and operates in different contexts, according to the individual. Increasing recognition of circumstances associated with HHT-related complications have led to recommendations to modify lifestyles and medical management. During pregnancy, the physiological increase in cardiac output approaches 50%. Small series and case reports highlighted pregnancy risks in HHT. A recent study reported thirteen pregnant women with life-threatening PAVM bleeds, strokes or myocardial infarction. Importantly, incorporation of the denominator from which the population was drawn allowed estimates of absolute risks, with 1.0% (95% confidence intervals 0.13, 1.9%) of HHT pregnancies resulting in maternal and fetal death. All deaths occurred in women previously considered well: prior awareness of the HHT or PAVM diagnosis was associated with improved survival in the event of a life-threatening complication. As a result, in British obstetric practice, general recommendations for management of HHT pregnancies now include maternal and clinician education to consider haemoptysis or sudden severe dyspnoea as a medical emergency prompting urgent hospitalization; management as 'high risk pregnancies'; and modifications of usual obstetric practice. Interpretation of these data for pregnancy and other potential stressors of the HHT phenotype such as flying, diving, and recreational activities, will differ according to individual circumstances, and prevailing health care systems. Determination of absolute complication rates is helpful in guiding informed recommendations and choices.

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(C05) ASSOCIATIONS OF IRON DEFICIENCY AND IRON TREATMENT WITH VENOUS THROMBOEMBOLISM AND BIOMARKERS IN REPLICATE HHT COHORTS

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Background: Pulmonary emboli and deep venous thrombosis (venous thromboemboli, VTE) are major medical problems, and in the UK are the most common direct cause of maternal death in pregnancy. We have previously shown that VTE occur surprisingly frequently in hereditary haemorrhagic telangiectasia (HHT), and that elevated levels of coagulation factor (F)VIII are a biomarker for recurrent VTE in HHT, as in the general population. **Methods:** To better understand VTE risk factors and potential mechanisms, replicate cohorts of individuals with definite HHT were recruited prospectively between 1999-2006 (n=309), and 2007-2010 (n=221). Associations between patient-specific variables; FVIII levels; and radiologically-proven VTE were assessed in univariate and stepwise multiple regression analyses. **Results:** In separate and pooled cohorts, basal FVIII levels were significantly higher when concurrent serum iron levels were low (linear multiple regression p values 0.025; 0.045; and 0.006 respectively). Overall, 30 (5.7%) of the 530 HHT patients had suffered a VTE, but this endpoint demonstrated no association with any of four markers of iron deficiency: Instead, in logistic regression analyses, once corrected for FVIII levels, VTE was associated with use of iron treatments (pooled series odds ratio 3.6 [95% confidence intervals 1.39, 9.45] p=0.008). **Conclusions:** These data suggest that iron deficiency is associated with elevated levels of the prothrombotic risk factor, coagulation factor VIII, further supporting the need for iron deficiency prevention/treatment programs in HHT and at-risk individuals in the general population. The possibility that there may also be prothrombotic risks of pharmaceutical iron replacement requires further study.

(B26) BRAIN ABSCESES IN PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Objective: Identifying causative micro-organisms for brain abscesses in patients with Hereditary Hemorrhagic Telangiectasia (HHT) and identifying procedures which impose an increased risk. **Methods:** Inclusion of patients in Dutch HHT Database, registered as having had a brain abscess in the past. Data of these patients were collected by chart review and telephone administered questionnaire regarding period before, during and after infection. **Results:** 25/966 patients with HHT (17 female, 8 male) had a brain abscess in the past, of who 4 had repeat brain abscesses. Average age at presentation of first abscess: 43.0 years (SD 13.4). Of 22/25 (88%) patients the genetic mutation was known: 21/22 Endoglin mutation, 1/22 ACVRL-1 mutation. Ten/25 (40%) patients mentioned preceding infection/procedure: 6 infection mouth/jaw, 3 dental care, 1 bronchitis. Average interval between proce-

dures/infection and presentation with brain abscess: 23.4 days (SD 23.6). None of these patients received endocarditis prophylaxis. Micro-organisms were cultured in 18/29 (62%) cases: 13 *Streptococcus milleri*, 1 *Staphylococcus aureus*, 1 alpha-hemolytic streptococ, 1 *Peptostreptococcus*, 2 multiple micro-organisms. After 22/29 (76%) brain abscess presentations patients were diagnosed with a PAVM with a significant feeding artery. Oxygen shunt measurement was done in 15/25 patients, with an average shunt of 23.75% (SD 5.3). **Patient outcomes:** 4/25 (16%) patients deceased secondary to brain abscess, 15/25 (60%) patients had remaining neurologic impairment, 3/25 (12%) patients had complete recovery, 3/25 (12%) unknown. **Conclusion:** Brain abscess is more common in patients with Endoglin mutation. Nine/25 (36%) brain abscesses were preceded by infection/intervention of oral origin.

(A18) REPRODUCIBILITY OF OXYGEN SATURATION MONITORING DURING SIX-MINUTE WALK AND EXERCISE TESTING IN PATIENTS WITH PULMONARY ARTERIOVENOUS MALFORMATIONS

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Background: Patients with pulmonary arteriovenous malformations (PAVM) are at risk for multiple complications and require close follow-up. We investigated the reproducibility of six-minute walk test (6MWT) and exercise stress test (EST) for the evaluation of low oxygen saturation in patients with PAVMs. **Methods and Results:** Twenty-two HHT patients with PAVMs participated in an HIC approved protocol. Patients were 9 to 74 years of age (mean 28) with broad spectrum of PAVM anatomic subtypes, including focal and diffuse. Standard 6MWT and cycle ergometry EST were both performed twice with adequate rest between tests. Heart rate (HR) and oxygen saturation was measured at the beginning and end of the tests. Distance walked and maximum resistance were recorded. Intraclass correlation coefficients at end of 6MWT were: HR (ri = 0.940; 95% CI, 0.863 - 0.975), oxygen saturation (ri = 0.973; 95% CI 0.933 - 0.989), distance (ri = 0.942; 95% CI, 0.867 - 0.975). Intraclass correlation coefficients at end of EST were: HR (ri = 0.941; 95% CI, 0.865 - 0.975), oxygen saturation (ri = 0.993; 95% CI, 0.982 - 0.997), and maximum resistance (ri = 0.941; 95% CI, 0.864 - 0.975). **Conclusions:** 6MWT and EST are reproducible measures of exercise capacity and oxygen saturation in patients with PAVMs. Both tests were safe even in patients with low oxygen saturations. Further study of these tests in patients with PAVMs, correlated with angiography and chest CT, may prove valuable in reducing repeated radiologic evaluation.

(C06) IS FLYING SAFE FOR INDIVIDUALS WITH HEREDITARY HAEMORRHAGIC TELANGIECTASIA?

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Introduction. Flight-related complications reported in individuals with hereditary haemorrhagic telangiectasia (HHT) have included deep venous thrombosis (DVT), ischaemic stroke (1) and haemorrhage from pulmonary AVMs (PAVMs)(2). In addition there are theoretical concerns that the reduced barometric pressure associated with flying might exacerbate PAVM-induced hypoxaemia (3). **Methods.** With ethical approval (NRES 10/H0806/8), individuals with definite HHT who had attended our hospital's HHT clinic were sent a questionnaire to document flights they had taken, and symptoms experienced during or shortly afterwards. Responses were correlated with sea level erect oxygen saturations (SaO₂), and haemoglobin concentration. **Results.** 159 replies were received from 308 questionnaires (response rate 51.6%). 147 individuals (97 [66%] with PAVMs) had flown. The median number of flights per individual was 25, totalling 18,943 flight hours in 3,950 flights. 111 (77%) respondents reported no complications during or after flights. 26 participants (18%) reported nosebleeds, often described as more severe than at ground level. Six (4%) experienced dyspnoea (breathlessness); two (1%) had DVTs, and one suffered an ischaemic stroke. There was no difference in erect SaO₂ at sea level or haemoglobin concentration between those who developed complications, and those who did not (Figure 1A-D).

Figure 1. Comparison of erect oxygen saturations (SaO₂) at sea level, and average haemoglobin (Hb) concentrations, of individuals who reported complications and those who did not. Horizontal bars denote median and interquartile range

Conclusion: Flight appears safe for the majority of individuals with HHT. It is difficult to predict who will experience complications based on clinic assessments. Recognition of potential thrombotic and haemorrhagic sequelae should influence pre-flight advice.

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2. Pelage JP, et al. Hematol Meeting Reports 2009;3:15
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(C01) LIVING WITH HEREDITARY HAEMORRHAGIC TELANGIECTASIA. COPING AND PSYCHOLOGICAL DISTRESS

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Purpose. To examine whether coping is important in accounting for variations in psychological distress in individuals with HHT and to examine whether coping mediate the relationship between experience of illness, and psychological distress. **Patients and methods:** 39 women and 27 men affected with HHT with a mutation in ENG or ALK1, were included. Coping was measured with the Coping Orientation to Problems Experienced Scale (COPE), psychological distress with Hospital Anxiety and Depression scale (HADS) and Becks hopelessness scale (BHS). Experience of illness was measured by asking questions due to frequency, duration and intensity of nose bleedings, time used on nose care each day, impact of nose bleeding on daily life, being afraid of other complications than nose bleedings, amount of blood transfusion and discomfort due to epistaxis. **Results:** Both experienced illness variables and coping strategies made independent significant contributions to the psychological distress factors, but experienced illness was responsible for the highest variance (43 % of the variance of anxiety (HADS-A), 36 % of the variance of depression (HADS-D) and of hopelessness (BHS) respectively). The findings indicated also that the coping strategies "behavioral disengagement" might have a mediating role on the association between experienced illness and psychological distress. **Conclusion:** The results substantiate that individuals that are affected of HHT must deal with psychological distress and the associations between experienced illness and psychological distress seems to be mediated by particular coping strategies.

(D10) TRAUMA CAN INDUCE TELANGIECTASES IN PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Background. The first international guidelines state that coagulation of endonasal telangiectases is a first line therapy. The long-term effect is not well known. According to self-observation of some patients, the treatment may eventually lead to an increase of epistaxis. This hypothesis is empirically supported by the observation that trauma can induce telangiectases in an animal model. Our hypothesis is that mechanical or sun-induced damage in humans can lead to formation of telangiectases, thus these would be found more on the dominant hand and the

lower lip. *Methods:* The number of telangiectases of the hands and lips of 72 consecutive patients with HHT was counted. *Results:* 3 patients were ambidextrous and 68 right-handed. In the latter, there were 51 cases where the right hand was affected more but in 8 cases was the left hand. In two cases the upper lip was more affected, in 48 the lower lip. In 18 cases the lips were not assessed, e.g. because after therapy. *Conclusion:* The results suggest that mechanical and light-induced trauma contribute to the formation of telangiectasia. It is not clear if this also applies to endonasal coagulation. There is probably also an overlay with other effects, e.g. scar formation. Pending other studies, we inform patients before elective endonasal coagulation on a possible long-term deterioration. Additionally, we discuss the concomitant therapy with drugs which might compensate for the genetic loss, e.g. tranexamic acid, during the period of wound healing.

Session XIII Summary

HEREDITARY HEMORRHAGIC TELANGIECTASIA HONORARY LECTURE: A CAREER DEVOTED TO HHT

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POSTERS

BASIC SCIENCE

(A15) ENDOGLIN REGULATES LEUKOCYTE INFILTRATION

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Hereditary Hemorrhagic Telangiectasia (HHT) is characterized by fragile vessels that develop into arteriovenous malformations (AVMs). Endoglin (Eng) haploinsufficiency is the cause of HHT type 1, and Eng plays a major role in vascular repair. Inflammation is an essential component of the repair process that requires leukocyte migration into the damaged tissues. We postulated that Eng heterozygous (Eng+/-) mice may show impaired leukocyte migration, which could contribute to the HHT phenotype. Eng+/- mice and their littermates (Eng+/+) were i.p. injected with 0.2 mL of carrageen (1.5mg/ml) or saline. Two days later, peritoneal cells were harvested and total leukocytes were analyzed by flow cytometry. In some experiments, mice received an antibody to VCAM-1 intravenously, to block endothelial-leukocyte interactions. Carrageen induced a marked increase in the number of total peritoneal leukocytes, but to a significantly lower extent in Eng+/- than Eng+/+ mice. All cell types were down-regulated except for eosinophils. Endothelial cells from Eng+/- mice showed lower VCAM-1 expression than those from Eng+/+ mice and anti-VCAM-1 injection markedly decreased the carrageen-induced peritoneal migration to similar levels in both groups. Our results suggest that endoglin regulates leukocyte entry into the tissues and that lower expression of VCAM-1 on the endothelium may account for the reduced number of infiltrating cells, leading to deficient vascular repair and generation of AVMs.

(A16) ENDOGLIN REGULATES RENAL INFLAMMATION AFTER ACUTE RENAL INJURY

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Inflammation plays a major role in tissue repair. Endoglin

(Eng) has been implicated in the tissue repair carried out by leukocytes. Acute renal injury is characterized by vascular endothelium activation and monocyte and neutrophil infiltration. The purpose of this study was to assess the effect of Eng haploinsufficiency on endothelial activation and leukocyte infiltration after renal injury induced by ischemia/reperfusion (I/R) or folic acid (FA) injection. Endoglin heterozygous (Eng+/-) mice and littermates (Eng+/+) were subjected to left kidney ischemia for 30 min followed by reperfusion. Different groups were i.p. injected with a 250 mg/Kg dose of FA. Four, 24 and 48 hours after I/R or FA administration, kidneys were removed and the presence of neutrophils (myeloperoxidase activity), and macrophages (CD68+ cells), and the expression of ICAM-1, VCAM-1 and PECAM were assessed by Western blot and immunohistochemistry. Plasma levels of urea and creatinine were measured to assess renal function. Both models of acute kidney injury showed increases in plasma levels of urea and creatinine, higher expression of adhesion molecules (ICAM-1, VCAM-1 and PECAM), and more neutrophil and macrophage infiltration. In the I/R model, Eng+/- mice showed lower plasma levels of urea and creatinine, reduced ICAM-1, VCAM-1 and CD68 renal levels, and less myeloperoxidase activity, compared with their Eng+/+ littermates. In the FA model, Eng+/- mice also showed lower plasma levels of urea and creatinine, and less renal VCAM-1 expression. Our results suggest that endoglin is involved in activation of the vascular endothelium and in neutrophil infiltration observed after acute kidney injury.

(B09) CHARACTERIZATION OF CHICKEN ENDOGLIN AND ITS TISSUE EXPRESSION

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Endoglin is a TGF-beta co-receptor expressed in endothelial cells, where it plays a crucial role in angiogenesis, cardiovascular development and vascular remodelling. In humans, mutations in the endoglin gene give rise to Hereditary Hemorrhagic Telangiectasia type 1 (HHT1), an autosomal dominant disorder associated with vascular lesions in skin, mucosa and internal organs. So far, endoglin cDNA has been sequenced in several species from mammals, amphibians and birds. While in mammals the characterization of endoglin protein expression and function is well documented, little is known about the protein homologue in birds. In silico analysis by multiple sequences alignment showed a low homology score of 30-33 between the full length chicken endoglin protein and several mammalian homologues. However, a high homology score (80-85) was observed with the cytoplasmic and transmembrane regions and the overall structure of the zona pellucida (ZP) and orphan domains of the extracellular region appear to be conserved. Transient expres-

sion of chicken endoglin allowed the identification of a 180-kDa disulfide linked homodimer similar to the mammalian homologues. To further characterize its tissue expression, the novel specific monoclonal antibody (mAb) 7H5A8 was generated against chicken endoglin transfectant cells. The mAb 7H5A8 specifically recognized chicken endoglin by western blot, immunoprecipitation, immunofluorescence flow cytometry as well as immunofluorescence microscopy assays and displayed a positive staining of the endothelium in veins and arteries from frozen tissue sections of lung and bursa of Fabricius. These results may help to further understand the endoglin expression in birds.

(B23) GENERATION OF ENDOGLIN KO MICE SPECIFIC OF THE MYELOID LINEAGE. INVOLVEMENT OF THE IMMUNE SYSTEM IN THE HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Mutations in Endoglin or ALK1 (HHT1 and HHT2) are responsible for more than 90% of HHT cases. Both genes are predominantly expressed in endothelial cells, and, at a lower extent, in macrophages and encode for proteins involved in the TGF- β signalling pathway. Although HHT is a vascular disease, there is evidence for the implication of the immune system in this pathology. Thus, some clinical observations show that HHT patients suffer frequent infections unusual in the general population, including recurrent infections of *Staphylococcus aureus* and subcutaneous abscesses. These infections suggest a functional role for endoglin in macrophages, involved in immune surveillance. To deepen into the possible relation between HHT and the immunological function, a specific myeloid-lineage-endoglin knock-out mouse was generated. A mouse strain expressing cre recombinase controlled by the myeloid specific Lysozyme promoter was crossed with a bifloxed endoglin strain. In the resulting mice carrying the cre gene and the loxP-flanked endoglin gene, cre recombinase specificity in the myeloid lineage was studied. The characterization was made by genomic DNA PCR, by RT-quantitative PCR, and by immunohistochemistry to check the specificity of Cre recombinase in the myeloid lineage. Other tests currently under study include the comparison of blood cell counts among wild type, heterozygous and homozygous endoglin KO mice. Moreover, the phagocytic activity in macrophages and granulocytes from those 3 different genotypes will be compared. The results of these studies will throw light on the role of endoglin deficiency in the myelocytic lineage and especially in the immune response.

(A09) CHARACTERIZATION OF BMP9 EXPRESSION, THE ALK1 LIGAND

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Bone Morphogenetic Protein 9 (BMP9) has been recently found to be the physiological ligand for the ALK1 receptor, and to be a major circulating vascular quiescence factor. However, not much is known concerning BMP9. This prompted us to investigate the human endogenous sources of this cytokine and to further characterize its circulating form(s) and its function. Analysis of BMP9 expression reveals that it is mainly produced by hepatocytes and intrahepatic biliary epithelial cells. It is interesting to note that the liver is the main organ affected in the Rendu-Osler type 2 Syndrome (ACVRL1 mutations, HHT2), thus suggesting a possible local role for BMP9 in the development of the disease. Gel filtration analysis combined with ELISA and biological assays demonstrate that BMP9 circulates in plasma both 1) as an unprocessed inactive form that can be further activated by the furin convertase protease family and 2) as a mature and fully active form (composed of the mature form associated with its propeptide). Analysis of BMP9 circulating levels during mouse development demonstrates that BMP9 peaks during the first three weeks after birth and then decreases to 2 ng/mL in adulthood. We also show that circulating BMP9 physiologically induces a constitutive Smad1/5/8 phosphorylation in endothelial cells. Taken together, our results argue for the role of BMP9 as a hepatocyte-derived hormone, circulating in inactive (40%) and active (60%) forms, the latter constantly activating endothelial cells to maintain them in a resting state.

(B03) IMMUNOSUPPRESSOR FK506 (TACROLIMUS) INCREASES ENDOGLIN AND ALK1 EXPRESSION AND MODULATES TGF- β 1 SIGNALLING IN ENDOTHELIAL CELLS

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Patients suffering from HHT show severe epistaxis, and/or gastrointestinal bleeding, both of which notably interfere with their quality of life. The two predominant types of HHT HHT1 and HHT2 are caused by mutations in Endoglin (ENG) and ACVRL1/ALK1 genes, respectively. ENG and ALK1 code for proteins involved in the TGF- β 1 signalling pathway and it is widely accepted that

HHT pathogenicity results from haploinsufficiency. No cure for HHT has been found, so identification of drugs able to increase the expression of these genes is essential when proposing new therapies. We report the efficacy of Tacrolimus (FK506) in increasing ENG and ALK1 expression. The rationale comes from a case report of an HHT patient who received a liver transplantation following hepatic failure due to a liver arteriovenous malformation. The liver was transplanted and the immunosuppressor FK506 was used to prevent the rejection. Since the first month of FK506 treatment, the internal and external telangiectases, epistaxes and anaemia disappeared. Here we find that the immunosuppressor FK506 (tacrolimus) increases the protein and mRNA expression of ENG and ALK1 in cultured endothelial cells by stimulating the TGF- β 1/ALK1 dependent signalling pathway, and repressing the TGF- β 1/ALK5 counterpart. As a consequence of ALK1 activation, endothelial cell functions depending on it, like tubulogenesis and cell migration in wound healing experiments are potentiated. These results suggest that the mechanism of action of FK506 involves a partial correction of Endoglin and ALK1 haploinsufficiency and may therefore be an interesting drug for use in transplanted HHT patients.

GENETICS

(A13) WHY IS GENETIC SCREENING FOR HEREDITARY HEMORRHAGIC TELANGIECTASIA UNDERUTILIZED IN FAMILIES?

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Appropriate management of HHT reduces morbidity and mortality, but relies on identifying which family members are affected. Genetic testing usually can identify which at-risk asymptomatic relatives will need follow-up. Despite its clinical utility and cost effectiveness, genetic testing is only infrequently performed. To investigate barriers to genetic testing for HHT, we conducted 12 on-line discussion groups with 119 people with HHT or their family members. We found that even though the majority of participants believed that people with HHT and at-risk relatives should have genetic testing, most had not been tested. Multiple barriers to HHT genetic testing were identified including lack of knowledge about genetic testing, problems with access, and emotional barriers. Many participants: did not understand the rationale for HHT testing or the benefits of early detection; believed that HHT genetic testing is expensive and not covered by insurance; and believed that primary care providers don't know how to order genetic testing. Access to HHT testing is limited by distance from an HHT Center or a genetics clinic. Emotional barriers to genetic testing include fear of insurance discrimination; fear of being diagnosed with HHT; denial of having HHT or being at risk; guilt and stigma. We recommend the HHT Foundation develop and

distribute brief educational materials for patients and providers that describe the rationale for genetic testing, and emphasize the benefits of early detection and treatment. In addition, laboratories offering genetic testing for HHT should provide support for primary care physicians to order and interpret genetic tests.

(A25) A DEEP-SEQUENCING APPROACH TO IDENTIFY NEW GENES INVOLVED IN HHT

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Mutations of the Endoglin (ENG) and Activin A receptor type II-like kinase-1 (ACVRL1) genes are known to be the major genetic factors of HHT. Mutations in SMAD4, causing HHT in association with juvenile polyposis, have also been described. However, mutations are not found in about 20% of HHT cases. We investigated a pedigree where the proband was reported to have epistaxis, telangiectasias, pulmonary arteriovenous malformation and a familial history of HHT with 8 affected or potentially affected relatives over 4 generations. Direct sequencing and large rearrangements analysis of ACVRL1, ENG and SMAD4 did not identify any mutation in these genes. A multipoint linkage analysis was performed in this pedigree and confirmed that these 3 gene regions are unlikely to explain the familial segregation of the disease. Following the proof-of-concept that new causal genes for mendelian disorders can be identified by exome sequencing of a small number of affected individuals, we designed a two-generational filtering strategy specially adapted to a genetic disease transmitted as an autosomal dominant trait with complete penetrance. This strategy includes an intra-familial filtering step comparing sequenced data from unaffected and affected parents to their affected offspring. We performed peripheral blood DNA sequencing of all protein-coding regions (exome) in 4 selected individuals of the family using Agilent SureSelect exome hybridization capture and the Illumina DNA sequencing platform. Around 35,000 variants (SNP and INDEL) were identified in each individual. Successive filtering steps applied to detect variants should designate candidate variants for HHT in this family.

(A26) ACVRL1 GERMINAL MOSAICISM WITH TWO MUTANT ALLELES IN A FAMILY WITH HEREDITARY HAEMORRHAGIC TELANGIECTASIA ASSOCIATED WITH PAH

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Germline mutations in genes encoding members of the TGF-beta/BMP superfamily are causal for two hereditary vascular disorders, hereditary hemorrhagic telangiectasia (HHT) and heritable pulmonary arterial hypertension (PAH). When the two diseases co-exist, Activin A receptor type II-like kinase-1 (ACVRL1, also known as ALK1) mutations are mainly involved. We report a remarkable ACVRL1 germline mosaicism characterized by the presence of two mutant alleles in a woman initially diagnosed with PAH at age 21. She also met the Curaçao diagnostic criteria for HHT based on additional findings of telangiectases and epistaxis. Mutation analysis of ACVRL1 identified two closely located heterozygous deleterious mutations within exon 10: c.1388del (p.Gly463fsX2) and c.1390del (p.Leu464X). The two mutations were transmitted independently to the three sons of the index case. One son received the c.1388delG and two sons received the c.1390del. They all present symptoms of HHT, to varying degrees which needs to be interpreted with regards to their young age. Allele-specific PCR analysis demonstrated that c.1388del is the predominant mutation in lymphocytes of the index case. Haplotype analysis revealed that both mutant alleles have a common origin. The presence of these two distinct alterations might result of an incorrect repair of a larger de novo mutational event that occurred in the early stage embryo. An alternative hypothesis is that these two distinct alterations might be explained by a revertant mosaicism mechanism.

(B05) MUTATIONS FOUND IN THE DANISH POPULATION CAUSING HEREDITARY HEMORRHAGIC TELANGIECTASIA.

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HHT is an autosomal vascular disorder caused by mutations of either of three loci ENG, ACVLR1 or MADH4. HHT is characterised by recurrent nosebleeds, mucocutaneous telangiectases, and more severe visceral malformations. During the last decade the Danish HHT centre has been performing genetic screening of patients and relatives

with HHT. The molecular genetic screening serves dual purposes, a) as part of the clinical management as genotype/phenotype correlations exists, b) to identify asymptomatic family members. *Materials and Methods:* Inclusion of patient's who were talking part in the general clinical HHT management preogram. Bi-directional sequencing of all exons and exon-intron boundaries of the three loci ENG, ACVLR1 and MADH4 are performed to characterize minor genetic variants. MLPA analysis is performed for all three loci to characterize large rearrangements. *Results:* In 61 families we found mutations in either ENG (N=35) or ACVLR1 (N=26). In ENG a total of 22 different mutations were found 16 was unreported. In ACVLR1 24 different mutations were found 13 was unreported. The mutations were mainly of a familial character all though in ENG a single mutation is present in 11 families and 2 mutations are represented in 2 families. Likewise in ACVLR1 2 mutations was found in 2 different families. I ENG 1 and in ACVLR1 3 families had major deletions found by MLPA. No mutations were found in MADH4. *Conclusion:* The majority of mutations found during clinical genetic screening in HHT will be of a family specific kind. Proper analysis requires performing investigation for major deletions as well.

(D03) DUTY TO RE-CONTACT IN THE GENOMIC AGE

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Molecular testing for HHT often yields uncertain results, either because no genetic variation is discovered, or the pathogenicity of a genetic variant is unknown. Whether health professionals have a duty to re-contact patients when new or re-interpreted information becomes available has been debated across many medical specialties. The only policy statement on this matter aimed specifically at medical geneticists did not have the full support of the Board of Directors of the American College of Medical Genetics (Genet Med 1999;1:171-2). This statement anticipated rapid advances in human genetics, and placed most of the responsibility on the patient/family to request updated information. The discussion that I will present re-examines the obligation to re-contact patients in several clinical circumstances relevant to HHT, and suggests processes by which this can be conducted. The broad spectrum of situations includes, but is not limited to: reinterpretation of whether a nucleotide sequence variation is likely to be pathogenic; discovery of a new gene that either causes or predisposes to a condition for which evaluation was originally sought; returning to research subjects potentially clinically actionable information; and abnormalities on genomic arrays, whole exome scanning or whole genome sequencing that were outside the original indication for performing the study. The need for re-examining the issue of re-contacting is accentuated, not only by novel genetic technologies, but by electronic health records that can facilitate the transfer of updated information to the health professional and even to the patient.

(D05) CHARACTERIZATION OF ACVRL1 DELETION IN A LARGE ITALIAN FAMILY

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We collect a series of HHT family (including patients and their unaffected relatives) in which our screening methods (coding regions sequencing for both genes) did not identify any mutations. We reevaluated the clinical picture for all the index cases, confirming the diagnosis according to the Curaçao criteria for all of them and selected for further investigations the larger families. In the index case of a 16 members Italian family we found a deletion in ACVRL1 gene with MRC Holland SALSA MLPA kit PO93-B1 HHT/PPH1. We analyzed with this kit all the family members and confirmed the deletion of ACVRL1 exons 3, 4, 5, 6, 7 and 8 in all affected patients. We found cosegregation of the deletion with the disease within the family. This deletion spanned a region of at least 4 kb. To identify the breakpoints, we first designed primers pairs near ACVRL1 probe 2 and ACVRL1 probe 9 which were useful to identify an abnormal band. We then designed new primers pairs to narrow down the breakpoints, which were identified breakpoints in exon 3 and intron 8 confirming MLPA results. The deletion size is 4594 bp.

CLINICAL

(A22) THALIDOMIDE-RELATED WORSENING OF PRE-EXISTING RENAL DYSFUNCTION IN HEREDITARY HEMORRHAGIC TELANGIECTASIA: CASE REPORT AND DISCUSSION OF MECHANISMS

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Thalidomide is a glutamic acid derivate that has recently gained interest as an angiogenesis inhibitor in the treatment of erythema nodosum leprosum, hematologic malignancies and solid tumors. Isolated case reports show a beneficial effect of thalidomide in chronic GI bleeding in HHT, as it has demonstrable effects on the circulating levels of VEGF. HHT, is due to mutations in endothelium-specific components of the TGF-beta signaling pathway. We report the case of a male patient with HHT, who had worsening of his pre-existing proteinuria shortly after starting thalidomide. The urine albumin: creatinine ratio increased from 250-400 to >2,000 mg/mmol. Concurrent with thalidomide treatment the patient was

receiving daily albumin infusion (25 g/day) for severe hypoalbuminemia with intractable peripheral edema and ascites due to liver dysfunction. Because of worsening proteinuria, the albumin infusion was discontinued. A renal biopsy was also performed because the underlying cause of proteinuria was unclear. The biopsy revealed substantial endothelial damage and reduplication of the glomerular basement membrane with a pattern suggestive of membranoproliferative glomerulonephritis. Proteinuria returned to baseline levels upon discontinuation of albumin infusion. We conclude that the main cause for the sudden worsening of proteinuria was albumin overload in the setting of underlying glomerular injury. It is tempting to speculate that thalidomide and/or HHT were causally related to glomerular endothelial damage found on biopsy. Therefore, it seems to be important to evaluate renal filtration and permselectivity (proteinuria) functions prior to initiation of thalidomide and/or other VEGF inhibitors in the context of HHT.

C14) TROPHIC DISORDERS WITH BEVACIZUMAB IN HHT PATIENT

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Hereditary Hemorrhagic Telangiectasia (HHT) can cause recurrent occult gastrointestinal hemorrhages resulting in severe anemia in some cases. A 76-year-old HHT woman was hospitalized for anemia. Platelet aggregation inhibiting therapy had been recently introduced for popliteal artery thrombosis. The patient had also a long standing history of left recurrent venous ulcer and post-phlebitic syndrom. Physical examination revealed few mucocutaneous telangiectasias and lateral and medial malleolus skin ulcers. Screening for arteriovenous malformation (AVM) disclosed one pulmonary AVM, which was embolized. During procedure, arteriography demonstrated a pulmonary embolism. No liver AVM was found but extensive intestinal telangiectasias were seen on CT-scan and endoscopic video capsule. Whereas platelet aggregation inhibiting therapy was replaced by oral anticoagulant therapy, anemia remained severe (hemoglobin level : 65g/l), requiring bi-monthly red-cell transfusions. Lantreotide was given for 10 months, and then tamoxifen for 8 months without any result. During this period, the malleolus ulcers improved slowly, but the patient had chronic anemia and received 116 packed red blood cell units over a 24 month period. At this time, the venous ulcers were nearly completely healed. Then, bevacizumab (BVZ) (5mg/kg/2weeks) was given. After the 1st BVZ perfusion, the transfusion requirement dramatically decreased and the hemoglobin level remained stable for 6 months. However, following the second perfusion, the venous ulcers extended and even worsened despite of the BVZ discontinuation. Eighth months later, they are still present. This case underlines the BVZ efficiency for digestive bleeding in HHT. However, trophic disorders must be considered as a contraindication to BVZ.

(D01) SUDDEN UNEXPLAINED DEATH IN EPILEPSY: FAILURE OF MRI TO DETECT CEREBRAL ARTERIOVENOUS MALFORMATIONS

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A 17 year old male with definite HHT presented with a generalized seizure three years after diagnosis and initial treatment. Neurologic examination was entirely normal. EEG, ECG, and echocardiogram were normal except for residual intrapulmonary shunt. MRI's after presentation and three years previously showed only a small venous angioma in the left internal capsule. Cerebral angiography revealed two additional telangiectasia, and two AVM's – one in the left temporal lobe, and one in the left parietal lobe. CT angiography showed the same AVM's. He was discharged on an anticonvulsant pending decision about treatment. Five days later he had a generalized seizure, and after returning to a normal alert and ambulatory state, he died suddenly. Autopsy demonstrated that the cerebral AVM's had not bled, and that there were no cardiac abnormalities, vascular or otherwise. Sudden unexplained death in epilepsy is an uncommon but increasingly recognized complication, the cause of which remains obscure. In the present case, standard screening with cerebral MRI with gadolinium failed to reveal the presumably causative vascular abnormalities. A review of the surprisingly sparse literature reveals that, though earlier papers reported near-equivalence of MRI and cerebral angiography,^{1,2} later, screening-based papers showed considerable discrepancies in detection efficacy.^{3,4} This fact, emphasized by the lethal outcome in our patient, suggests that the HHT community should re-examine current CNS screening recommendations, and consider more extensive studies of cerebral AVM detection by MRI, CT angiography, and cerebral angiography.

(A11) NAILFOLD CAPILLAROSCOPY FINDINGS IN AN HHT SPANISH POPULATION

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Objective: To evaluate characteristics of nailfold capillary microscopy findings in a Spanish cohort of patients. **Methods:** One hundred and eighteen HHT patients were studied by 100x digital microscopy of hand nailfold inspection. Statistical analyses of epidemiological data, presence of telangiectasias in other typical locations and contrast echocardiography results were performed. **Results:** Sixty men and fifty eight women (medium age 47.3±14.455) were studied. Forty-two individuals were HHT type 1 and 76 type 2. Pathological findings were disclosed in 67 (56.8%) patients (60% males and 53.4% women) with increasing prevalence regarding age (90% of patients over 65 had anomalies) (p=0.024). Presence of megacapillaries was the most typical observation (49 cases). Differences regarding genetics were globally

significant with higher incidence of pathological findings in type 1 (81%) and 43.4% in type 2 (p < 0.001); after stratified analysis considering age, this difference remained significant in groups 31-50 (p=0.06) and 51-65 (p < 0.001). Presence of telangiectasias in additional locations was higher in patients with pathological capillaroscopy but only significant regarding lips (61.9% of patients with telangiectasias on lips had nailfold anomalies, p=0.017). Considering contrast echo, 60.4% of patients with pathological results showed capillary anomalies compared with 44.4% of patients with normal test (p=0.141). Comparing echo bubble pass, 55.1% of grade 1-2 and 77.3% of grade 3-4 had nailfold anomalies (p=0.063). **Conclusions:** HHT type 1 patients in middle ages present significant higher prevalence of capillaroscopy anomalies, this could be useful to develop management strategies in absence of molecular diagnoses based on capillary microscopy findings.

(A19) HEREDITARY HEMORRHAGIC TELANGIECTASIA: AN UNDER-DIAGNOSED DISEASE?

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Background. The prevalence of Hereditary Hemorrhagic Telangiectasia (HHT) is estimated to be at least 1 in 10,000, though there are few studies to date. Experts agree that HHT is under-diagnosed in North America, and that diagnosis is often delayed. **Objectives:** 1) To estimate prevalence of HHT in Ontario, Canada and 2) To study variations in prevalence across regions and age groups, as evidence of under-diagnosis. **Methods:** 556 patients with definite HHT diagnosis, seen at Toronto HHT Centre, were geocoded with postal codes. Prevalence rates in Ontario cities were calculated using Canadian census data, and stratified by age. **Results:** The overall prevalence was 0.3 cases per 5000. Prevalence rates varied amongst cities, from 0.06 to 1.1 cases per 5000. There were no significant differences between urban and rural prevalence rates. Prevalence variation was seen across age groups, with greater prevalence in older adults (50+ years) (0.40 per 5000), compared to the 20-49 age group (0.23 per 5000). Using the maximum prevalence of 1.1 per 5000, we estimate that as many as 70% of cases of HHT are undiagnosed. **Discussion and Conclusions:** HHT prevalence is estimated to be as high as 1 in 5000, but variation across regions and age groups suggests that under-diagnosis is an issue.

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(B24) CLINICAL AND GENETIC CHARACTERISTICS OF PATIENTS WITH HHT - THE NATIONAL ISRAELI CENTER EXPERIENCE

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Introduction: A national HHT center was established in Israel in 2006. Clinical and genetic characteristics of HHT and the prevalence of visceral AVMs in Israeli patients were not previously described. **Objectives:** To characterize the genetic and clinical manifestations of Israeli HHT patients. **Methods:** All patients referred to the Israeli HHT center were included. Diagnosis of definite and suspected HHT was made using the Curacao diagnostic criteria or genetic analysis when informative. Bubble echocardiography followed by chest CT when indicated was performed to detect pulmonary AVMs. Cerebral AVMs were detected by head MRI. **Results:** 193 patients (2-75y, median-37y; 32% < 19y) were included. 103 patients were diagnosed with definite HHT and 22 with suspected HHT. Familial genetic analysis confirmed the diagnosis of HHT1 in 21 patients and HHT2 in 33. Novel mutations were described in three families. No mutation was found in 28%. Pulmonary AVMs were detected in 40% of patients with definite HHT. The AVMs were asymptomatic in 50% of patients. Nineteen patients demonstrated significant pulmonary complications (cyanosis, hemothorax, brain abscesses, strokes). Pulmonary AVMs were more common in HHT1 than HHT2 ($p < 0.05$). Cerebral AVMs were detected in 10%, mostly small and asymptomatic. 27 patients with pulmonary AVMs were treated with pulmonary artery embolization with improvement and no sequelae. **Conclusions:** Visceral AVMs, frequently asymptomatic, are common in Israeli HHT patients. Patients with HHT or with a family history of HHT should be screened for visceral AVMs, since morbidity is significant and treatment is usually successful.

(C17) MID-INFRARED SPECTROSCOPY, AN ALTERNATIVE IN HHT DIAGNOSTICS

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HHT diagnostic is laborious, time consuming and costly. A patient is considered to have HHT with at least 3 out of the following 4 criteria: spontaneous, recurrent epistaxis; multiple telangiectases (lips, oral cavity, fingers, nose); visceral lesions (GI-telangiectasia, PAVMs, HAVMs, CAVMs); or an HHT-affected first degree relative. The disease shows an inter- and intra-family penetrance variability, and penetrance is also depending on age. Therefore, not all typical symptoms may be present, especially at a younger age. Aside of clinical diagnosis by different methods, genetic testing for the disease genes endoglin,

ALK1 or Smad4 is a further but also costly option. The standard of care requires a knowledge of the disease among general clinicians and access to appropriate diagnostic tests. Therefore, it would be desirable to have a diagnostic tool at hand that facilitates diagnosis. A tool that will lead to an earlier, faster, and cheaper diagnosis. Infrared (IR)-spectroscopy has emerged as an alternative diagnostic tool for different diseases and other purposes. IR-spectroscopy is based on the detection of molecular vibrations in different chemical groups of i.e. proteins, lipids, nucleic acids. The idea behind using IR-spectroscopy in diagnostic is that the disease leads to a change in group composition of the different molecules, in for example blood plasma or serum, generating a disease-specific IR-fingerprint, different from the spectrum of healthy people. In preliminary experiments with 86 patients, an HHT-specific IR-fingerprint was established with a sensitivity and specificity of 98%. These analyses are on going and results will be presented.

IR-spectra of 86 HHT patients and 91 healthy non-HHT persons. Spectra of non-HHT people are in blue and HHT patients are in red. A. Shown are the spectra of all plasma samples after 2. derivation analysis. B. Shown is the average spectrum of 91 non-HHT people (blue) and the average spectrum for 86 HHT patients (red) after 2. Derivation analysis. Clear differences between the reference group and HHT patients are encircled.

(C19) IS SUPINE AND UPRIGHT PULSE OXIMETRY A USEFUL SCREENING TOOL FOR PULMONARY ARTERIOVENOUS VENOUS MALFORMATIONS IN CHILDREN WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA?

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Different screening procedures for pulmonary arteriovenous malformations (pAVMs) including testing for orthodeoxia have been proposed in HHT patients. Pulse oximetry is often used as a noninvasive tool, but limited data are available about its diagnostic role in children. We here report our experience with measuring oxygen saturation in both supine and upright position in children with HHT. To date, 37 pediatric patients with genetically confirmed HHT (mean age $\pm$ SD: 7.4 $\pm$ 4.3 years; range 0.8 to 16 years) were included in this analysis. Oxygen saturation in both the supine and upright position was studied using a Nellcor oxymeter with either a pediatric spot check clip or probe. Comparisons were made between 17 patients with pAVM based on positive contrast echocardiogram subsequently confirmed by CT and 20 patients with negative imaging studies. 6 patients with positive studies subsequently underwent embolisation procedures and were

analyzed as a subgroup. Oxygen saturations in the supine position were not significantly different between the two groups overall, but significantly lower in the subgroup that subsequently underwent embolisation procedures ($p < 0.01$, t-test). Median oxygen concentrations were 99% in both supine and upright position in HHT children without pulmonary AVMs and 98% in both positions in children with pulmonary AVMs. No patient had a positional change in oxygen saturation of more than 2%. Based on this preliminary analysis testing oxygen saturations supine and upright does not offer significant information to identify pediatric patients with pAVMs.

(A12) OPHTHALMOLOGICAL ANOMALIES IN HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Objective. To describe ophthalmological findings in a Spanish cohort of HHT patients by current external examination of conjunctiva and iris and direct ophthalmoscopy after pupil dilatation with slit lamp and not contact lense to observe vascularization of the retina. **Results.** One hundred and eighty seven HHT patients (95 men and 92 women) were evaluated from 2004 to 2010 by ophthalmologist in the HHT unit in Hospital Sierrallana. Medium age was 47.04 ± 14.905 (range 6-79). Considering genetics, 69 individuals were HHT type 1 and 118 type 2. Pathological findings were disclosed in 81 patients (43,3%) with no significant differences regarding sex (44,2% men and 42,4% women), and presented in all cases with conjunctival telangiectasias (isolate or multiple) with no retinal involvement. Bleeding was reported by four patients. Prevalence significantly increased with age (11,1% in patients under 31, 45,3% in the range 31-50, 47,8% in 51-65 and 68,8% in older than 65) ($p=0.001$). Ocular lessions were slightly more prevalent in HHT1 patients (47.8%) than in HHT2 individuals (40.7%), with not significant difference ($p=0.341$). Presence of telangiectasias in additional locations (face, oral, lips, fingers, nose) was always higher in patients with pathological ophthalmological exploration but never significant. **Conclusions:** Ophthalmological involvement with conjunctival telangiectasias is common in HHT and increases with age. No differences regarding sex or genetic type are observed. Bleeding is unusual and retinal lessions were not observed in this series.

(A10) EVALUATION OF CORRELATION BETWEEN HEREDITARY HEMORRHAGIC TELANGIECTASIA EPISTAXIS SEVERITY SCORE (HHT-ESS) AND SADICK SCALE

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Objective. To evaluate correlation of the validated HHT-ESS with Sadick scale used currently up to date in our HHT unit compared also with self perception in severity of epistaxis and quality of life. **Methods:** One hundred HHT patients chosen at random from database were interviewed by phone evaluating HHT-ESS, Sadick scale (frequency and quantity ranging from 1 to 3, categorized in mild, moderate or severe), self reported epistaxis severity and quality of life by using termometer of Euroqol 5D. **Results:** Forty four men and fifty six women were included with mean age $49.66 (\pm 13.873)$; 46 patients were HHT type 1, 52 patients HHT 2 and two had no identified mutation. Self perception of severity was mild in 48%, moderate in 42%, and severe in 10% of cases. Mean HHT-ESS was of $3.66 (\pm 2.37)$. Ponderated Kappa for cathegoric variables showed moderate-substantial correlation between HHT-ESS and Sadick scale (0.58997 with 95% confidence interval 0.652 to 0.7147) better in men with no differences regarding genetics. Sadick scale showed 18% discordant exceeding severity values, 19% in women and 27% in patients over 65. Correlation between self perception and Sadick scale was moderate-substantial as it was with HHT-ESS (better for extreme ages). Correlation of Euroqol 5D was significative ($p=0.001$) with Sadick scale ($r=0.63$) and HHT-ESS ($r=0.55$). **Conclusions:** Sadick scale seems to overestimate severity of epistaxis mainly in women and elderly population compared with HHT-ESS, although correlation of both scales is moderate.

(B10) DISCUSSION ON THE ITALIAN GUIDELINES FOR THE TREATMENT OF HHT EPISTAXIS

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Epistaxis is the most frequent symptom in HHT affecting more than 95% of patients. In 2009 an italian experts board has developed guidelines for the treatment of HHT epistaxis based on literature review and clinical experiences in order to structure decisional algorithms in case of acute or chronic nosebleeding. In case of acute not self-limiting epistaxis, nose pinching and nasal medications with fat gauces or haemostatic ointments are the first line therapeutical procedures to consider, leaving nasal packing, nasal embolization or other invasive surgical procedures in case of failment. Authors have proposed different grading scores for the severity of nosebleeds varing from epistaxis limited to few blood spots on an handkerchief to

severe haemorrhages. The treatment of chronic epistaxis must consider both the severity of epistaxis and the influence of epistaxis on patient's quality of life. Mild epistaxis generally benefit from daily nasal medications with lipidic ointments and fat gauces. In case of moderate epistaxis not responsive to these medications, minimally-invasive treatments with lasers, Argon Plasma, antifibrinolytic and hormonal therapies must be considered. Invasive procedures such as septodermoplasty, brachytherapy and Young's procedure represent the last choice to consider when severe epistaxis failed to benefit from the other therapeutic options exposed.

(B11) ENDOSCOPIC TREATMENT OF HHT EPISTAXIS: COMPARATIVE ANALYSIS OF FIVE SURGICAL DEVICES

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The treatment of the HHT epistaxis is an unsolved clinical problem. Multiple therapeutic approaches have been proposed and, as suggested by Geithoff et al., they can be distinguished in destruction of telangiectasia, reduction of blood flow, reduction of trauma, improvement of protection and control of fibrinolytic activity. Nowadays the management of chronic recurrent epistaxis in HHT has mainly focused on procedures that maintain integrity of the nasal fossa but although that, there have been no controlled or well designed comparative studies of any of these procedures. Since 1996 to 2010, 380 HHT patients, diagnosed according to the Curaçao criteria, have been hospitalized in the ENT Unit of IRCCS "Policlinico S. Matteo" in Pavia. Of these, 252 underwent surgical procedures for their epistaxis. Here we present our first impressions about five different devices that we recently tested. We show the characteristics of ArgonPlasmaCoagulation, diode-laser, thulium-laser, PK-Diego shaver, Tissuelink monopolar, underlining the advantages and disadvantages of each one.

(B28) THE NATURAL HISTORY OF EPISTAXIS IN PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGECTASIA IN THE NORWEGIAN POPULATION

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Background: Epistaxis is usually the first and most common symptom in hereditary hemorrhagic telangiectasia (HHT). The severity of HHT associated epistaxis is highly

variable and can affect the patient's quality of life. In the literature, the natural history of epistaxis in HHT patients has been described in a few countries but not from the Norwegian population. **Objective:** This work focused on the natural history of epistaxis in the Norwegian population. **Material and Methods:** 98 patients with 3 or 4 Curaçao criteria were included. The severity of epistaxis was graded depending on epistaxis intensity, frequency and the amount of blood transfusion during a period of 4 weeks. The epistaxis grades were studied in association to age, gender, gene mutation, age of onset and whether the patient had or had not been treated for epistaxis during the last 2 years. **Results:** 90% of the patients complained of mild to moderate epistaxis. 77% of the patients started epistaxis by or before the age of 20 years. The progression of HHT associated epistaxis with age could not be proved. There was no significant difference in the grades of epistaxis between HHT1 and HHT2 type, neither between females and males. There was a significant difference in the grade of epistaxis between non ENG, non ALK1 carrier patients and ENG or ALK1 carrier patients. **Conclusion:** Compared with other populations, the grading of epistaxis in Norwegian patients with HHT gave generally similar results. A multi-centre epidemiological study is required to get a larger study population.

(D20) FEASIBILITY OF PROMOTING HHT DIAGNOSIS IN ENT CLINICS

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Background: Experts agree: HHT is under-diagnosed and often delayed. From recent survey of HHT patients, 40% consulted ENT for epistaxis, mean 14 years pre-diagnosis. **Long-term goal:** Improve rates of diagnosis through ENT physicians, specifically through, pilot project educating two ENT clinics and displaying poster entitled "Recognizing HHT, Easy as 1-2-3" in waiting rooms. Current study goals: develop poster, obtain feedback from relevant stakeholders, review feasibility. **Methods:** HHT Centre team developed draft poster. Orally presented at the HHT Foundation, 15th National Patient and Family Conference and displayed publicly during conference. Feedback requested by questionnaire from all attendees and informally (verbal) welcomed. **Results:** Received feedback from 67 individuals: 61% patients, 22% health care providers, 31 % family members. Respondents said YES that poster would help physicians diagnose HHT (91%), that poster would help patients ask doctor about HHT (95%), that poster would help recognize HHT in themselves (82%) or relatives (77%). 94% said NO, poster was not too simple. Some suggested poster should show subtle telangiectasia, others felt it was physician focused. The poster was modified accordingly. We polled our HHT ENT physicians and pilot centre; they agreed the proposed project feasible. In summary, proposed approach to promoting HHT diagnosis in ENT

clinics appears feasible and therefore will proceed with pilot in ENT clinics.

Funding (MEF): Nelson Arthur Hyland Foundation, Li Ka Shing Knowledge Institute

(C29) FAMILY MEMBERS OF PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA: HHT CENTER PARIS EXPERIENCE IN 343 RELATIVES FROM 116 FAMILIES WITH GENOTYPING

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Objective: to assess the clinical and genetic characteristics of family members of patients with hereditary hemorrhagic telangiectasia (HHT). **Setting.** HHT Center in Paris. **Participants:** 343 members family of 116 genotyping HHT patients screened from January 2004, through December 2010. **Interventions:** volunteers previously well informed directly by means of educational course of the proband about the intrafamilial variability of symptoms and potential severe visceral complications; screened during one half a day by mean of a stepped multidisciplinary clinical screening protocol based upon Curaçao criteria and genetic screening systematically proposed. **Main outcome measures:** age, sex, prevalence of cutaneo mucous telangiectases, visceral arteriovenous malformations (AVMs), genetic characteristics and proposed therapeutics. **Results:** 182 relatives were found with manifestations of HHT. Below twenty years old: 34%, between twenty and forty: 31%, over forty: 35%. Sex ratio: 0.75. All four Curaçao criteria were present for 123 cases, three criteria for 30 cases, two criteria for 17 cases and 12 cases (6,6%) were asymptomatic with only the familial criteria and found by mean of the genetic focused screening. 108 were relatives of the 66 HHT1 probands. 40 of them were referred for embolization of large pulmonary arterio venous malformations (PAVMs); 74 were of the 50 HHT2 probands, one of them, proposed for cerebral AVMs embolization. Antibioprophylaxis was proposed for 123 patients with small PAVMs. **Conclusions.** More than half of HHT family members present with visceral AVMs which can lead to life-threatening events. They should be encouraged to engage in a screening program, from earliest age, since the prevalence of potentially serious localizations is higher than previously thought.

(D19) DYSPLASIA AND MALIGNANCY IN THE LUMINAL GASTROINTESTINAL TRACT IN PATIENTS WITH SMAD4 MUTATIONS AND JUVENILE POLYPOSIS-HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Hereditary Hemorrhagic Telangiectasia (HHT) is an autosomal dominant disease which is usually associated with mutations in Endoglin and activin receptor like kinase 1 genes, characterized by telangiectasias and arteriovenous malformations. Juvenile polyposis (JP) is a syndrome characterized by hamartomatous polyps throughout the gastrointestinal tract, with increased risk of malignancy. SMAD4 and BMPR1A mutations can be identified in JP. A recently described SMAD4 mutation-associated syndrome combines the features of HHT and JP. This cohort of 16 JP-HHT patients, we report six patients (three male, three female, average age 27.5 years) who developed dysplastic and malignant colonic lesions. Two patients had focal dysplasia within juvenile polyps. Two presented with colorectal carcinoma with no previous diagnosis of dysplasia. Two patients developed invasive adenocarcinoma 1 year after dysplastic lesions were identified on colonoscopy. One of these patients also had one juvenile polyp with a focus of signet-ring type intramucosal carcinoma. Most of the patients had only few juvenile polyps detected (3-5 polyps), but one patient had multiple (>50 polyps). Though patient numbers are small, the carcinoma rate appears elevated and is presenting at an early age. Also, in two patients, the time period between the detection of colonic dysplastic lesions and adenocarcinoma was short (1 year). These results emphasize the importance of early recognition of the combined JP-HHT syndrome due to the need for close surveillance of these patients for the development of gastrointestinal malignancies at early ages.

(A01) HEPATIC MICROBUBBLE ULTRASONOGRAPHY CONTRAST IN HEMORRHAGIC HEREDITARY TELANGIECTASIA WITH LIVER INVOLVEMENT. A PROMISING TOOL?

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Introduction. Liver vascular malformations are present in 50-78 % of HHT patients usually screened with Doppler Ultrasound, but CT scan has a better sensitivity. We proposed to use contrast-enhanced ultrasonography (CEUS) to improve depiction of vascular abnormalities. **Methods:** Prospective single-center study. Three groups were defined: group 1 (non HHT patients without liver disease), group 2 HHT patients without liver involvement (normal hepatic ultrasound and hepatic artery (HA) diameter < 6 mm) and group 3 (HHT patients with liver involvement

(abnormal hepatic ultrasound and HA diameter ≥ 6 mm)). CEUS used a low-MI technique with i.v boluses of SonoVue® (Bracco, Italy; Toshiba AplioXG®). After injection, the following data were recorded: vascular spots, arterial contrast enhancement, transit time between the hepatic artery and hepatic veins, transit time between the hepatic artery and portal veins, enhancement curve (during a minute record on a sagittal section including hepatic artery, portal vein, and supra-hepatic veins), enhancement evaluation, maximum enhancement time, area under the curve, decreasing curve, target resetting and weighting by intensity enhancement. **Results:** 10 patients were in group 1, 11 in group 2 and 38 patients in group 3. vascular spots, arterial contrast enhancement, transit time between the hepatic artery and hepatic veins and parenchyma enhancement curve were significantly different in these groups ($p < 0.001$ for all). **Conclusion:** CEUS is a promising approach in the non-invasive characterization of HHT liver lesions and can be useful for evaluating anti-angiogenic treatments.

(C26) IMAGING FOR HEPATIC MANIFESTATIONS AND COMPLICATIONS OF HEREDITARY HEMORRHAGIC TELANGIECTASIA: A PICTORIAL REVIEW

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Hereditary hemorrhagic telangiectasia (HHT), an autosomal dominant disorder of variable penetrance, is characterized by the presence of mucocutaneous and visceral vascular malformations (VMs). Hepatic imaging is undertaken in HHT patients with suspected complications of liver VMs, but is also useful to document visceral VMs for the purpose of confirming a clinical diagnosis of HHT. This project reviews the sonographic, CT, and MR imaging features of the hepatic manifestations of HHT including intrahepatic shunting (arteriportal, arteriosystemic and portosystemic), vascular lesions (telangiectasia, confluent vascular masses and focal nodular hyperplasia), and potential complications (high output cardiac failure, portal hypertension, cholangiopathy and atypical cirrhosis).

(D11) IS THE DIAMETER OF THE HEPATIC ARTERY A GOOD PREDICTOR OF LIVER INVOLVEMENT IN HEREDITARY HEMORRHAGIC TELANGIECTASIA PATIENTS WITHOUT CLINICALLY SIGNIFICANT LIVER DISEASE?

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Objective: To evaluate the usefulness of the common hepatic artery (CHA) diameter as a predictor of liver

involvement in patients without significant liver disease according to analytical and radiological findings. **Material and methods:** 112 HHT patients (66.7% women, mean age 48.7 years, 76% type 2 HHT) with a CT performed to detect liver vascular malformations (LVM) defined as: telangiectases, confluent vascular masses (CVM), perfusion anomalies and/or intrahepatic shunts (IS). The diameter of the CHA was measured 1 cm distal to its origin in the celiac trunk. **Results:** 53.6% patients had LVM: telangiectasias (66.7%), CVM (41.7%), perfusion anomalies (41.7%) and IS (28.3%). 79 of the 112 patients had a measurement of the CHA diameter on CT; 37 in the group of LVM. When we compared the diameter of the CHA between patients with and without LVM we found a statistically significant difference between the two groups (6.9 and 5.9 mm respectively, $p=0.011$). An association between the diameter of the CHA and the presence of more than one type of LVM was also found. However a clear cut off to differentiate between patients with or without LVM was not assessed. 7 mm diameter of the CHA had a sensitivity, specificity, PPV and NPV of 48.6%; 76.2%; 64.3% and 62.7 % respectively. **Conclusions:** In patients without significant liver disease there is an increase in CHA diameter when LVM are present. However, it is difficult to establish a clear cut off to differentiate between patients with or without LVM.

(D12) ARE VASCULAR ANATOMIC VARIANTS A FREQUENT FINDING IN PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA?

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Objective: To evaluate the prevalence of vascular anatomic variants in the origin of the hepatic artery or its branches. **Material and methods:** 112 HHT patients (66.7% women, mean age 48.7 years, 76% type 2 HHT) with a CT performed to detect abdominal vascular malformations. Vascular anatomic variants in the origin of the hepatic artery (HA) and its branches were classified according to Michels classification. **Results:** 47.3% of the patients had an anatomic variant in the origin of the HA or its branches. According to Michels classification, type III (the HA has its origin in the superior mesenteric artery) and type II (left HA has its origin in the left gastric artery) were the most frequent types. When compared patients with or without vascular anatomic variants in the origin of the HA, we found that patients with Type II HHT have more frequently vascular anatomic variants than those with type I HHT: (58.5% and 38.3% respectively). This difference was statistically significant ($p = 0.026$). **Conclusions:** Vascular anatomic variants in the origin of the HA or its branches are more frequent than previously described. Patients with HHT type II appear to have this finding more frequently.

(A21) COMPARISON OF TRANSCRANIAL DOPPLER BUBBLE STUDY TO ECHO BUBBLE STUDY FOR SCREENING OF PULMONARY ARTERIOVENOUS MALFORMATION IN PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Approximately 30-50% of patients with HHT have pulmonary arteriovenous malformations (AVMs), which can cause hypoxemia, hemorrhage, and paradoxical embolization. All patients with HHT should be screened for pulmonary AVMs, but the optimal screening method remains uncertain. The currently recommended screening test is transthoracic echocardiography with intravenous microbubble injection (TTE bubble study). A positive study is followed by a chest CT for confirmation and characterization. Although minimally invasive and sensitive for pulmonary AVM, TTE bubble studies can be difficult to interpret due to cardiac motion, and false positive results are frequent. This can result in unnecessary CT scans in patients who do not harbor detectable pulmonary AVMs. Transcranial Doppler ultrasound with intravenous microbubble injection (TCD bubble study) is an alternative screening test, wherein the ultrasound probe insonates the carotid artery from a transcranial approach. Recent studies suggest that the TCD bubble study is superior to the TTE bubble study for detection of right-to-left shunting from atrial septal defects. Our hypothesis is that the TCD bubble study may be more accurate than the TTE bubble study for the detection of right-to-left shunting from pulmonary AVMs in HHT patients. In this study, 10 patients with known HHT will receive both TTE and TCD bubble studies. Patients with a positive result on either test will undergo a confirmatory CT scan, which will act as the gold standard for presence or absence of pulmonary AVM. A comparison between the two screening modalities will then be performed.

(A27) DETECTION OF REPERFUSED PAVM IN CONTRAST-ENHANCED MAGNETIC RESONANCE ANGIOGRAPHY AFTER CATHETER EMBOLIZATION IN PATIENTS WITH HHT

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Purpose: To demonstrate the detectability of reperfused PAVM after coil embolization by contrast-enhanced magnetic resonance angiography (CE-MRA) in patients with HHT. **Methods and Materials:** 259 patients (mean age 46.3, male 106, female 153) with confirmed HHT according to Curacao criteria or their first degree relatives underwent screening pulmonary CE-MRA (Gadolinium-BOPTA 0.1 mmol/kg bodyweight) for PAVM. Detection of rPAVM was based on CE-MRA (Gadolinium-BOPTA 0.1 mmol/kg bodyweight) and confirmed by pulmonary catheter angiography (DSA). **Results:** 67 patients under-

went embolization and initial complete occlusion of 152 PAVMs. Follow-up CE-MRA at 3, 12, 24 months and up to 7 years revealed reperfusion of 22 embolized PAVMs in 19 patients. All patients with rPAVMs underwent re-embolization, which detected no additional rPAVMs. Reperfusion was caused by corrosion of tungsten filaments which are no longer used; insufficiently packing of the embolization coils; and opening of collateral feeding vessels, which were not present on screening CE-MRA and 3 months after embolization. Due to overlying embolization materials CE-MRA was especially helpful for the re-embolization of complex PAVMs to identify reperfused vessels. **Conclusion:** Embolization-therapy is safe and efficient. Tight packing is a prerequisite in coil-embolization for sufficient and durable occlusion. Despite initial successful embolization, reperfusion may occur at later time, so regular follow-up is mandatory. CE-MRA revealed high accuracy for this purpose as compared to catheter angiography considered as the gold standard and may be preferable to CT-angiography due to lack of radiation and beam hardening artifacts.

(B04) AGITATED SALINE VERSUS GELOFUSINE FOR ECHOCARDIOGRAPHIC SCREENING OF PULMONARY ARTERIOVENOUS MALFORMATIONS IN PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Aim: Agitated saline solution (SS) transthoracic contrast echocardiography (TTCE) has been recommended as the screening test of choice for pulmonary arteriovenous malformations (PAVMs) in hereditary hemorrhagic telangiectasia (HHT). Gelofusine (GF) is an alternative contrast, which exhibits a higher density and stability of microbubbles. We aimed to compare both contrasts in a HHT population to prospectively determine the predictive value to detect PAVMs on high-resolution chest CT. **Methods and results:** 76 consecutive HHT patients underwent both SS and GF TTCE for PAVMs screening. Positive TTCE tests were categorized into to four grades according to the classification model of Barzilai et al. Two patients with patent foramen ovale were excluded. Mean age was 47±18 years; 57% were female; 41.8% had HHT type1 mutation, 36.4% HHT type2 and 21.8% an undetermined or unknown mutation. SS TTCE was positive in 52 patients (70.3%) and in 65 (87.8%) with GF. Chest CT showed PAVMs in 17 (23%) patients. All patients with negative TTCE had a normal CT (NPV 100% for both SS and GF). A positive SS TTCE had 100% sensitivity and a PPV of 33.7 %, and a positive GF TTCE showed a sensitivity of 100% and a PPV of 26.2%. When the grades were considered, the PPV for SS and GF were 3.65% y 0% for grade 1, 45.5% and 21% for grade 2, 71.4% and 40% for grade 3, and 100% and 81.8% for

grade 4 respectively. *Conclusions:* SS TTCE appears to be superior to GS as a screening tool for PAVMs.

(B20) RADIATION EXPOSURE IN HHT PATIENTS WITH DOCUMENTED PULMONARY ARTERIOVENOUS MALFORMATIONS

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Hereditary hemorrhagic telangiectasia (HHT) is a rare hereditary disorder which may be complicated by clinically significant arteriovenous malformations (AVMs) in the brain, lung, gastrointestinal tract and liver. Recently published International HHT Guideline recommendations, as well as HHT Centre clinical protocols involve imaging with ionizing radiation, for diagnosis and long-term follow-up of patients with pulmonary AVMs. As such, patients with HHT may undergo repeated imaging procedures over a lifetime and may be exposed to a cumulative dose of radiation much higher than the average population. Radiation exposure is an established cause of many cancers, and there is evidence from observational studies of excess risks from fractionated exposures in the dose range that would be received from repeated computed tomography (CT) scans. The purpose of this study is to determine the radiation exposure from diagnostic and therapeutic imaging procedures in a population of patients with HHT who have PAVMs. We have modeled radiation exposure for a standard HHT patient with an identified PAVM. The patients would undergo five thoracic CTs, three chest x-rays and 1.1 pulmonary angiography procedures (as 10% will require repeat angiography for reperfusion of PAVMs) over a ten year period, resulting in an expected effective dose of 28.1 mSv, with a probable range of 16.1 - 50.1 mSv. We will present the current model as well as validation of this model in a population of 200 HHT patients with PAVMs in our HHT Centre Database.

(B22) PULMONARY INVOLVEMENT IN HEREDITARY HEMORRHAGIC TELANGIECTASIA: PRE-THERAPEUTIC AND POST-EMBOLIZATION ASSESSMENT USING MULTIDETECTOR COMPUTED TOMOGRAPHY AND ANGIOGRAPHY

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Purpose. To describe imaging findings before and after embolization of pulmonary arteriovenous malformations (PAVMs). To illustrate the blood supply to embolized PAVMs and the possible mechanisms of recanalization. *Materials and methods.* Pictorial review based on our pluridisciplinary consultation for HHT patients. All consecutively seen patients with HHT were evaluated using enhanced multidetector computed tomography (MDCT).

All embolized patients were evaluated using MDCT, and occasionally with angiography when re-embolization was indicated. *Results.* During a 10-year period, a total of 767 patients with HHT belonging to 435 HHT families were evaluated at our center. From our database, 345 patients presented PAVMs detected using MDCT and 188 were embolized. According to the classification of PAVMs, we differentiated simple, complex, telangiectatic, multiple discrete and diffuse PAVMs. Special attention had been paid to accessory feeding arteries, initial systemic supply, thrombosis, calcifications, stenosis, signs of rupture, and signs of pulmonary hypertension. After embolization, the retraction and the contrast enhancement of the embolized PAVM were analyzed, and an enlargement of systemic arteries was evaluated. After embolization, hemoptysis may be related to traumatic injury, a pulmonary infarction, and a systemic supply, originating from bronchial and non-bronchial arteries. *Conclusion.* A contrast-enhanced MDCT is useful for the pre-and post-embolization assessment of PAVMs. MDCT facilitates the distinction between a persistent right-to-left shunt through the PAVM, and a left-to-left shunt towards the draining vein of the PAVM originating from systemic arteries.

(C02) IMPACT OF PULMONARY ARTERIOVENOUS MALFORMATIONS ON RESPIRATION-RELATED QUALITY OF LIFE IN PATIENTS WITH HHT

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To evaluate the impact of pulmonary arteriovenous malformations (PAVM) on the respiration-related quality of life in patients with HHT, we prospectively recruited 28 consecutive patients. The quality of life was measured using the respiratory Saint Georges' respiratory questionnaire (SRGQ; expressed as percent). Participants were divided into 3 groups: group A comprised patients with no PAVM detected on CT-scan (n= 6; 3 females; age: 47.7±15.4 yrs (mean + SD); group B included patients with one or more PAVM on the CT-scan, not suitable for embolisation based on the diameter of their feeding artery (n= 8; 6 females; age: 37.9±12.0 yrs); group C was composed of patients with one or more PAVM on CT-scan who underwent a procedure of embolisation of at least one PAVM (n= 14; 10 females; age: 45.2±20.0 yrs). In this group, quality of life was evaluated before embolisation. The SGRQ score was 7.5±6.2%, 10.0±9.7% and 23.9±17.3% in groups A, B and C respectively (p < 0.03). There was a significant difference between groups C and A, and between groups C and B, but not between groups A and B. We conclude that the presence of PAVM necessitating embolisation but not of "small" PAVM impairs the respiration-related quality of life in patients with HHT.

(Supported by AMRO)

(C04) PULMONARY THROMBOEMBOLI MODIFYING THE NATURAL HISTORY OF PULMONARY ARTERIOVENOUS MALFORMATIONS

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Introduction: It has been presumed that auto-embolization of pulmonary arteriovenous malformations (PAVMs) by pulmonary emboli (PE) should occur, but radiological and physiological data has not been captured. **Case history:** A 64-year-old man was referred for PAVM embolization. CT scans delineated multiple large PAVMs particularly at the right base. Resting oxygen saturations (SaO₂) on air had been 70-75% for at least 5 years. Acute pulmonary thromboemboli were diagnosed at the time of admission for pulmonary angiography 26 days later (Figure 1). The SaO₂ had risen to 90% on air, accompanied by a symptomatic improvement. Embolization of small non-occluded PAVMs was performed without complication. Further investigations over the next 24 hours identified no source of thrombus or evidence of malignancy, but there was CT evidence of paradoxical emboli, with new splenic and renal infarcts. The decision regarding anticoagulation was difficult, but in view of a renal vein thrombosis in his single, partially infarcted, kidney, and acute kidney injury, he was anticoagulated with tinzaparin. Eighteen days later, he presented with abnormal neurological signs, and SaO₂ 70% breathing room air. Subsequent pulmonary angiography demonstrated recanalization of the previously auto-embolized PAVMs. Six feeding arteries ranging in diameter from 3 to 9 mm were embolized, leading to SaO₂ of 92%, maintained four months post embolization. **Conclusion:** The striking images in this case provide the clearest evidence to date for auto-embolization of PAVMs by pulmonary thromboemboli, illustrating potential consequences from the interplay between pathological thrombi and PAVMs.

Roked et al, AJRCCM 2011 in press.

(C27) EXPERIENCE WITH THE USE OF AZUR HYDROCOIL EMBOLIZATION SYSTEM IN PULMONARY ARTERIO-VEIN MALFORMATIONS

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We evaluated our experience with the use of Azur hydrocoil embolization system (Terumo). Eleven pulmonary arterio-venous malformations (PAVMs) were treated in eight patients. Five malformations had been previously treated and data suggesting recanalization appeared during follow-up. Complete occlusion of all PAVMs was achieved in all patients (Figures 1a & 1b).

Preliminary results suggest that Azur hydrocoil offers a good option to treat PAVMs.

(D25) PARTIAL OXYGEN PRESSURE IS NO PREDICTOR FOR MIGRAINE WITH AURA IN PATIENTS SCREENED FOR HEREDITARY HEMORRHAGIC TELEANGIECTASIA

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Objective: Migraine with aura (MA) is associated with the presence of pulmonary arteriovenous malformations (PAVM) in patients with hereditary hemorrhagic teleangiectasia (HHT). We evaluated whether the partial oxygen pressure (pO₂) was a predictor for MA. **Methods:** All persons, screened for HHT between May 2004 and December 2010, who filled in the validated headache questionnaire and underwent pO₂ measurement, were included. **Results:** In total 703 persons were screened for HHT during the study period. A headache questionnaire was filled in correctly by 626 persons (89.0%). A pO₂ was measured in 585 out of the 626 persons, and these were included for analysis. The mean age was 44.0±15.3 years and 61.8% were female. The mean pO₂ was

12.1±1.8 kPa and 11.5% suffered MA. Using univariate analysis, female sex (OR 2.28: 95%CI 1.25–4.17, p=0.007), low pO₂ (OR 0.76: 95%CI 0.76–1.00, p=0.05), and PAVM on chest CT scan (OR 3.80: 95%CI 2.20 – 6.58, p<0.001) were all predictors for the presence of MA in patients screened for HHT. Only female sex (OR 2.29: 95%CI 1.22–4.30, p=0.01) and PAVM on chest CT scan (OR 3.47: 95%CI 1.87–6.41, p<0.001) were independent predictors for MA in patients screened for HHT. *Conclusion:* The partial oxygen pressure was no independent predictor for migraine with aura in patients screened for hereditary hemorrhagic teleangiectasia.

(D29) GRADE OF RIGHT-TO-LEFT SHUNT USING CONTRAST TRANSTHORACIC ECHOCARDIOGRAPHY AND THE COMPARISON WITH PARTIAL OXYGEN PRESSURE IN PATIENTS SCREENED FOR HEREDITARY HEMORRHAGIC TELEANGIECTASIA

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Objective: The presence of a pulmonary right-to-left shunt can be diagnosed by contrast transthoracic echocardiography (TTE). We evaluate whether the partial oxygen pressure (pO₂) is associated with the grade of pulmonary RLS measured by contrast TTE in patients screened for hereditary hemorrhagic teleangiectasia (HHT). *Methods:* All persons, screened for HHT between May 2004 and December 2010, who underwent a contrast TTE and pO₂ measurement were included. The shunt using contrast TTE was graded as none, minimal, moderate, and large. *Results:* In total 703 persons were screened for HHT during the study period. A pO₂ was measured in 656 persons. A contrast TTE was performed in 601 out of the 656 persons. The mean age was 43.8±15.2 years and 62.3% were female. The mean pO₂ was 12.1±1.7 kPa. Using contrast TTE, a minimal, moderate or large shunt was present in 17.8%, 9.8%, and 17.8%, respectively. The pO₂ was 12.3±1.6 kPa in patients without a shunt, 12.5±1.6 kPa in the presence of minimal shunt (p=0.19 versus no shunt), 12.8±1.5 kPa in the presence of a moderate shunt (p=0.01 versus no shunt), and 10.7±1.9 kPa in the presence of a large shunt (p<0.001 versus no shunt). The pO₂ was a predictor for the presence of any shunt diagnosed by contrast TTE (OR 0.84: 95%CI 0.77 – 0.93, p=0.001). *Conclusion:* In patients with large shunts on contrast transthoracic echocardiography the partial oxygen pressure was significantly lower compared to those without a shunt. The partial oxygen pressure was a predictor for the presence of any shunt found by contrast echocardiography.

(A06) FIRST HEREDITARY HEMORRHAGIC TELANGIECTASIA ARGENTINEAN UNIT AT THE HOSPITAL ITALIANO DE BUENOS AIRES (HIBA) AND A REFERENCE CENTER FOR SOUTH AMERICA

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HHT is a complex and rare disease that often leads to multi-organic affection and health impairment. Multidisciplinary approach in specialized units with trained staff is mandatory. The HIBA created on February 2010 the first global and specialized HHT unit in Argentina and South America. We report the creation of the HIBA-HHT Unit, its organization, aims and working procedures. HIBA is a first level reference and university hospital which counts with over 700 beds and with 2,500,000 consultations/year, equipped with the technological and highest scientific resources to provide a complete medical care and research within HHT. The main goal of the unit is to offer specialized, comprehensive and coordinated assistance, accurate diagnosis and genetic counseling. Other objectives include updating and training of local professionals, collaboration with patient associations and promotion of clinical and basic research. The unit provides a global assistance; an internist coordinates consultations with specialties as ENT, angiography, gastroenterology, hematology, neurosurgery, pediatrics, dermatology, pneumonology, cardiology, hepatology, obstetrics and genetics. Part of the communication among staff involved is through an e-learning online platform. It also provides an updated site (www.hospitaliano.org.ar/renduoslerwber) containing information for patients and professionals. Patients are followed by a structured online health record based on an electronic health chart. Future plans include research projects and collaboration to better understand the nature of the disease. The creation of this new unit at HIBA provides the HHT Argentinean patients and families with a multidisciplinary team, offering adequate and integral assistance and promoting knowledge and scientific advances in the area.

(C13) LEISHMANIASIS IN HEREDITARY HEMORRHAGIC TELANGIECTASIA PATIENT: CASE REPORT

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Infectious susceptibility of HHT patients is described and usually explained by mechanical factor like epistaxis treatment in case of staphylococcal disease. Mrs. S., a 78-year-old HHT woman with treated cerebral AVM and multiple nasal grafts, is hospitalized in June 2010 for severe epistaxis with anemia. Despite of embolization of nasal arteries and red-cell transfusion, she was hospitalized again in late July 2010 for anemia. After five days she has fever, abdominal pain then septic shock. The blood cells count revealed pancytopenia (hemoglobin 80 g/L, neutrophils 960/mm³ and platelets 85000/mm³). The body CT-scan did not show any infection but a pulmonary AVM.

Leishmanii infantum were found in the bone marrow aspirate and *Enterobacter cloacae* was identified in 2 blood cultures. With liposomal amphotericin B and antibiotic therapy, the clinical condition quickly improved. Any entry route of bacteria nor factor of immunodeficiency were found. She did not relapsed to date. Even in endemic areas like the South of France, visceral leishmaniasis remains a rare disease that affects children and immunocompromised individuals. It does usually not complicate by severe sepsis despite of frequent neutropenia. Dupuis-Girod described an high infection rate in a retrospective cohort of 353 HHT patients, and concluded to the probable role of mechanical factors associated with moderate abnormalities of the immune system. Since no mechanical factor can explain these two simultaneous severe infections, it highlights a possible intrinsic immune deficiency related to the HHT condition.

(C15) PREGNANCY IN HEREDITARY HEMORRHAGIC TELANGIECTASIA: RETROSPECTIVE STUDY OF 129 PREGNANCIES IN 59 WOMEN

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Hereditary hemorrhagic telangiectasia (HHT) affects women by the time they reach child bearing age. As we noted that venous thrombosis (VT) occurred in HHT women during pregnancy or post partum, we conducted a retrospective study to evaluate VT in HHT pregnant women and describe route of pregnancy. We included women from CIROCO database of Lyon and Montpellier, born between 1954 and 1991, who had one or more pregnancies. Patients were informed by postmail to contact the National Reference Center for answer by phone to a structured questionnaire. We included 59 women with 129 pregnancies, 50 have been screened: 48% had pulmonary arteriovenous malformation (PAVM), 48.8% an hepatic involvement, 4 had cerebral AVM. Median age at the time of pregnancy was 29-year old. In 58 pregnancies, HHT disease was known but only 64% of women underwent a thoracic CT scanner and 39% had PAVM. In this group, only 60% controlled PAVM after delivery. Increase of cutaneous telangiectasia occurred in 15.5%, epistaxis were stable. Only one case of VT is reported. All women received thromboprophylaxis, well tolerated, according on usual recommendations. Only 2 hemoptysis was observed: one in a patient who were embolized for one PAVM before pregnancy. Others complications were: 1 pulmonary edema, 1 vasovagal syncope at delivery, 1 eclampsia, 8 hemorrhage of delivery, 2 hydramnios, 1 retroplacenta hemorrhage, 1 macrosomia, 1 stillbirth. In this limited cohort, VT is unfrequent and pregnancies well tolerated. We need to reassure these women and inform of red flag symptoms to avoid serious complications.

(C28) LOW PREVALENCE AND NUMBER OF TELANGIECTASIA IN FEET OF PATIENTS HAVING HHT

I Bourgault Villada, J Roume, B Raffestin, JH Blondel, S Blivet, D Cobarzan, T Chinet, C Fagnou, G Lesur, A Ozanne, JP Pelage, F Soubrier, P Lacombe

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Background: HHT is characterized by Curaçao criteria which include the presence of mucosal and cutaneous telangiectases localized both on the face and extremities. **Objective:** To determine the number of patients presenting with telangiectases on the feet and to characterize their clinical particularities. **Materials and method:** 310 HHT patients were carefully examined by the same dermatologist between January 1, 2004 and December 31, 2010. In each of them telangiectases in the oral cavity and on the face, hands, fingers and feet were counted. Results are given as mean±SD. **Results:** Only 15 patients (9 women and 6 men) had telangiectases on their feet. Eleven had mutation on ALK1 and four on ENG genes. In this group of patients, age was 70±11 years and total number of telangiectases 402±217, from which 228±134 on the hands and 25±28 on the feet. **Conclusions:** The presence of telangiectases on the feet is a rare event (5%), observed in aged patients having numerous telangiectases. They should be carefully identified, in particular on the sole of feet, in order to prevent further complications including bleeding of cutaneous arteriovenous malformations, hyperkeratosis and corns. Daily change of shoes should be recommended to prevent these complications.

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