Figure 1

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Figure 2

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Figure 3

Figure 4

Elevated Factor VIII in hereditary haemorrhagic telangiectasia (HHT): association with venous thromboembolism

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ABSTRACT

Introduction: Hereditary haemorrhagic telangiectasia (HHT) causes chronic nasal and gastrointestinal haemorrhage. Prothrombotic agents are commonly used for severe haemorrhage. Thrombotic risks have not been defined.

Methods To identify prothrombotic variables in HHT patients, and assess their potential functional significance, a pilot ELISA-based study comparing plasma proteins in healthy individuals with HHT to age/sex-matched non-HHT controls was validated in a full study of 309 consecutive HHT-affected individuals.

Results In the pilot study, Factor VIII (FVIII) and Von Willebrand Factor antigen concentrations were elevated in the HHT group compared to non-HHT controls ($p \leq 0.0013$, Mann-Whitney). Service laboratory measurements confirmed high FVIII:Ag in 125 HHT-affected individuals with no recent ill-health, intervention or venous thromboemboli. FVIII:Ag levels increased with age. Logistic regression also suggested an age-independent association with HHT-associated pulmonary arteriovenous malformations (PAVMs). No association was demonstrated between FVIII:Ag and acute phase response, disseminated intravascular coagulation, ABO group, pulmonary artery pressure or markers of HHT haemorrhage. Elevated FVIII:Ag were associated with shortened activated partial thromboplastin times (APTTs), and VTE: VTE affected 20/309 (6.5%) HHT-affected individuals, at median age 61(36-71)yr. Four VTE occurred in Factor V Leiden heterozygotes in the months following PAVM-associated brain abscess. The strongest association with VTE was with log-transformed FVIII:Ag measured 10-132 months from VTE (odds ratio 2.41 (95% confidence intervals 1.254, 4.612, $p=0.008$). Age made no additional contribution to VTE risk once adjusted for FVIII:Ag.

Conclusions HHT-related elevation of FVIII:Ag levels may influence thrombotic risk in HHT. Individualised risk-benefit considerations may be helpful in the management of individuals with HHT.

Key words: brain abscess, DIC, pulmonary AVMs, tranexamic acid, VWF

INTRODUCTION

Co-existence of haemorrhagic and thrombotic events is well recognised, for example following the consumptive coagulopathy of disseminated intravascular coagulation (DIC). In the management of chronic haemorrhagic conditions however, concern regarding possible deep venous thromboses (DVT) and pulmonary emboli (PE) may not be paramount.

Hereditary haemorrhagic telangiectasia (HHT, also known as Osler-Weber-Rendu syndrome) leads to chronic haemorrhage due to primary vascular wall pathology. Affecting at least 1 in 5,000-8,000 in Europe ^{1,2}, HHT is classically recognised by nose bleeds, characteristic mucocutaneous telangiectasia on the lips, oral mucosa and fingertips, and anaemia secondary to chronic haemorrhage from telangiectasia in the nose and gastrointestinal tract. HHT also leads to the development of large arteriovenous malformations (AVMs) in pulmonary, cerebral and hepatic circulations ^{3,4}. Concurrent coagulation defects are only rarely described ⁵⁻¹⁰. In contrast, all patients have abnormal vascular structures at the sites of haemorrhage. HHT telangiectasia display focal dilatations, asymmetric wall development, and turbulent blood flow due to microscopic arteriovenous communications ^{11,12}. The causative gene defects confirm a primary vascular pathology, as the most commonly mutated genes (*endoglin* causing HHT type 1 ¹³; *ALK-1* causing HHT type 2 ¹⁴) encode endothelial cell-expressed proteins that transmit or modulate signals by members of the transforming growth factor (TGF)- β superfamily. HHT is also caused by mutations in *MADH4* ¹⁵, and currently unidentified genes on chromosomes 5 ¹⁶ and 7 ¹⁷.

Thrombotic events in individuals with HHT have been reported. Disseminated intravascular coagulation (DIC) was described in 24 selected HHT patients referred due to an undiagnosed

haemostatic disorder ¹⁸, and there are occasional case reports of deep venous ¹⁹ and mesenteric vein ²⁰ thromboses in HHT patients. Furthermore, HHT-related pulmonary AVMs are frequently complicated by paradoxical thromboembolic strokes ^{3,21}.

We hypothesised that HHT might result in disturbances to the tightly regulated dynamics involved in regulation of coagulation and fibrinolysis at the endothelial cell surface, and examined whether there were any HHT-specific factors that may exacerbate or modify conventional risk factors for thrombosis ^{22,23}.

METHODS

All studies were ethically approved by the Hammersmith, Queen Charlotte's, Chelsea, and Acton Hospital Research Ethics Committee (LREC 00/5792, LREC 00/5908 and LREC 02/6289).

Study design and patient populations

The study was performed in two phases: A pilot study was first performed in otherwise healthy HHT patients and age/sex matched controls in order to identify processes perturbed as a consequence of HHT itself. In the second phase, a study in the full HHT population was performed to determine whether any perturbations were likely to influence thrombotic risk.

The full study population were the 309 individuals with definite HHT ²⁴, reviewed in our specialised HHT clinic between 1 May 1999 and 31 May 2005, with follow up until 30 April 2006. The pilot study was conducted between 2000 and 2001, in 38 HHT-affected individuals from this population, selected because they were otherwise healthy, and had never had a suspected or proven deep venous thrombosis or pulmonary embolus. Non-HHT controls for the pilot study were recruited from spouses or hospital staff.

HHT assessment

Structured assessments documented the severity of HHT. Full blood count, global coagulation parameters, fibrinogen, and routine biochemistry including liver function tests and C-reactive protein (CRP) were measured by the hospital service laboratories. All HHT-affected individuals underwent screening for asymptomatic pulmonary AVMs. From 2001, thoracic CT scans using dedicated protocols were used routinely for the screen. The right-to-left (R-L) shunt was quantified directly by radionuclide scans ²⁵, and indirectly by measurement of arterial oxygen saturation (SaO₂) standing ²⁵. Pulmonary artery pressures were recorded at pulmonary angiography performed to embolise PAVMs. Measurements were made prior to contrast injection, and only included for the purpose of this study if obtained within a year of FVIII:Ag measurements.

Assignment of VTE status

HHT patients reporting previous VTE were assigned to the HHT/VTE group if VTE had been confirmed by formal radiological investigations: doppler ultrasound, CT-pulmonary angiography, other contrast studies, or ventilation-perfusion scanning resulting in mismatched perfusion defects not explained by the presence of pulmonary AVMs. Four events occurred following patient review: a sudden death in which PE was confirmed as the cause of death at post-mortem, and three in which DVTs were confirmed by doppler ultrasound.

FVLeiden/G20210A genotyping

Conventional genetic risk factors for thrombosis, Factor V (FV) Leiden ²⁶ and prothrombin mutation G20210A ²⁷ were assessed in HHT/VTE patients and age-matched HHT/noVTE controls. All surviving HHT/VTE individuals were invited to provide DNA samples; 15 consented. HHT/noVTE controls (two per HHT/VTE patient, plus three for age-matching) were selected from unrelated individuals with definite HHT but without a personal history of deep venous thrombosis. The age distributions of the HHT/VTE patients and 33 unrelated

HHT/noVTE controls were closely matched (lower quartile [Q_1] 43/45, median [Q_2] 54/60, upper quartile [Q_3] 71/71) yr. After DNA extraction, FV 1691 (Leiden) and PT 20210 genotypes were determined by PCR amplification and sequencing, blinded to patient status (primer sequences available on request).

Pilot study

A pilot study comparing plasma proteins from HHT-affected individuals to age and sex-matched non-HHT controls was designed with the power to detect a difference of 0.6 standard deviation for tests at the 5% significance level (two-tailed). Strict exclusion criteria to reduce confounding influences were current or previous thromboses, intercurrent ill health/intervention, and hormonal or prothrombotic treatment in the previous year. Venous blood samples were collected for enzyme-linked immunosorbant assays (ELISA) of proteins that might be influenced by HHT gene mutations or aberrant haemodynamics in HHT vascular lesions. ELISAs were performed according to the manufacturers' instructions for plasma D-dimers (Biopool); plasminogen activator inhibitor 1 (PAI-1)²⁸ (Biopool); soluble P-selectin/CD62P (R and D Systems); soluble thrombomodulin/ sCD141²⁹ (Diacalone Research), and Von Willebrand Factor, VWF³⁰, (Helena Biosciences). In view of the VWF results, Factor VIII (FVIII):Ag (Diagnostica Stago), was analysed. All levels were expressed as a percentage of the non-HHT control mean.

Validation study

In view of possible implications of elevated FVIII:Ag levels³¹, FVIII:Ag measurements were included in routine HHT outpatient clinic assessments from 2001. Since FVIII is an acute phase protein,³² known to rise post-thrombosis, in pregnancy, after exercise³³ and in some forms of pulmonary hypertension (ref), FVIII:Ag was not measured for the purposes of this study if the individual was within four months of a known infective illness, medical intervention or pregnancy, or if they were known to have significant pulmonary hypertension. Samples were taken towards the end of an afternoon clinic visit. Participants in the pilot

study were not specifically recalled for the full study. Conversely, pilot study participants who were reviewed again medically after 2001 were not excluded from the full study.

Statistics

Basic statistics, including tests for Gaussian distribution, univariate analyses, correlations and linear regression analyses of single variables were performed using Prism 4 (Graph Pad Software Inc, San Diego). Continuous variables were compared by two-sided Mann-Whitney test, binary variables using Fisher's exact test. FVIII:Ag values varied widely with a skew to the right, and numerous outliers; log transformation resulted in a Gaussian distribution. To investigate the simultaneous effect of covariates, regression analyses of continuous (log-transformed FVIII:Ag) and binary (DVT) dependent variables were performed with stepwise addition of variables (SPLUS6).

RESULTS

Pilot study

Plasma levels of VWF:Ag were age-related (Figure 1A) and significantly higher in the HHT/noVTE individuals than in age-matched non-HHT controls (Figure 1B). Plasma levels of FVIII:Ag were also age-related (Figure 1C) and significantly higher in HHT/noVTE individuals, compared to age-matched non-HHT controls (Figure 1D). Plasma concentration of VWF:Ag and FVIII:Ag were highly correlated in both groups (Figure 1E).

Possible explanations were explored. There was no significant difference in the acute phase proteins CRP and fibrinogen between the groups (data not shown), and none of the HHT/noVTE group had abnormal liver function tests, or evidence of overt DIC (Figure 1F). There was no difference between the groups in the proportion of blood group O individuals (who have significantly lower plasma concentrations of VWF and hence FVIII:Ag³⁴; $\chi^2 =$

1.975, $p=0.16$), nor in PAI-1, soluble thrombomodulin or soluble P-selectin, the other proteins which had been measured in the pilot study (data not shown).

FVIII:Ag in general HHT population

In the full study, service laboratory FVIII:Ag was measured at least 4 months from any ill-health/intervention, and at least 10 months from any VTE. In this selected healthy HHT group, FVIII:Ag ranged from 0.52-4.87 (Q_1 1.51, Q_2 1.77, Q_3 2.27) u/ml. 87/125 (70%) of individuals had FVIII:Ag measurements exceeding the upper limit of the normal laboratory range of 0.45-1.58 u/ml, supporting the pilot study observation that FVIII:Ag levels were higher than the age-matched control group. The results were not significantly different excluding 16 individuals who had participated in the pilot study, or five individuals whose CRP exceeded 10 u/ml associated with FVIII:Ag 1.11-2.60 u/ml (p values of unpaired t-tests 0.90 and 0.96 respectively).

Factors that might contribute to elevated FVIII:Ag levels in HHT were explored as they could confound analyses of FVIII:Ag contribution to thrombotic risk in HHT. In univariate analyses, log-transformed FVIII:Ag measured ≥ 10 months from VTE was significantly correlated with age, fibrinogen levels, presence of pulmonary AVMs, and use of oral iron supplements (a marker of HHT-related haemorrhage), but not with Hb or CRP (Table 1).

Pulmonary hypertension, particularly thromboembolic pulmonary hypertension, is associated with elevated FVIII levels (ref). Pulmonary hypertension may occur in HHT (ref tremb). In our overall cohort, pulmonary hypertension was rare (Figure 2), only one of the 78 patients with FVIII measurements had significant pulmonary hypertension (associated with FVIII:Ag of 1.77u/ml, the median value for population), and there was no correlation between PAP and FVIII:Ag (Table 1).

Table 1. Univariate associations of log-transformed FVIII:Ag

<i>Variables</i>	<i>Cases</i>	<i>Median (Q1, Q3)</i>	<i>Spearman R (95% CI)</i>	<i>P value</i>
Age (yr)	124	51 (40, 61.5)	0.41 (0.25, 0.55)	p<0.0001*
Gender (females)	124	84 (67.8)	0.016 (-0.17, 0.20)	0.86
Pulmonary AVMs	124	114 (91.9)	0.22 (0.45, 0.39)	0.01*
Pulmonary AVM symptoms [†]	113	26 (23)	-0.13 (-0.31, 0.06)	0.18
Right-to-left shunt (%)	68	9.6 (4.8, 17.7)	0.046 (-0.20, 0.29)	0.71
SaO ₂ (%)	106	94 (88, 96)	0.057 (-0.14, 0.25)	0.57
PAP§ systolic (mmHg)	78	23.5 (20, 27)	0.14 (-0.09, 0.36)	0.21
PAP mean (mmHg)	78	13 (11, 16.5)	0.19 (-0.038, 0.40)	0.09
PAP diastolic (mmHg)	78	7 (5, 9)	0.12 (-0.11, 0.34)	0.29
Iron treatment for anaemia	122	46 (37.2)	0.20 (0.015, 0.37)	0.030*
Hb (g/dl)	120	14.5 (12.5, 15.8)	-0.12 (-0.30, 0.064)	0.19
CRP (iu/ml)	64	1 (1, 2)	0.11 (-0.14, 0.36)	0.37
Fibrinogen (g/L)	120	3.09 (2.7, 3.5)	0.3 (0.11, 0.46)	0.0014*
Platelets x10 ⁹ /l	113	267 (234, 437)	0.13 (-0.062, 0.31)	0.17
Prothrombin time (s)	118 [‡]	10.8 (10.4, 11.1)	-0.1 (-0.29, 0.08)	0.26

Legend: med, median; Q1, Q3, interquartile range; Rx, treatment; fib, fibrinogen; plt, platelets. * *P* value significant at FDR=0.05 level³⁵. [†] dyspnoea, haemoptysis; [‡] one patient on warfarin; § PAP, pulmonary artery pressure

Since HHT-related haemorrhage increases with age³⁶, multiple regression analyses were performed (Table 2). Again, there was no association with CRP, and once adjusted for age, neither fibrinogen nor oral iron use correlated with log-transformed FVIII:Ag (data not shown). The best model suggested however, that the unexpected association of pulmonary AVM with the level of FVIII:Ag might be independent of age (Table 2).

Table 2: Multiple regression of log transformed FVIII:Ag

Source	Type III sum of squares	Degrees of freedom	Mean square	variance ratio (F)	Odds ratio (95% confidence intervals)	Standard Error	T test	P value
Corrected model	3.36	3 [‡]	1.19	9.47	-	-	-	-
Intercept	0.11	1	0.11	0.86	0.385 (0.047, 0.73)	0.17	2.26	0.355
PAVM [†]	0.69	1	0.69	5.51	-0.276 (-0.51, -0.043)	0.117	-2.31	0.021
Age	1.64	1	1.64	13.11	0.008 (0.004, 0.013)	0.002	3.62	0.00045

Legend: Analyses performed in all 125 patients with FVIII:Ag measurements. FVIII:Ag assayed at least 4 months from any known infective illness, medical intervention or pregnancy, and ≥10 months from VTE. Parameter estimates =0 for [†] PAVM. Corrected model with 3 degrees of freedom resulted in r²=0.21, adjusted r²=0.18, type III sum of squares =3.56, and included VTE (p=0.035), as discussed further below.

FVIII:Ag and APTT

High FVIII:coagulant activity is associated with shortening of the activated partial thromboplastin time (APTT)³⁷, reflecting the proximal position of FVIII in the cascade reactions measured by the APTT (Figure 3A). To validate the FVIII:Ag measurements used for this study, correlations between FVIII:Ag and APTT were performed. In both pilot (Figure 3B) and full (Figure 3C) studies, FVIII:Ag levels inversely correlated with the APTT. The inverse correlation with APTT was maintained following log transformation of FVIII:Ag (Figure 3D).

Venous thromboemboli in full HHT population

To address whether FVIII:Ag influenced prothrombotic risk, association with VTE was examined. In the full study population, 20/309 (6.5%) individuals from 19 different HHT families gave a history of venous thromboembolic event either prior to review (n=16, including three with more than one event), or in the period of follow-up (n=4). Ages at the time of VTE ranged from 28-72 (Q_1 38, Q_2 50, Q_3 6) yr. 7/20 HHT/VTE patients had needed transfusions reflecting the high proportion of the overall HHT population with evidence of significant haemorrhage (Table 1). Fourteen had pulmonary AVMs.

Clinical risk factors for thrombosis were present in 13/20 HHT/VTE patients and included commencement on oestrogens in the preceding month (n=2), protein S deficiency (n=1; the sole HHT/VTE individual with detected abnormalities in protein C, protein S and antithrombin III), and recent long distance flight (n=1). Nine HHT patients developed VTE while hospital inpatients; five while recovering from a brain abscess secondary to pulmonary AVMs, two with liver failure or recent hepatic embolisation, and two with indwelling venous catheters for other conditions.

Univariate analyses did not suggest that transfusion need or other markers of HHT-related haemorrhage were protective against VTE (Table 3). The majority of individuals had pulmonary AVMs but there was no difference in the severity of pulmonary AVMs as quantified by right-to-left shunt, or oxygen saturation, between those experiencing and not experiencing thrombosis (Table 3). The univariate analyses did suggest that older individuals with HHT were at higher risk of VTE, as in the general population ^{22,23} (Table 3).

Table 3: Venous thromboemboli (VTE) univariate associations

	VTE	VTE	No VTE	No VTE	P value
<i>Continuous variable</i>	<i>Cases</i>	<i>Median (Q1, Q3)</i>	<i>Cases</i>	<i>Median (Q1, Q3)</i>	<i>Mann-Whitney</i>
Age (yr)	20	61 (48, 66)	283	48 (35, 59)	0.015*
SaO ₂ (%)	18	95 (90, 96.5)	229	95 (91, 97)	0.78
R-L shunt (%)	10	12.3 (8.7, 22)	122	8.9 (4.7, 19.9)	0.23
Hb (g/dl)	19	14.4 (10.8, 15.4)	252	14.4 (12.7, 15.5)	0.66
Platelets x10 ⁹ /l	11	283 (231, 363)	175	259 (231, 328)	0.64
Prothrombin time(s)	12	10.9 (10.3, 11.5)	161	10.8 (10.4, 11.2)	0.75
Fibrinogen (g/L)	13	3.27 (2.48, 3.73)	131	3.02 (2.62, 3.50)	0.68
<i>Binary variable</i>	<i>Cases</i>	<i>Number (%)</i>	<i>Cases</i>	<i>Number (%)</i>	<i>Fisher's exact</i>
Females	20	14 (70)	286	179 (62.6)	0.58
Pulmonary AVMs	20	17 (85)	286	193 (67.5)	0.089
Iron treatment	18	10 (56)	261	76 (29)	0.059
Transfused	18	4 (22)	261	26 (10)	0.20
Hormone treatment	17	4 (23.5)	249	47 (19)	0.75
Tranexamic acid treatment	18	2 (11)	259	15 (5.8)	0.70

Legend: * significant at FDR=0.05 level ³⁵, other abbreviations as in Table 1.

FV Leiden and PT20210A genotypes were performed on 15 consenting HHT/VTE patients and 33 age-matched controls. The overall frequency of heterozygous FV Leiden mutations was 5/48 (10.4%) tested individuals with HHT, in-keeping with the prevalence of 8% in a control UK population ³⁸ (p=0.59, one sample t test). All FV Leiden mutations occurred in the individuals with HHT/VTE group (5/15; 33%) and none in the 33 controls. This difference was statistically significant (p=0.0018, Fisher's exact test). In all cases heterozygous for FV Leiden, there had been an additional clinical precipitant of VTE (brain abscess n=4; female hormones n=1). One of the 48 tested patients with a previous history of VTE was heterozygous for both FV Leiden and PT20210A.

FVIII:Ag and VTE

Log-transformed baseline FVIII:Ag measurements were compared in HHT individuals who experienced a thrombosis ≥ 10 months from FVIII:Ag measurement, and in HHT individuals who had never experienced a thrombosis. In univariate analyses, log-transformed FVIII:Ag was significantly higher in HHT/VTE individuals compared to the HHT/noVTE group (Figure 4). Binary logistic regression analyses confirmed the association of baseline log-transformed FVIII:Ag levels measured at an interval of ≥ 10 (range 10-132, median 30) months from VTE with VTE risk (Table 4). The odds ratio of experiencing DVT was 2.41 (95% confidence intervals 1.25, 4.61) for a unit increase in baseline log transformed FVIII:Ag. Age made no additional contribution once adjusted for FVIII:Ag (Table 4).

Table 4: Venous thromboemboli (VTE) logistic regression analyses

Analysis	Variable	Odds ratio	Standard error	Wald test	degrees of freedom	Exp odds ratio (95% CI)	P value
Step1	Age	0.033	0.024	1.797	1	1.033 (0.985, 1.084)	0.18
-	FVIII	0.732	0.249	4.386	1	2.078 (1.048, 4.122)	0.036*
-	Constant	-5.436	1.476	13.559	1	0.004	0
Step 2	FVIII	0.878	0.332	6.98	1	2.405 1.254, 4.612)	0.008*
-	Constant	-3.95	0.841	22.052	1	0.019	0

Legend: Analyses performed in all 125 patients with FVIII:Ag measurements. Variables entered on step one: age, log transformed FVIII:Ag. Exp: exponential.

DISCUSSION

This study reports an elevation of FVIII:Ag levels in HHT, an elevation of probable functional significance for long term thrombotic risk. The findings imply that the possibility of thrombotic events cannot be ignored in HHT patients, even when transfusion-dependent. A similar concern has been raised for individuals with other haemorrhagic disorders including haemophilia³⁹.

The strengths of this study include the large numbers examined in a condition where many clinicians see only isolated cases. In addition, the study design attempted to minimise bias from independent factors leading to high FVIII levels, and included assessments of pulmonary artery pressure to exclude pulmonary hypertension as a cause for elevated FVIII:Ag in this population with predominantly normal pulmonary artery pressures. One potential weakness was the retrospective nature of the VTE assessments in most individuals. A further important factor was the high frequency of pulmonary AVMs in our population. The population was screened using a highly sensitive screening tool (thoracic CT scan with dedicated protocol). In a recent CT- and angiography-verified study⁴⁰, pulmonary AVMs affected at least 48.6% of a French HHT population compared to 5-15% in less well-screened populations^{3,36}). Nevertheless, a pulmonary AVM bias was undoubtedly present in our population.

Before assessing whether elevated FVIII:Ag might be associated with VTE risk, we explored mechanisms that may contribute to elevation of FVIII:Ag levels in the HHT population. There was no evidence for involvement of DIC, impaired fibrinolysis or platelet activation. CRP measurements confirmed that our study design had generally excluded individuals with significant intercurrent infections or inflammatory responses, and in any case, there was no association between FVIII:Ag and CRP in this study. Since HHT is caused by mutations in proteins expressed on vascular endothelial cells, we anticipated that aberrant endothelial cell function in HHT may stimulate the endothelium to release VWF, resulting in elevation of plasma FVIII:Ag. We were initially surprised by the association between pulmonary AVMs and baseline FVIII:Ag levels. The recent demonstration that pulmonary microvascular endothelial cells synthesise FVIII⁴¹ raises the possibility that such synthesis is dysregulated in the pulmonary vascular endothelial cells of individuals with HHT and pulmonary AVMs.

Activated FVIII is an essential component of intrinsic Xase in the common and thrombin-amplified coagulation cascades. To confirm the validity of FVIII:Ag measurements when many thrombotic studies measure FVIII:coagulant (FVIII:c), functional effects on global

coagulation parameters were examined. Our study demonstrated that FVIII:Ag, and log-transformed FVIII:Ag, inversely correlated with the APTT. These findings parallel those in the general population demonstrating elevated FVIII:c is associated with shortened APTT ³⁷.

Furthermore, in the HHT study population, we demonstrated that a single baseline service laboratory measurement of plasma FVIII:Ag was independently associated with the risk of VTE approximately 1-11 years from the measurement. Again, these findings are in-keeping with evidence emerging from the general population that high FVIII levels are associated with first and recurrent episodes of venous thrombosis ^{31,42}. Intriguingly, in the best model of our data, age made no additional contribution to VTE risk. We speculate that age-dependent increases in FVIII as seen in our study, and in the general population ⁴¹, may be a major contributor to the increased risk of VTE with age ^{22,23}.

FVIII:Ag levels were likely to be only one of multiple factors contributing to the thrombotic profile of individuals with HHT. Where the diagnosis of underlying HHT had been recognised, it was generally considered a relative contraindication to prophylactic and subsequent therapeutic anticoagulation of individual patients. As expected, inheritance of HHT did not protect against chance co-inheritance of independent prothrombotic genetic polymorphisms which occurred at frequencies which corresponded to those reported for a control UK population. There was however, a significant excess of FV Leiden genotype in HHT patients who experienced thromboses, as in the general population for whom a 2.7 fold increase in VTE risk has been cited ²⁷. FV Leiden heterozygosity appeared to be associated particularly with VTE in the period following a pulmonary AVM-induced brain abscess, a condition resulting in an acute phase response, neurosurgical intervention, and prolonged intravenous antibiotics.

The findings of elevated FVIII:Ag and other prothrombotic risk factors in HHT pose difficult clinical management issues. High proportions of HHT-affected individuals require iron therapy, and some are transfusion-dependent due to chronic nasal and gastrointestinal

haemorrhage. Conventional VTE prophylaxis and treatment are often withheld in view of the risk of precipitating active bleeding from these sites or visceral AVMs. The multiplicity of HHT telangiectatic lesions and their progressive development limit the efficacy of endoscopic, embolisation and surgical treatments for nasal and gastrointestinal haemorrhage⁴³. To limit blood loss, therapeutic manipulation of coagulation and fibrinolytic pathways is often employed using conjugated oestrogens⁴⁴, or prothrombotic agents such as tranexamic acid⁴⁵ and aminocaproic acid⁴⁶.

The data presented here lead us to conclude that individuals with HHT may be at a higher risk for thrombotic events than previously suspected. Further studies are needed to elucidate the mechanisms leading to elevated FVIII:Ag in HHT. Pending such studies, routine measurement of FVIII, FV Leiden, and other thrombophilic markers in HHT patient assessments may assist individualised risk-benefit considerations.

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LEGENDS TO FIGURES

Figure 1. Pilot study plasma protein screen: Age-related plasma levels of A) VWF:Ag, and C) FVIII:Ag in 38 HHT/noVTE individuals (triangles and solid regression line), and 38 non-HHT controls (squares and dotted regression line). B) VWF:Ag and D) FVIII:Ag levels in non-HHT controls (squares) and HHT/noVTE (triangles), *p* values calculated by Mann

Whitney. E) Correlations of FVIII:Ag with VWF:Ag. F) Plasma D-dimer levels in non-HHT controls and HHT/noVTE individuals. The HHT/noVTE group had no other evidence of overt DIC (platelet counts $136-528 \times 10^9/l$; prothrombin time 10.0-14.0 seconds).

Figure 2. Pulmonary artery pressure measurements in population

Systolic (sys), mean and diastolic (d/s) PAP measurements presented for full population (open symbols) and 78 patients with FVIII measurements (filled symbols, circled for one individual with significant pulmonary hypertension). Stippled areas represent normal range.

Figure 3: APTT and FVIII: A: Schematic of coagulation pathway highlighting sites of FVIII and FV activity, and cascade reactions contributing to the APTT (stippled area). B: Linear regression of APTT with FVIII:Ag in the pilot study of 38 HHT/noVTE (triangles and solid regression line) and 38 non-HHT controls (squares and dotted regression line). C, D: Linear regression and 95% confidence limits of regression line of APTT with C) FVIII:Ag, and D) log-transformed FVIII:Ag in the full study of 125 HHT/ $\pm$ VTE individuals. Light stippled areas indicate APTT normal laboratory range (24-32 seconds).

Figure 4. Plasma FVIII:Ag and VTE. Distribution of log transformed FVIII:Ag in HHT/noVTE, and HHT/VTE subgroups. Light stippled areas indicate log-transformed FVIII:Ag normal laboratory range. *: unpaired t test.

REFERENCES

1. Bideau A, Brunet G, Heyer E, Plauchu H, Robert J-M. An abnormal concentration of cases of Rendu-Osler disease in the Valserine valley of the French Jura: a geneological and demographic study. *Annals of Human Biology*. 1992; 19: 233-247.

2. Kjeldsen AD, Vase P, Green A. Hereditary hemorrhagic telangiectasia (HHT): a population-based study of prevalence and mortality in Danish HHT patients. *J Intern Med.* 1999; 245: 31-39.
3. Guttmacher AE, Marchuk DA, White RI. Hereditary hemorrhagic telangiectasia. *New Engl J Med.* 1995; 333: 918-924.
4. Begbie ME, Wallace GMF, Shovlin CL. Hereditary haemorrhagic telangiectasia (Osler-Weber-Rendu syndrome): A view from the 21st century. *Postgrad Med J.* 2003; 79: 18-24.
5. Girola SS, Cusa C, DiGiulio G, Marti A, Mazzone A. Lung angiodysplasia and von Willebrand's disease in a family with Rendu-Osler disease. *Recenti Prog Med.* 1996; 87: 589-91.
6. Maggi CA, Ballarin E, Comin U, Mannucci PM. Hemorrhagic telangiectasia and hemophilia A: an occasional association? *Haematologica.* 1983; 68(3): 399-404.
7. Sudarshan A, Natelson EA, Gordon C. Hereditary hemorrhagic telangiectasia and factor VIII inhibitor. *South Med J.* 1985; 78: 623-4.
8. Ferran M, Arderiu A, Vilardell M, Tornos J. Hereditary hemorrhagic telangiectasia and congenital factor XI deficiency. *Med Clin (Barc).* 1986; 86(10): 425-7.
9. Muckle JJ. Low in-vivo adhesive platelet count in hereditary haemorrhagic telangiectasia. *Lancet.* 1964; 2: 880-882.
10. Larsson SO. Osler's disease with impaired adhesion and aggregation of platelets. *Acta Medica Scandinavica.* 1974; 196: 133-136.
11. Maire R, Schnewlin G, Bollinger A. Videomikroskopische Untersuchungen von Telangiektasien bei Morbus Osler und Sclerodermie. *Schweiz. med. Wschr.* 1986; 116: 335-338.
12. Braverman IM, Keh A, Jacobson BS. Ultrastructure and three-dimensional organization of the telangiectases of hereditary hemorrhagic telangiectasia. *J Invest Dermatol.* 1990; 95: 422-427.
13. 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, Haitjema T, Westerman CJJ, Porteous ME, Guttmacher AE, Letarte M, Marchuk DA. Endoglin, a TGF- β binding protein of endothelial cells, is the gene for hereditary haemorrhagic telangiectasia type 1. *Nature Genetics*. 1994; 8: 345-351.
14. Johnson DW, Berg JN, Baldwin MA, Gallione CJ, Marodel I, Yoon S-J, Stenzel TT, Speer M, Pericak-Vance MA, Diamond A, Guttmacher AE, Jackson CE, Attisano L, Kucherlapati R, Porteous MEM, Marchuk DA. Mutations in the activin receptor-like kinase 1 gene in hereditary haemorrhagic telangiectasia type 2. *Nature Genetics*. 1996; 13: 189-195.
 15. Gallione C, Repetto GM, Legius E, Rustgi AK, Schelley SL, Tejbar S, Mitchell G, Drouin E, Westermann C, Marchuk D. A combined syndrome of juvenile polyposis and hereditary haemorrhagic telangiectasia is associated with mutations in MADH4 (SMAD4). *Lancet*. 2004; 363: 852-859.
 16. Cole SG, Begbie ME, Wallace GMF, Shovlin CL. A new locus for hereditary haemorrhagic telangiectasia (HHT3) maps to chromosome 5. *J Med Genet*. 2005; 42: 577-582.
 17. Bayrak-Toydemir P, McDonald J, Akarsu N, Toydemir RM, Calderon F, Tuncali T, Tang W, Miller F, Mao R. A fourth locus for hereditary hemorrhagic telangiectasia maps to chromosome 7. *Am J Med Genet*. 2006; 140: 2155-62.
 18. Bick RL. Hereditary hemorrhagic telangiectasia and disseminated intravascular coagulation: a new clinical syndrome. *Annals of the New York Academy of Sciences*. 1981; 370: 851-854.
 19. Blaustein A. Treatment of thrombosis in individuals with hereditary hemorrhagic telangiectasis: report of three cases including two members of one family. *Angiology*. 1956; 7: 55-60.
 20. Sturm W, Gaenzer H, Heiniger-Rothbucher D, Neumayr G, Kirchmair R, Patsch J, Weiss G. Unsuspected mesenteric vein thrombosis in a patient with a hereditary bleeding disorder. *Blood Coag Fibrinolysis*. 2003; 14: 599-600.

21. Shovlin CL, Jackson JE, Pulmonary arteriovenous malformations, in Pulmonary Circulation, 2nd edn, A. Peacock, L. Rubin, Editors. 2004, Arnold: London.
22. Oger E. Incidence of venous thromboembolism: a community-based study in Western France. *Thrombosis and Haemostasis*. 2000; 83: 657-60.
23. Heit JA, Silverstein MD, Mohr DN. The epidemiology of thromboembolism in the community. *Thromb Haemost*. 2001; 86: 452-63.
24. Shovlin CL, Guttmacher AE, Buscarini E, Faughan M, Hyland R, Westermann CJJ, Kjeldsen AD, Plauchu H. Diagnostic criteria for hereditary haemorrhagic telangiectasia (Rendu-Osler-Weber syndrome). *Am J Med Genet*. 2000; 91: 66-67.
25. Gupta P, Mordin C, Curtiss J, Hughes JMB, Shovlin CL, Jackson JE. PAVMs: Effect of embolisation on right-to-left shunt, hypoxaemia and exercise tolerance in 66 patients. *Am J Roent*. 2002; 179: 347-55.
26. Dahlback B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognised mechanism characterised by poor anticoagulant response to activated protein C: Prediction of a co-factor to activated protein C. *Proc Natl Acad Sci*. 1993; 90: 1004-1008.
27. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood*. 1996; 88: 3698-703.
28. Letamandía A, Lastres P, Botella L, Raab U, Langa C, Velasco B, Attisano L, Bernabeu C. Role of endoglin in cellular responses to transforming growth factor- β . *J Biol Chem*. 1998; 273: 33011-33019.
29. Seigneur M, Constans J, Blann A, Renard M, Pellegrin JL, Amiral J, Boisseau M, Conri C. Soluble adhesion molecules and endothelial cell damage in HIV infected patients. *Thrombosis and Haemostasis*. 1997; 77: 646-649.
30. van Mourik JA, Boertjes R, Huisveld IA, Fijnvandraat K, D DP, van Genderen PJ, Fijnheer R. von Willebrand factor propeptide in vascular disorders: A tool to distinguish between acute and chronic endothelial cell perturbation. *Blood*. 1999; 94: 179-185.

31. Kraaijenhagen RA, in'tAnker PS, Koopman MM, Rietsma PH, Prins M H, van den Ende A, Buller HR. High plasma concentration of Factor VIIIc is a major risk factor for venous thromboembolism. *Thrombosis and Haemostasis*. 2000; 83: 5-9.
32. Begbie M, Notley C, Tinlin S, Sawyer L, Lillicrap D. The Factor VIII acute phase response requires the participation of NFkappaB and C/EBP. *Thrombosis and Haemostasis*. 2000; 84: 216-22.
33. van den Burg PJ, Hospers JE, van Vliet B, Mosterd WL, Bouma BN, Huisveld IA. Changes in haemostatic factors and activation products after exercise in healthy subjects with different ages. *Thromb Haemost*. 1995; 74: 1457-64.
34. O'Donnell J, Laffan MA. The relationship between ABO histo-blood group, factor VIII and von Willebrand factor. *Transfus Med*. 2001; 11: 343-51.
35. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: a practical and powerful approach to multiple testing. *J Roy Stats Soc (B)*. 1995; 57: 289-300.
36. Plauchu H, de Chadarevian J-P, Bideau A, Robert J-M. Age-related profile of hereditary hemorrhagic telangiectasia in an epidemiologically recruited population. *Am J Med Genet*. 1989; 32: 291-297.
37. Tripodi A, Chantarankul V, Martinelli I, Bucciarelli P, Mannucci PM. A shortened activated partial thromboplastin time is associated with the risk of venous thromboembolism. *Blood*. 2004; 104: 3631-4.
38. Bowen DJ, Bowley S, John M, Collins PW. Factor V Leiden (G1691A), the prothrombin 3'-untranslated region variant (G20210A) and thermolabile methylenetetrahydrofolate reductase (C677T): a single genetic test genotypes all three loci- determination of frequencies in the S.Wales population of the UK. *Thromb Haemost*. 1998; 79: 949-954.
39. Dargaud Y, Meunier S, Negrier C. Haemophilia and thrombophilia: an unexpected association! *Haemophilia*. 2004; 10(4): 319-26.
40. Cottin V, Plauchu H, Bayle JY, Barthelet M, Revel D, Cordier JF. Pulmonary arteriovenous malformations in patients with hereditary hemorrhagic telangiectasia. *Am J Respir Crit Care Med*. 2004; 169(9): 994-1000.

41. Rumley A, Emberson JR, Wannamethee SG, Lennon L, Whincup PH, Lowe GD. Effects of older age on fibrin D-dimer, C-reactive protein, and other hemostatic and inflammatory variables in men aged 60-79 years. *J Thromb Haemost*. 2006; 4: 982-7.
42. Kyrle PA, Eichinger S. The risk of recurrent venous thromboembolism: the Austrian study on recurrent venous thromboembolism. *Wien Klin Wochenschr*. 2003; 115(13-14): 471-4.
43. Sargeant IR, Loizou LA, Rampton D, Tulloch M, Brown SG. Laser ablation of upper gastrointestinal vascular ectasias: long term results. *Gut*. 1993; 34: 470-475.
44. Van Cutsem E, Rutgeerts P, Vantrappen G. Treatment of bleeding gastrointestinal vascular malformations with oestrogen-progesterone. *Lancet*. 1990; 335: 953-955.
45. Sabba C, Gallitelli M, Palasciano G. Efficacy of unusually high doses of tranexamic acid for the treatment of epistaxis in hereditary hemorrhagic telangiectasia. *N Engl J Med*. 2001; 345: 926.
46. Saba HI, Morelli GA, Logrono LA. Treatment of bleeding in hereditary hemorrhagic telangiectasia with aminocaproic acid. *New Engl J Med*. 1994; 330(25): 1789-1790.