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The *ACVRL1* c.314-35A>G Polymorphism is Associated with Organ Vascular Malformations in Hereditary Hemorrhagic Telangiectasia Patients with *ENG* Mutations, but not in Patients with *ACVRL1* Mutations

Ludmila Pawlikowska[1,2], **Jeffrey Nelson**[1], **Diana E. Guo**[1], **Charles E. McCulloch**[3], **Michael T. Lawton**[4], **William L. Young**[1,4,5,†], **Helen Kim**[1,2,3], **Marie E. Faughnan**[6,7,*], and **the Brain Vascular Malformation Consortium HHT Investigator Group**[‡]

[1]Center for Cerebrovascular Research, Department of Anesthesia and Perioperative Care, University of California, San Francisco, San Francisco, California

[2]Institute for Human Genetics, University of California, San Francisco, San Francisco, California

[3]Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, California, USA

[4]Department of Neurological Surgery, University of California, San Francisco, San Francisco, California

[5]Department of Neurology, University of California, San Francisco, San Francisco, California

[6]Division of Respirology, Department of Medicine and Li Ka Shing Knowledge Institute, St. Michael's Hospital, Toronto, Canada

[7]Division of Respirology, Department of Medicine, University of Toronto, Toronto, Canada

Abstract

Hereditary hemorrhagic telangiectasia (HHT) is characterized by the presence of vascular malformations (VMs) and caused by mutations in TGFβ/BMP9 pathway genes, most commonly *ENG* or *ACVRL1*. Patients with HHT have diverse phenotypes related to skin and mucosal telangiectases and organ VMs, including arteriovenous malformations (AVM). The clinical heterogeneity of HHT suggests a potential role for genetic modifier effects. We hypothesized that the common polymorphisms *ACVRL1* c.314-35A>G and *ENG* c.207G>A, previously associated with sporadic brain AVM, are also associated with organ VM in HHT. We genotyped *ACVRL1* c. 314-35A>G and *ENG* c.207G>A in 716 patients with HHT recruited by the Brain Vascular Malformation Consortium and evaluated association of genotype with presence of any organ VM, and specifically with brain VM, liver VM and pulmonary AVM, by multivariate logistic

[*]Correspondence to: Marie E. Faughnan, MD, MSc, St. Michael's Hospital, 30 Bond Street, Toronto, Ontario M5B 1W8, Canada, Phone: 416-864-5412, Fax: 416-864-5922, Faughnanm@smh.ca.

[‡]Brain Vascular Malformation Consortium HHT Investigator Group: Murali Chakinala, Marie E. Faughnan, James R. Gossage, Katharine Henderson, Vivek Iyer, Raj Kasthuri, Helen Kim, Timo Krings, Michael T. Lawton, Doris Lin, Johannes Jurgen Mager, Justin McWilliams, Jamie McDonald, Ludmila Pawlikowska, Jeffrey Pollak, Felix Ratjen, Karen Swanson, Karel terBrugge, Dilini Vethanayagam, Andrew White, Robert I. White Jr, Pearce Wilcox, William L. Young†

[†]deceased

regression analyses stratified by HHT mutation. Among all patients with HHT, neither polymorphism was significantly associated with presence of any organ VM; *ACVRL1* c. 314-35A>G showed a trend toward association with pulmonary AVM (OR=1.48, p=0.062). *ACVRL1* c.314-35A>G was significantly associated with any VM among patients with HHT with *ENG* (OR=2.66, p=0.022), but not *ACVRL1* (OR=0.79, p=0.52) mutations. *ACVRL1* c.314-35A>G was also significantly associated with pulmonary AVM and liver VM among *ENG* mutation carriers. There were no significant associations between *ENG* c.207G>A and any VM phenotype. These results suggest that common polymorphisms in HHT genes other than the mutated gene modulate phenotype severity of HHT disease, specifically presence of organ VM.

Keywords

hereditary hemorrhagic telangiectasia; vascular malformation; arteriovenous malformation; phenotype; genetic modifier

INTRODUCTION

Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant Mendelian disorder caused by mutations in several genes encoding proteins in the TGFβ/BMP9 signaling pathway [McDonald et al., 2011]. Approximately 85% of patients with HHT have a mutation in *ENG* [McAllister et al., 1994] or *ACVRL1* [Johnson et al., 1996] and 3% have a mutation in *SMAD4* [Gallione et al., 2004]. *BMPR2* [Rigelsky et al., 2008] and *BMP9* [Wooderchak-Donahue et al., 2013] mutations have been reported in syndromes with phenotypic overlap. Patients with HHT have skin and mucosal telangiectases and vascular malformations (VM), including arteriovenous malformations (AVM), in visceral organs including brain, liver and lung, which can lead to chronic bleeding, anemia, life-threatening hemorrhage, stroke and heart failure[Faughnan et al., 2011; McDonald et al., 2011]. Pulmonary AVM and brain VM are more common in patients with *ENG* mutations, while liver VM are more common in patients with *ACVRL1* mutations [Bayrak-Toydemir et al., 2006; Letteboer et al., 2006]. The considerable clinical heterogeneity of HHT phenotypes, even among patients with the same mutated HHT gene and within families, suggests a possible role for genetic modifier effects.

Two common polymorphisms in HHT genes, *ACVRL1* c.314-35A>G (*ACVRL1* IVS3-35A>G) and *ENG* c.207G>A, have been reported to be associated with sporadic brain AVM [Pawlikowska et al., 2005]; the *ACVRL1* c.314-35A>G association has been replicated in 2 independent cohorts [Simon et al., 2006]. *ENG* c.207G>A was also associated with surgical outcomes in sporadic brain AVM [Shen et al., 2014]. We hypothesized that these polymorphisms are also associated with organ VM in HHT and tested this hypothesis in an HHT cohort recruited by the Brain Vascular Malformation Consortium (BVMC) [Akers et al., 2013].

METHODS

Cohort Recruitment

Patients with a confirmed clinical HHT diagnosis by the Curaçao criteria [Faughnan et al., 2011] were enrolled by the BVMC HHT Project as previously described (http://rarediseasesnetwork.epi.usf.edu/BVMC) [Akers et al., 2013]. We analyzed 716 patients enrolled at 11 sites between June 2010 and December 2013. All patients provided written informed consent, including for genetic studies. The study protocol was approved by each institutional review board. Data collected included age, sex, family relationships, genetic testing results (mutated gene: *ACVRL1*, *ENG*, *SMAD4*, unknown), clinical presentation and symptoms. Patients were screened for organ VM and other clinical features according to standard clinical practice and International HHT Guidelines [Faughnan et al., 2011], including: comprehensive history, physical, routine blood tests, screening for pulmonary AVM by contrast echocardiography, brain VM screening by magnetic resonance imaging, clinical screening for liver VM (chronic right upper quadrant pain, portal hypertension, high-output heart failure, liver bruit on examination, abnormal liver function tests) and clinical screening for recurrent spontaneous epistaxis and HHT-related gastrointestinal bleeding. If screening was positive for pulmonary AVM or brain VM, patients underwent further diagnostic imaging and treatment, where appropriate. If clinical assessment was suggestive of symptomatic liver VM, diagnostic imaging was recommended and therapy where appropriate. The BVMC HHT cohort targets 25% brain VM-positive patients; other cohort characteristics are similar to other cohorts [Letteboer et al., 2006; Nishida et al., 2012]. The majority of the cohort is Caucasian (96%) reflecting patient populations at participating centers.

Patients provided blood or saliva (Oragene, DNA Genotek, Ontario, Canada) samples, which were sent for DNA extraction and banking to the NINDS Repository at Coriell Institute (http://ccr.coriell.org/Sections/Collections/NINDS), or to the University of California, San Francisco (UCSF) (saliva).

Genotyping

Genotyping was performed by staff blinded to clinical phenotype. *ACVRL1* c.314-35A>G (formerly *ACVRL1* IVS3-35A>G, rs2071219) and *ENG* c.207G>A (rs11545664) were genotyped using Taqman™ assays (C__15868502_10 and C__25592400_10, Applied Biosystems, Foster City, CA). Both SNPs had genotype call rates >98% and were in Hardy Weinberg equilibrium among Caucasians ($p>0.05$).

Statistical Analysis

Phenotype frequencies were compared by Fisher's exact test. Genotypes were collapsed for analysis into risk genotype carriers (*ACVRL1* c.314-35A>G: AA+AG, *ENG* c.207G>A: GG) vs. non-carriers [Pawlikowska et al., 2005]. Phenotypes evaluated included pulmonary AVM, liver VM, brain VM and the composite phenotype "any organ VM", defined as presence of any organ VM or AVM. Association of genotype with phenotype was evaluated by logistic regression analyses adjusted for age, gender and clustering within known families and further stratified by HHT mutation status (*ACVRL1* or *ENG*) for the two most frequently

mutated genes. Since liver VM analysis restricted to *ENG* subjects produced an infinite estimated odds ratio (OR) for *ACVRL1* c.314-35A>G, we calculated a one-sided 95% confidence interval (CI) based on the profile likelihood and derived a p-value from the likelihood ratio test comparing models with and without the polymorphism. To ensure familial clustering could be disregarded for this specific analysis, we tested the effect of family with a mixed-effects logistic regression model. The statistical significance threshold was set at p=0.025 after Bonferroni correction for 2 polymorphisms. We also evaluated associations using a co-dominant genetic model testing each genotype compared to the lowest-risk genotype [Pawlikowska et al., 2005], and restricted to Caucasians.

RESULTS

Of 716 patients with HHT studied, 93.7% were Caucasian and 59% were female; 71% had at least one VM of any type, 50% had pulmonary AVM, 20% had liver VM and 20% had brain VM (Table I). Known family relationships were recorded for 177 patients (2–4 patients per family, except for 1 family of 17 distant relatives). Among the 436 (61%) patients with mutation data, 48% had *ENG* mutations, 43% had *ACVRL1* mutations and 4% had *SMAD4* mutations (Table I). As reported for other cohorts [Bayrak-Toydemir et al., 2006; Letteboer et al., 2006], patients with *ENG* mutations were more likely to have any organ VM, brain VM and pulmonary AVM (all p<0.0001), while patients with *ACVRL1* mutations were more likely to have liver VM (p<0.0001) and anemia (p=0.003) (Table I). Compared to patients without genetic testing results, patients with *ACVRL1* or *ENG* mutations were more likely to have any organ VM (p=0.002), pulmonary AVM (p<0.0001) and anemia (p=0.001), likely due to more extensive clinical evaluation of patients from multiplex families.

Among 689 patients with HHT with VM data, neither polymorphism was significantly associated with organ VM, although *ACVRL1* c.314-35A>G showed a trend toward association with pulmonary AVM (OR=1.48, 95%CI=0.90–2.22, p=0.062) and liver VM (OR=1.46, 95%CI=0.85–2.50, p=0.17) (Table II).

When stratifying by HHT mutation, *ACVRL1* c.314-35A>G was significantly associated with organ VM among the 211 *ENG* mutation carriers (overall: OR=2.66, 95%CI=1.15–6.13, p=0.022; Caucasians: OR=2.83, 95%CI=1.18–6.76, p=0.020), but not among the 189 *ACVRL1* mutation carriers (OR=0.79, CI=0.38–1.63, p=0.52). In *ENG* mutation carriers, *ACVRL1* c.314-35A>G was also significantly associated with pulmonary AVM (OR=2.45, p=0.016) and liver VM (p=0.001, all 21 liver VM-positive patients carried the A risk allele), but not with brain VM (Table II). When restricted to Caucasians, all significant associations were consistent (data not shown).

There were no statistically significant associations between *ENG* c.207G>A and VM of any type. However, among *ACVRL1* mutation carriers only, the effect direction was consistent with association of *ENG* c.207G>A with VM overall (OR=1.29, p=0.5) and with brain VM (OR=3.09, p=0.16) (Table II). Again, no associations with *ENG* c.207G>A were observed among *ENG* mutation carriers.

DISCUSSION

We report here the first evidence that common polymorphisms in HHT genes other than the mutated gene are associated with differences in HHT phenotype severity, and specifically presence of organ VM. In a well-characterized cohort of patients with HHT, the common polymorphism *ACVRL1* c.314-35A>G, previously reported as associated with sporadic brain AVM [Pawlikowska et al., 2005; Simon et al., 2006] was also associated with pulmonary AVM, liver VM and overall, with presence of any organ VM in patients with *ENG* mutations, but not in patients with *ACVRL1* mutations. Conversely, there was also a pattern, although not statistically significant, for association of the *ENG* c.207G>A with any VM and brain VM in patients with *ACVRL1*, but not *ENG* mutations. These findings suggest that common genetic variation in HHT genes other than the mutated gene may modify HHT phenotype severity.

Common polymorphisms acting as genetic modifiers have been reported in other Mendelian diseases, such as cystic fibrosis [Wright et al., 2011] and there are other reports of phenotype-modifying polymorphisms near the gene that when mutated causes disease: a common polymorphism in *KCNQ1*, rs2074238, is associated with symptomatic status in patients with long-QT syndrome, who are heterozygous for *KCNQ1* or *KCNH2* (disease causative) mutations [Duchatelet et al., 2013]; and 2) a common polymorphism in *TERT* is associated with survival and with disease recurrence in bladder cancer caused by somatic mutations in the *TERT* promoter [Rachakonda et al., 2013]. In patients with HHT, common variants in *PTPN14* and *ADAM17*, genes in loci originally mapped as phenotype modifiers in TGFβ knockout mice, have been reported to be associated with pulmonary VM [Benzinou et al., 2012; Kawasaki et al., 2014]. These associations have not yet been replicated in independent cohorts.

The association with the common polymorphism *ACVRL1* c.314-35A>G was originally reported for sporadic brain AVM in two independent cohorts [Pawlikowska et al., 2005; Simon et al., 2006] and for dural arteriovenous fistulae in one cohort [Simon et al., 2006]. Extension of these associations to organ VMs in HHT suggests common mechanisms of VM development in different organs (brain, lung, liver) in both sporadic and HHT disease.

The functional effects of *ACVRL1* c.314-35A>G are unknown. It is located 35 base-pairs from an intron-exon junction and is hypothesized to affect splicing [Pawlikowska et al., 2005]. As with sporadic brain AVM disease, the observed HHT VM association is with the major A allele carried by the majority of patients (GG genotype carriers have fewer VMs). ENG c.207A>G is a synonymous exonic variant, however it is also a cis eQTL linked to ENG mRNA expression in monocytes [Zeller et al., 2010]. Thus both polymorphisms could result in reduced expression or expression of an abnormal protein, further impairing TGFβ/BMP9 pathway signaling.

We acknowledge several limitations of our study. The genetic association results require replication in an independent HHT cohort and functional studies to determine their molecular mechanism. Family relationships (known and cryptic) within the cohort are a confounder; however, the statistical adjustment we used for known relatedness is

conservative, and cryptic relationships should be rare. The findings are currently limited to Caucasians, as there are not enough patients of other ethnicities in the BVMC cohort to evaluate the effect in non-Caucasians. The *ENG* c.207A>G associations did not reach statistical significance and require a larger cohort to confirm or rule out. Unlike for sporadic brain AVM, we found no strong evidence for *ACVRL1* c.314-35A>G association with brain VM in HHT, however the number of brain VM-positive patients is small resulting in low statistical power. The prevalence of brain VM in the BVMC cohort is targeted by design (25% planned, 20% observed), but similar (top end of the reported range) to frequencies observed in other HHT cohorts [Letteboer et al., 2006; Nishida et al., 2012]. Screening for liver VM was based on clinical assessment, and therefore the liver VM-positive group includes patients with more severe, clinically evident liver VM, consistent with the overall focus on organ VM involvement as a marker of more severe HHT disease. The strengths of our study include the large size of the HHT cohort and the comprehensive phenotype information collected.

In conclusion, we report for the first time that common polymorphisms in HHT genes other than the mutated gene may modify the HHT phenotype, and that genetic associations previously reported in sporadic brain AVM extend to the Mendelian form of the disease. Our results suggest a multiple-hit mechanism, where diverse genetic hits to multiple HHT genes may predispose to the most severe phenotypic manifestations. The findings provide insights into potential mechanisms underlying the marked clinical heterogeneity of HHT, where some patients suffer from severe VM organ involvement with devastating complications, while others have only skin telangiectases. A better understanding of which patients with HHT are at highest risk of complications would aid in clinical management, risk stratification and therapy development.

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References

Akers AL, Ball KL, Clancy M, Comi AM, Faughnan ME, Gopal-Srivastava R, Jacobs TP, Kim H, Krischer J, Marchuk DA, McCulloch CE, Morrison L, Moses MA, Pawlikowska L, Young WL. Brain Vascular Malformation Consortium: Overview, progress and future directions. J Rare Disord. 2013; 1:5. [PubMed: 25221778]

Bayrak-Toydemir P, McDonald J, Markewitz B, Lewin S, Miller F, Chou LS, Gedge F, Tang W, Coon H, Mao R. Genotype-phenotype correlation in hereditary hemorrhagic telangiectasia: mutations and manifestations. Am J Med Genet A. 2006; 140:463–470. [PubMed: 16470787]

Benzinou M, Clermont FF, Letteboer TG, Kim JH, Espejel S, Harradine KA, Arbelaez J, Luu MT, Roy R, Quigley D, Higgins MN, Zaid M, Aouizerat BE, van Amstel JK, Giraud S, Dupuis-Girod S, Lesca G, Plauchu H, Hughes CC, Westermann CJ, Akhurst RJ. Mouse and human strategies identify PTPN14 as a modifier of angiogenesis and hereditary haemorrhagic telangiectasia. Nat Commun. 2012; 3:616. [PubMed: 22233626]

Duchatelet S, Crotti L, Peat RA, Denjoy I, Itoh H, Berthet M, Ohno S, Fressart V, Monti MC, Crocamo C, Pedrazzini M, Dagradi F, Vicentini A, Klug D, Brink PA, Goosen A, Swan H, Toivonen L, Lahtinen AM, Kontula K, Shimizu W, Horie M, George AL Jr, Tregouet DA, Guicheney P, Schwartz PJ. Identification of a KCNQ1 polymorphism acting as a protective modifier against arrhythmic risk in long-QT syndrome. Circ Cardiovasc Genet. 2013; 6:354–361. [PubMed: 23856471]

Faughnan ME, Palda VA, Garcia-Tsao G, Geisthoff UW, McDonald J, Proctor DD, Spears J, Brown DH, Buscarini E, Chesnutt MS, Cottin V, Ganguly A, Gossage JR, Guttmacher AE, Hyland RH, Kennedy SJ, Korzenik J, Mager JJ, Ozanne AP, Piccirillo JF, Picus D, Plauchu H, Porteous ME, Pyeritz RE, Ross DA, Sabba C, Swanson K, Terry P, Wallace MC, Westermann CJ, White RI, Young LH, Zarrabeitia R. International guidelines for the diagnosis and management of hereditary hemorrhagic telangiectasia. J Med Genet. 2011; 48:73–87. [PubMed: 19553198]

Gallione CJ, Repetto GM, Legius E, Rustgi AK, Schelley SL, Tejpar S, Mitchell G, Drouin E, Westermann CJ, Marchuk DA. A combined syndrome of juvenile polyposis and hereditary haemorrhagic telangiectasia associated with mutations in MADH4 (SMAD4). Lancet. 2004; 363:852–859. [PubMed: 15031030]

Johnson DW, Berg JN, Baldwin MA, Gallione CJ, Marondel I, Yoon SJ, Stenzel TT, Speer M, Pericak-Vance MA, Diamond A, Guttmacher AE, Jackson CE, Attisano L, Kucherlapati R, Porteous ME, Marchuk DA. Mutations in the activin receptor-like kinase 1 gene in hereditary haemorrhagic telangiectasia type 2. Nat Genet. 1996; 13:189–195. [PubMed: 8640225]

Kawasaki K, Freimuth J, Meyer DS, Lee MM, Tochimoto-Okamoto A, Benzinou M, Clermont FF, Wu G, Roy R, Letteboer TG, Ploos van Amstel JK, Giraud S, Dupuis-Girod S, Lesca G, Westermann CJ, Coffey RJ Jr, Akhurst RJ. Genetic variants of Adam17 differentially regulate TGFbeta signaling to modify vascular pathology in mice and humans. Proc Natl Acad Sci U S A. 2014; 111:7723–7728. [PubMed: 24812125]

Letteboer TG, Mager JJ, Snijder RJ, Koeleman BP, Lindhout D, Ploos van Amstel JK, Westermann CJ. Genotype-phenotype relationship in hereditary haemorrhagic telangiectasia. J Med Genet. 2006; 43:371–377. [PubMed: 16155196]

McAllister KA, Grogg KM, Johnson DW, Gallione CJ, Baldwin MA, Jackson CE, Helmbold EA, Markel DS, McKinnon WC, Murrell J, McCormick MK, Pericak-Vance MA, Heutink P, Oostra BA, Haitjeman T, Westerman CJ, Porteous ME, Guttmacher AE, Letarte M, Marchuk DA. Endoglin, a TGF-beta binding protein of endothelial cells, is the gene for hereditary haemorrhagic telangiectasia type 1. Nat Genet. 1994; 8:345–351. [PubMed: 7894484]

McDonald J, Bayrak-Toydemir P, Pyeritz RE. Hereditary hemorrhagic telangiectasia: An overview of diagnosis, management, and pathogenesis. Genet Med. 2011; 13:607–616. [PubMed: 21546842]

Nishida T, Faughnan ME, Krings T, Chakinala M, Gossage JR, Young WL, Kim H, Pourmohamad T, Henderson KJ, Schrum SD, James M, Quinnine N, Bharatha A, terBrugge KG, White RI Jr. Brain arteriovenous malformations associated with hereditary hemorrhagic telangiectasia: Genotype-phenotype correlations. Am J Med Genet A. 2012; 158A:2829–2834. [PubMed: 22991266]

Pawlikowska L, Tran MN, Achrol AS, Ha C, Burchard EG, Choudhry S, Zaroff J, Lawton MT, Castro RA, McCulloch CE, Marchuk DA, Kwok PY, Young WL. Polymorphisms in transforming growth factor-beta-related genes ALK1 and ENG are associated with sporadic brain arteriovenous malformations. Stroke. 2005; 36:2278–2280. [PubMed: 16179574]

Rachakonda PS, Hosen I, de Verdier PJ, Fallah M, Heidenreich B, Ryk C, Wiklund NP, Steineck G, Schadendorf D, Hemminki K, Kumar R. TERT promoter mutations in bladder cancer affect patient survival and disease recurrence through modification by a common polymorphism. Proc Natl Acad Sci U S A. 2013; 110:17426–17431. [PubMed: 24101484]

Rigelsky CM, Jennings C, Lehtonen R, Minai OA, Eng C, Aldred MA. BMPR2 mutation in a patient with pulmonary arterial hypertension and suspected hereditary hemorrhagic telangiectasia. Am J Med Genet A. 2008; 146A:2551–2556. [PubMed: 18792970]

Shen F, Degos V, Chu PL, Han Z, Westbroek EM, Choi EJ, Marchuk D, Kim H, Lawton MT, Maze M, Young WL, Su H. Endoglin deficiency impairs stroke recovery. Stroke. 2014; 45:2101–2106. [PubMed: 24876084]

Simon M, Franke D, Ludwig M, Aliashkevich AF, Koster G, Oldenburg J, Bostrom A, Ziegler A, Schramm J. Association of a polymorphism of the ACVRL1 gene with sporadic arteriovenous

malformations of the central nervous system. J Neurosurg. 2006; 104:945–949. [PubMed: 16776339]

Wooderchak-Donahue WL, McDonald J, O'Fallon B, Upton PD, Li W, Roman BL, Young S, Plant P, Fülöp GT, Langa C, Morrell NW, Botella LM, Bernabeu C, Stevenson DA, Runo JR, Bayrak-Toydemir P. BMP9 mutations cause a vascular-anomaly syndrome with phenotypic overlap with hereditary hemorrhagic telangiectasia. Am J Hum Genet. 2013; 93:530–537. [PubMed: 23972370]

Wright FA, Strug LJ, Doshi VK, Commander CW, Blackman SM, Sun L, Berthiaume Y, Cutler D, Cojocaru A, Collaco JM, Corey M, Dorfman R, Goddard K, Green D, Kent JW Jr, Lange EM, Lee S, Li W, Luo J, Mayhew GM, Naughton KM, Pace RG, Pare P, Rommens JM, Sandford A, Stonebraker JR, Sun W, Taylor C, Vanscoy LL, Zou F, Blangero J, Zielenski J, O'Neal WK, Drumm ML, Durie PR, Knowles MR, Cutting GR. Genome-wide association and linkage identify modifier loci of lung disease severity in cystic fibrosis at 11p13 and 20q13.2. Nat Genet. 2011; 43:539–546. [PubMed: 21602797]

Zeller T, Wild P, Szymczak S, Rotival M, Schillert A, Castagne R, Maouche S, Germain M, Lackner K, Rossmann H, Eleftheriadis M, Sinning CR, Schnabel RB, Lubos E, Mennerich D, Rust W, Perret C, Proust C, Nicaud V, Loscalzo J, Hubner N, Tregouet D, Munzel T, Ziegler A, Tiret L, Blankenberg S, Cambien F. Genetics and beyond--the transcriptome of human monocytes and disease susceptibility. PloS One. 2010; 5:e10693. [PubMed: 20502693]

TABLE I

Demographic and Clinical Characteristics of HHT Subjects

Characteristic	All Subjects	*ACVRL1*	*ENG*	P*
Female sex	425 / 716 (59%)	107/189 (57%)	127/211 (60%)	0.48
Age at baseline (y)	46.1 ± 19.1	45.2 ± 21.3	43.1 ± 18.8	0.30
Caucasian	671 / 700 (96%)	180/184 (98%)	201/208 (97%)	0.52
HHT mutation				
ACVRL1	189 / 436 (43%)	189/189 (100%)	-	
ENG	211 / 436 (48%)	-	211/211 (100%)	
SMAD4	16 / 436 (4%)	-	-	
All negative	20 / 436 (5%)	-	-	
VM	490 / 689 (71%)	90/181 (50%)	172/209 (82%)	**<0.001**
Brain VM	140 / 716 (20%)	21/189 (11%)	66/211 (31%)	**<0.001**
Pulmonary AVM	346 / 690 (50%)	34/183 (19%)	140/208 (67%)	**<0.001**
Liver VM	138/ 677 (20%)	47/182 (26%)	21/203 (10%)	**<0.001**
Epistaxis	678 / 700 (97%)	177/187 (95%)	201/208 (97%)	0.46
GI Bleeding	115 / 682 (17%)	22/183 (12%)	34/205 (17%)	0.25
Anemia	331 / 675 (49%)	92/180 (51%)	74/206 (36%)	**0.003**
ACVRL1* c.314-35A>G *genotype				0.68
GG	141 / 716 (20%)	37/189 (20%)	46/211 (22%)	
AG	337 / 716 (47%)	82/189 (43%)	97/211 (46%)	
AA	238 / 716 (33%)	69/189 (37%)	68/211 (32%)	
***ENG* c.207G>A genotype**				0.27
AA	9 / 716 (2%)	6/189 (3%)	3/211 (1%)	
AG	121 / 716 (17%)	34/189 (18%)	30/211 (14%)	
GG	581 / 716 (81%)	149/189 (79%)	178/211 (84%)	

Values are the number observed over the total number of non-missing (and the percent) or mean ± standard deviation.

*P-values are from comparison of subjects with known *ACVRL1* and *ENG* mutations with Fisher's exact test or a two-sample t-test. HHT, hereditary hemorrhagic telangiectasia; VM, any HHT organ vascular malformation including: brain VM, pulmonary arteriovenous malformation (AVM) or liver VM; GI, gastro-intestinal.

TABLE II

Association of *ACVRL1* c.314-35A>G and *ENG* c.207G>A with VM Phenotypes in Patients with HHT

		All Subjects				Subjects with *ACVRL1* Mutation				Subjects with *ENG* Mutation			
Outcome	**SNP**	**n**	**OR**	**95% CI**	**p**	**n**	**OR**	**95% CI**	**p**	**n**	**OR**	**95% CI**	**p**
VM	***ACVRL1* c.314-35A>G**	689	1.34	(0.87, 2.07)	0.178	181	0.79	(0.38, 1.63)	0.520	209	2.66	(1.15, 6.13)	**0.022**
	***ENG* c.207G>A**	689	1.10	(0.70, 1.72)	0.682	181	1.29	(0.57, 2.93)	0.537	209	0.41	(0.12, 1.40)	0.155
Brain VM	***ACVRL1* c.314-35A>G**	716	1.11	(0.68, 1.80)	0.675	189	1.03	(0.33, 3.16)	0.960	211	0.74	(0.36, 1.52)	0.411
	***ENG* c.207G>A**	716	1.19	(0.73, 1.93)	0.482	189	3.09	(0.63, 15.1)	0.163	211	0.62	(0.29, 1.32)	0.215
Pulmonary AVM	***ACVRL1* c.314-35A>G**	690	1.48	(0.90, 2.22)	0.062	183	0.57	(0.25, 1.33)	0.192	208	2.45	(1.18, 5.06)	**0.016**
	***ENG* c.207G>A**	690	1.29	(0.86, 1.93)	0.220	183	0.91	(0.36, 2.27)	0.835	208	1.80	(0.86, 3.77)	0.122
Liver VM	***ACVRL1* c.314-35A>G**	677	1.46	(0.85, 2.50)	0.170	182	1.57	(0.65, 3.81)	0.320	203	n/a*	(3.10, ∞)*	**0.001***
	***ENG* c.207G>A**	677	1.05	(0.62, 1.79)	0.856	182	1.17	(0.44, 3.10)	0.758	203	0.55	(0.16, 1.85)	0.332

Results from multivariable logistic regression analysis for carriers of the risk genotypes adjusted for age, sex and family clustering (except*, see below) are shown. P values meeting the statistical significance threshold (0.025) are in bold.

* The odds ratio (OR) cannot be determined, because 100% (21/21) of liver VM-positive patients carry the A risk allele, compared to 75% of liver VM-negative patients. We show a one-sided 95% confidence interval (CI) based on the profile likelihood and a p-value from the likelihood ratio test comparing models with and without the polymorphism. A mixed-effects logistic regression model found no estimated family effect; hence, the model was not adjusted for familial clustering. VM, any HHT organ vascular malformation including: brain VM, pulmonary arteriovenous malformation (AVM), liver VM; SNP, single nucleotide polymorphism.