LETTER TO JMG

Genotype-phenotype relationship in hereditary haemorrhagic telangiectasia

T G W Letteboer, J J Mager, R J Snijder, B P C Koeleman, D Lindhout, J K Ploos van Amstel, C J J Westermann

J Med Genet 2006;**43**:371–377. doi: 10.1136/jmg.2005.035451

Hereditary haemorrhagic telangiectasia (HHT) is an autosomal dominant disorder characterised by vascular malformations in multiple organ systems, resulting in mucocutaneous telangiectases and arteriovenous malformations predominantly in the lungs (pulmonary arteriovenous malformation; PAVM), brain (cerebral arteriovenous malformation; CAVM), and liver (hepatic arteriovenous malformation; HAVM). Mutations in the *ENG* and *ALK-1* genes lead to HHT1 and HHT2 respectively. In this study, a genotype-phenotype analysis was performed. A uniform and well classified large group of HHT patients and their family members were screened for HHT manifestations. Groups of patients with a clinically confirmed diagnosis and/or genetically established diagnosis (HHT1 or HHT2) were compared. The frequency of PAVM, CAVM, HAVM, and gastrointestinal telangiectases were determined to establish the genotype-phenotype relationship. The analysis revealed differences between HHT1 and HHT2 and within HHT1 and HHT2 between men and women. PAVMs and CAVMs occur more often in HHT1, whereas HAVMs are more frequent in HHT2. Furthermore, there is a higher prevalence of PAVM in women compared with men in HHT1. In HHT1 and HHT2, there is a higher frequency of HAVM in women. HHT1 has a distinct, more severe phenotype than HHT2. There is a difference in the presence of symptoms between men and women. With these data, genetic counselling can be given more accurately when the family mutation is known.

Hereditary haemorrhagic telangiectasia (HHT or Rendu-Osler-Weber disease) is an autosomal dominant disorder characterised by vascular malformations in multiple organ systems. Estimates of the frequency of the disease vary widely, the prevalence in the Netherlands is estimated 1 in 10 000, while in the Dutch Antilles the prevalence is at least 1 in 1330.[1]

The clinical symptoms of HHT are caused by direct arteriovenous connections without an intervening capillary bed. The resulting mucocutaneous teleangiectases can occur anywhere, but particularly in the oral cavity (lips, tongue), nose, and conjunctivae, and on the fingertips. Telangiectases in the nasal mucosa can result in epistaxis, usually the first and most common symptom, present in more then 90% of patients with HHT.[2–4]

Larger arteriovenous malformations (AVM) are mostly located in the lung (pulmonary arteriovenous malformations; PAVM), brain (cerebral arteriovenous malformations; CAVM) and liver (hepatic arteriovenous malformations; HAVM).[3–7] PAVMs are estimated to develop in 15–35% of patients,[3 8] resulting in a right to left shunt. PAVMs can cause hypoxaemia, bleeding (haemothorax), and bypass of emboli or septic material, which can lead to serious systemic complications such as cerebral abscess and infarction.[3 5 9] Screening for PAVMs is therefore advised, and treatment of PAVMs is justified even when asymptomatic.

Cerebral arteriovenous malformations (CAVMs) are less common (5–13% of patients), but are probably under recognised.[6 10–12] Although they are often silent, they can cause headache, seizures, ischaemia, and bleeding.[6 10] The bleeding risk ranges from <1% to 1.5–2% per year per patient.[10 13]

The frequency of hepatic involvement in HHT varies considerably, mainly because HHT patients have not been routinely screened for HAVM. The frequency is estimated to be up to 32%.[8 14–17] Liver involvement predominantly concerns shunts between the hepatic artery and hepatic veins. HAVMs are often asymptomatic, but may lead to a high cardiac output with heart failure and eventually to portal hypertension and biliary disease.

Gastrointestinal (GI) bleeding is usually present at an older age, due to telangiectases in the GI tract, which can cause severe anaemia. The estimated prevalence is 15–45%.[4 6 8 18]

It should be emphasised that there is considerable interfamilial and intrafamilial variability with respect to age related penetrance and pattern of clinical expression.

Mutations in the endoglin (*ENG*; OMIM #131195) or activin A receptor type-like kinase 1 (*ACVRL-1*, *ALK-1*; OMIM #601284) genes cause HHT. Expression studies in human umbilical vein endothelial cells and peripheral blood monocytes have confirmed haploinsufficiency as the causative mechanism in both forms of HHT.[8 19–21]

In 2004, patients with clinical features of both HHT and juvenile polyposis were shown to carry mutations in the *MADH4* gene.[22] To date, mutations in the *MADH4* gene have not been reported in patients with HHT without juvenile polyposis. Recently, a new locus has been mapped to chromosome 5, associated with classical HHT.[23]

Mutations in *ENG* and *ALK-1* result in HHT1 and HHT2 respectively. The identification and characterisation of mutations in HHT patients revealed extensive molecular heterogeneity.[20 24 25] As a result of different selection criteria, populations, and detection methods for the mutation analysis, different groups report different mutation detection rates. In a national study of Dutch HHT patients, pathogenic mutations were detected in 93% of the families, of which 53% were in the *ENG* gene and 40% in the *ALK-1* gene.[24]

The genetic heterogeneity in HHT explains part of the phenotypic variability. A higher prevalence of PAVMs and CAVMs was suggested in HHT1, while families with HHT2

Abbreviations: CAVM, cerebral arteriovenous malformation; CT, computed tomography; GI, gastrointestinal; HAVM, hepatic arteriovenous malformation; HHT, hereditary haemorrhagic telangiectasia; MRI, magnetic resonance imaging; PAVM, pulmonary arteriovenous malformation

generally tend to show a later onset of the symptoms and a milder phenotype.[3 4 7 8] Accurate data on the prevalence of the symptoms in HHT1 and HHT2 are, however, scarce. No extensive studies on the clinical manifestations in relation to the gene involved and the type of pathogenic mutation have been reported.

Although Berg *et al*[4] were the first to compare patients with HHT1 and HHT2 and to report differences in clinical features between the two groups, the clinical data were obtained using a questionnaire. The presence of symptoms was provided by the participants, and data were not cross checked with a review of the medical records. The participants were from the UK and the USA, areas with different screening protocols and population backgrounds. In 83 participants, this group found a PAVM significantly more often in patients with HHT1 (35%) compared with HHT2 (0%).

Abdalla *et al*[8] reported the analysis of patients with *ALK-1* documented in the literature (281). They found PAVM in only 5% of patients, CAVM in 2%, HAVM in 13%, and GI manifestations in 12%. Although the visceral manifestations are reported more frequently in HHT1, publications on this subject are limited.

We report on the frequencies of the visceral manifestations in HHT1 and HHT2 in a large Dutch cohort. This is the second study to compare the clinical data of patients from families with *ENG* (HHT1) and *ALK*-1 (HHT2) mutations. This is the first study to use clinical data obtained from one national HHT centre covering a circumscribed region in north western Europe with equal access to healthcare facilities.

MATERIALS AND METHODS

Patients

The patients were selected from a panel consisting of all probands and family members screened for HHT. Family members of index cases were advised to attend the hospital. Subjects referred until August 2004 were included in a database. This date was also used to calculate the age of each person; the ages depicted are not the ages at diagnosis, but the age at the time of the analysis. Most probands and family members were screened for visceral manifestations at St. Antonius Hospital, which specialises in the diagnosis and treatment of HHT. Clinical data of a minority of the patients ($n = 57$) were obtained through medical records from elsewhere; these patients were included after re-evaluation of the medical records. All manifestations of HHT were recorded in the database, for both probands and family members. At the time of analysis, the database consisted of 1291 people screened for the presence of HHT.

The clinical diagnosis HHT was established according to the Curaçao criteria.[26] At least three of the following four criteria were required for a clinical diagnosis: spontaneous and recurrent epistaxis, telangiectases at characteristic sites, visceral manifestations (PAVM, CAVM, HAVM, or GI telangiectases) and a first degree relative with HHT. In the presence of two criteria, the diagnosis was considered possible.

Molecular analysis

Mutation analysis was performed as reported.[24] In short, DNA was isolated from each of the probands, and exons 1–14 of *ENG* and exons 1–10 *ALK-1* and their flanking intronic sequences were amplified using PCR. The PCR products were purified and sequenced. Once the mutation was identified, relatives were tested for the disease causing mutation.

A genetic diagnosis was considered to be positive when the family mutation was present or when the patient was an obligate carrier of the mutation. All patients with a clinically and/or genetically confirmed diagnosis and who were older than 16 years at the time of the screening were included in the study.

When a pathogenic mutation was found in the proband, apparently affected and unaffected relatives were offered genetic counselling and DNA analysis, which was performed for most relatives but not all. Patients not tested but with a proven HHT and with one or more affected family members with a known pathogenic mutation were considered to have the same mutation. The affected patients were divided in three groups, HHT1, HHT2, or HHT? on the basis of the mutation findings. The group HHT? consisted of probands and their relatives for whom DNA was either not available (66 patients from 37 families) or in whom a pathogenic mutation was not found (10 patients from 7 HHT? families).

Screening for the presence of a PAVM was performed routinely by chest radiography and by measuring partial oxygen pressure in arterial blood and, if abnormal, followed by the 100% oxygen right to left shunt test.[27] A normal result excluded a PAVM. Patients with a suspected PAVM were offered subsequently a conventional angiography, a digital subtraction angiography of the pulmonary arteries, or computed tomography (CT) of the chest. When an abnormal chest radiography and or a pathological right to left shunt (>5%) was found, but confirmation through subsequent angiography or CT analysis was not performed, the PAVM was classified as doubtful.

Until 2001, screening for CAVMs was performed using intravenous digital subtraction angiography; when a CAVM was suspected, conventional cerebral angiography was also performed. Since 2001, this screening was performed with CT or magnetic resonance imaging (MRI) only when, after counselling, the patient requested it or because of symptoms. The screening for HAVM was not done routinely. Ultrasonography, CT or MRI was performed in cases of elevated alkaline phosphatase level or gamma glutamyl transpeptidase, or the presence of a murmur over the liver, heart failure, or abdominal pain. Between 1996 and 2003, screening for an HAVM was also performed before embolisation of a PAVM, in order to avoid embolisation complications.[28]

The search for GI involvement was only performed (by means of a regular diagnostic endoscopy or videocapsule endoscopy) when unexplained iron deficiency anaemia was detected or in cases of overt bleeding. Only when multiple GI telangiectases were detected was GI involvement considered confirmed.

Statistical analysis

The proportion of subjects in each group with visceral manifestations was calculated. The statistical analysis was performed using 2×2 table analysis with the χ^2 test. To compensate for multiple testing, the p value for individual tests was multiplied by the number of comparisons made (Bonferroni correction, p_c).

RESULTS

In total, 1291 people (558 men, 733 women) were included in the database containing patients and their family members screened for HHT symptoms. Of these, 1130 were older than 16 years at the time of the screening. Four people were excluded from the analysis because *MADH4* mutations were detected as a cause of HHT and juvenile polyposis. The 1126 people were 100 probands (32 men and 68 women), 484 affected family members, and 542 family members in whom the diagnosis could not be established or was excluded. Thus, 584 (242 men and 342 women) were older than 16 years and had been diagnosed clinically or genetically with HHT (table 1).

Telangiectases detected on physical examination or in the nose (rhinoscopy) were present in 98.4% of patients, and 97.2% of patients were known to have epistaxis (data not shown).

A clinically doubtful but genetically confirmed diagnosis was found in 19 patients in HHT1 (5%) and in 11 patients with HHT2 (8.6%). Numbers, ages, sex, and mean ages of different groups are depicted in table 1. There was a preponderance of women in the database, both as affected and unaffected family members. There was no significant difference in sex ratio between the three groups. The frequency of visceral manifestations found in HHT1, HHT2, and HHT? is depicted (tables 2 and 3).

PAVM

In the HHT1 group, 359 patients were examined clinically. Of these, 16 patients (4.4%) had a doubtful result. Of the remaining 343 patients, 167 (48.7%) were diagnosed clinically with a PAVM. In the HHT2 group, 12 of the 126 examined patients (9.5%) had a doubtful result. The frequency of PAVM in the HHT2 group was 5.3% (6/114 patients).

In total, 151 men with HHT1 were screened for PAVM, of whom eight had a doubtful result. Of the remaining 143 men, 58 men had a PAVM (40.6%). Of the 208 women screened for a PAVM, eight had an uncertain result. Of the remaining 200 women, 109 had a PAVM (54.5%). Therefore, PAVM occurred not only significantly more often in HHT1 than in HHT2 ($p = 6\times10^{-16}$), but also occurred more often in HHT1 women than HHT1 men, although this difference was not significant after correction for multiple testing.

In the HHT? group, 71 patients were examined for the presence of a PAVM, of whom 11 had a doubtful result. A PAVM was detected in 27 out of the remaining 60 patients (45%); 10 of 20 men and 17 of 40 women.

CAVM

In total, 268 HHT1 patients were investigated for a CAVM. In eight patients (four men, four women) the presence of a CAVM could not be definitely determined. The frequency of CAVM in HHT1 was 14.6% (38/260). Of 76 HHT2 patients (28 men, 48 women) screened for CAVM, none had a doubtful result and only one woman had a CAVM (1.3%).

In the HHT1 group, of the 109 men 14 had a CAVM (12.8%) compared with 24/151 of the women (15.9%). In the HHT? group, two patients had a dubious result. Of the remaining 42 patients (16 men, 26 women), four had a CAVM (9.5%).

The combination of PAVM and CAVM in the same patient was found only in the HHT1 group. Patients were included who were screened for both manifestations—that is had screening performed for PAVM and CAVM and had definite absence or presence of the manifestations. In patients with only one manifestation, the other one excluded, the combination PAVM/CAVM was considered absent. In HHT1, the combination was present in 22 (6 men and 16 women) of 253 patients (8.7%). Of the 231 patients without the combination, 120 had only PAVM, 13 had CAVM only, and 98 had neither PAVM or CAVM. In the HHT2 patients, the combination of CA1 had a CAVM, and 60 had no manifestation. In the HHT? group, the combination was detected in one of 38 patients (2.6%).

HAVM

In the HHT1 group, 162 patients (61 men, 101 women) were screened for HAVM, of whom 18 had a doubtful result. Of the remaining 144 patients (56 men, 88 women) 1 man and 10 women were diagnosed with an HAVM. In the HHT2 group, the liver was examined in 38 patients, of whom six had a doubtful result. An HAVM was detected in 13 (2 men, 11 women) of the remaining 32 patients (12 men, 20 women). Significantly more HAVMs were detected in HHT2 (40.6% versus 7.6%, $p = 0.0004$). Furthermore, in both groups HAVMs were present more often in women then in men. However, this difference was not significant after Bonferroni correction. In the HHT? group, there was a frequency of 21.2% (7/33) for HAVM.

GI localisation of HHT (telangiectases) was investigated in 78 HHT1 patients. In 56 (23 men, 33 women) multiple telangiectases were detected. Screening of the GI tract was undertaken in 29 HHT2 patients, and in 19 GI telangiectases were found (11 men, 8 women). In the HHT? group, the intestines were investigated in 16 patients, of whom 11 were diagnosed with HHT of the bowels. In an attempt to correct for possible referral bias, we performed a second analysis, in which we excluded the proband of each of the families, the

Table 1 Proportion of male (M) and female (F) subjects for HHT1, HHT2 and HHT?, with mean (SD) ages.

	HHT1	HHT2	HHT?
Probands and family			
No. of subjects (M:F ratio)	735 (0.74)	216 (0.77)	175 (0.68)
Mean age (SD), years	44.8 (17.2)	46.2(16.6)	47.8 (18.1)
HHT present (M:F ratio)	380 (0.74)	128 (0.71)	76 (0.58)
Mean age (SD), years	48.4 (18.2)	51.2 (16.2)	53.7 (15.5)
HHT possible (M:F ratio)	84 (0.83)	25 (1.27)	60 (0.67)
Mean age (SD), years	40.0 (16.8)	38.0 (14.8)	43.8 (20.9)
HHT absent (M:F ratio)	271 (0.73)	63 (0.75)	39 (0.95)
Mean age (SD), years	41.2 (14.7)	39.1 (16.8)	42.6 (15.2)
HHT present			
No. of subjects	380	128	76
No. of families	63	40	44
Members per family	6	3.2	1.7
Men	161 (42.4%)	53 (41.4%)	28 (36.8%)
Women	219 (57.6%)	75 (58.6%)	48 (63.1%)
Mean age (SD), years	48.4 (18.2)	51.2 (16.2)	53.7 (15.5)
Mean age M (SD), years	47.9 (18.8)	53.5 (13.6)	51.9 (13.6)
Mean age F (SD), years	48.8 (17.7)	49.7 (17.7)	54.8 (16.5)

Family members with a clinical and or genetic certain diagnosis, ascertained from the database, are shown. The "HHT presentW group consists of patients with a clinically and or genetically confirmed diagnosis. Patients were deemed "possible" when two of the four criteria were present. Patients with HHT in combination with juvenile polyposis were excluded.

Table 2 Prevalence of visceral manifestations in HHT1, HHT2, and HHT?

	HHT1	HHT2	HHT?	p
PAVM	167/343 (48.7%)	6 /114 (5.3%)	27/60 (45%)	1.2×10^{-16} ($p_c=6\times10^{-16}$)
CAVM	38/260 (14.6%)	1/76 (1.3%)	4/42 (9.5%)	0.0015 ($p_c=0.007$)
CAVM + PAVM	22/253 (8.7%)	0/67 (0%)	1/38 (2.6%)	0.012 ($p_c=0.062$)
HAVM	11/144 (7.6%)	13/32 (40.6%)	7/33 (21.2%)	8.7×10^{-7} ($p_c=4.4\times10^{-6}$)
GI telangiectasia	56/78 (71.8%)	19/29 (65.5%)	11/16 (68.8%)	NS (NS)

Patients with a doubtful result were not included in the analysis. Denominators varied between categories because not all patients underwent all examinations for all visceral organs. The statistical analysis was performed comparing HHT1 and HHT2. The p values are shown, with p values after correction for multiple testing in brackets.

first of the family who was referred. The results are given in table 4. The significant differences between HHT1 and HHT2 for PAVM and HAVM remained after exclusion of the probands. In HHT1, significantly more women have an HAVM (table 5). An increased frequency of CAVM was again observed in HHT1 compared with HHT2, but this trend was not significant after Bonferroni correction, nor was the difference in PAVM in HHT1 between men and women significant.

DISCUSSION

This study is the first analysis based on a national HHT population evaluated by use of a standard protocol applied within a single national HHT centre. In this study, we compared patients from families with *ENG* mutations with patients from families with *ALK-1* mutations. We report on the frequencies of disease manifestations in HHT1, HHT2, and HHT?. The results reveal differences between HHT1 patients and HHT2 patients and between men and women.

The three patient groups, HHT1, HHT2, and HHT?, were comparable with respect to age and age distribution, which is important when comparing age dependent disease expressions. The groups are also large from the viewpoint of statistical power. The proportion of family members with a certain diagnosis in the database was slightly different for HHT1 and HHT2. For HHT1, 51.7% of the family members were diagnosed with HHT, while for HHT2 this was 59.3%. In the HHT2 group, obviously fewer unaffected family members older than 16 years are known in the clinic. This may be due to the fact that family members of HHT1 patients are more likely to attend hospital because of the more severe phenotype in their relatives, even when they themselves are asymptomatic. This may also explain the higher number of relatives referred or examined from families with HHT1 compared with HHT2.

In the HHT1 and HHT2 groups, the percentage of family members with possible diagnosis was 11.4% and 11.5% respectively. The mean ages of these groups were lower than the mean ages of the total group. This probably reflects the age related penetrance, but has no influence on the comparison of the two groups.

In all three HHT groups, there was a significant female preponderance. The female preponderance was uniformly present in the database of 1291 people (56.7% women), after selection for the family members above 16 years (57.5% women) and in the group with clinically or genetically confirmed HHT (58.6% women). A female preponderance was also found among unaffected family members and among the groups that were screened but with an uncertain diagnostic result. Only in the HHT2 ''possibly affected'' group was there a male preponderance, but this is a small group. This finding may reflect the notion, held by both families and physicians, that women have an AVM more often than men. Therefore, women in a family are more aware of HHT or are stimulated to have screening performed. Another explanation might be that a different attitude towards healthcare exists between men and women. This was suggested to be the cause for female preponderance in, for example, families with colon cancer.[29] The fact that there is a female preponderance in both the affected and unaffected cohorts also raises the question as to whether there is a difference in genetic fitness between men and women. When there is a disadvantage for male fetuses in the early embryonic period, more girls will be born, resulting in a female preponderance. Thorough family investigations will shed light on this aspect.

Phenotypic differences between HHT1 and HHT2

A PAVM was significantly more frequent in HHT1 (48.7%) than in HHT2 (5.3%). This concurs with earlier reports. Berg *et al*[4] reported PAVM in 34.7% of HHT1 patients and no PAVM in HHT2 patients. The combined data published by Abdalla *et al*[8] show a frequency of PAVMs in HHT2 patients of 5%, very similar to our findings. Our screening technique with chest radiography and arterial blood gas is not as sensitive in the detection of PAVM as the echo bubble technique,[30] therefore, small PAVMs may have been missed in our study.

Table 3 Prevalence of visceral manifestations in HHT1 and HHT2, for men and women

	Men	Women	p
HHT1			
PAVM	58/143 (40.6%)	109/200 (54.5%)	0.011 ($p_c=0.054$)
CAVM	14/109 (12.8%)	24/151 (15.9%)	0.49 (NS)
PAVM + CAVM	6/105 (5.7%)	16/148 (10.8%)	0.16 (NS)
HAVM	1/56 (1.8%)	10/88 (11.4%)	0.035 ($p_c=0.174$)
GI telangiectases	23/33 (69.7%)	33/45 (73.3%)	NS (NS)
HHT2			
PAVM	2/50 (4%)	4/64 (6.3%)	0.593 (NS)
CAVM	0/28 (0%)	1/48 (2.1%)	0.442 (NS)
PAVM + CAVM	0/27 (0%)	0/40 (0%)	NS (NS)
HAVM	2/12 (16.7%)	11/20 (55%)	0.033 ($p_c=0.162$)
GI telangiectases	11/16 (68.8%)	8/13 (61.5%)	NS (NS)

Patients with a doubtful result were not included in the analysis. Denominators varied between categories because not all patients underwent all examinations for all visceral organs. The statistical analysis was performed comparing men and women. The p values are shown, with p values after correction for multiple testing in brackets.

Table 4 Prevalence of visceral manifestations in HHT1, HHT2, and HHT? after exclusion of the proband of each family (first patient referred and ascertained) in order to correct for possible referral bias

	HHT1	HHT2	HHT?	p
PAVM	133/300 (44.3%)	3/88 (3.4%)	16/42 (38.1%)	1.5×10^{-12} ($p_c=6\times10^{-12}$)
CAVM	31/225 (13.8%)	1/50 (2.0%)	2/27 (7.4%)	0.019 ($p_c=0.094$)
CAVM + PAVM	16/218 (7.3%)	0/45 (0%)	0/27 (0%)	0.06 (NS)
HAVM	9/119 (7.6%)	8/21 (38.1%)	6/19 (31.6%)	7.8×10^{-5} ($p_c=0.0004$)
GI telangiectasia	44/62 (71%)	12/18 (66.7%)	7/11 (63.3%)	NS (NS)

Patients with a doubtful result were not included in the analysis. Denominators varied between categories because not all patients underwent all examinations for all visceral organs. The statistical analysis was performed comparing HHT1 and HHT2. The p values are shown, with p values after correction for multiple testing in brackets

As the same screening method was used in HHT1 and HHT2 and there is no evidence that patients with HHT2 have smaller PAVMs than patients with HHT1, our results probably reflect the proportional difference between HHT1 and HHT2, and provide a good estimate of the frequency of PAVM.

CAVM was detected in 14.6% of patients with HHT1 and 1.3% of the HHT2 patients ($p_c = 0.007$). Although the significance was lost after correction for referral bias, caused by the smaller number of patients, the difference remains striking. The gold standard for diagnosing CAVM is carotid angiography, but this technique is too invasive for screening asymptomatic patients. Therefore, small CAVMs could have remained undetected. The prevalence of CAVM in HHT1 and HHT2 in our study is comparable with other reports.[4 8 11 12] In the literature, very few reports found significant different frequencies for HHT1 and HHT2, but owing to low numbers, the power to detect significant differences was low. Two earlier reports found CAVMs in 8.2% of cases in HHT1[4] and 2–3% in HHT2.[4 8]

In this study, the combination of PAVM and CAVM in the same patient was found only in the HHT1 cohort, in 8.7% of patients. This is very similar to the expected frequency that can be calculated by multiplying the separate frequencies from PAVM and CAVM solely (7.1%). This suggests that PAVM and CAVM occur independently of each other, are not due to a common pathogenic factor such as specific HHT1 mutations and may be due to different interacting factors that are genetic, environmental, or both.

There is a highly significant difference in the prevalence of HAVM between HHT1 (7.6%) and HHT2 (40.6%). A potential source of bias is the fact that in HHT1 relatively more asymptomatic patients have been screened because of the high number of embolisations. When all patients with a PAVM were excluded, only 2.4% (1/41) of the HHT1 patients had an HAVM compared with 40.7% (11/27) of the HHT2 patients, which is still significantly different ($p_c = 2.5\times10^{-4}$).

Telangiectases of the GI tract were found in similar proportions in HHT1 and HHT2. The high prevalence is probably the result of the fact that only patients with unexplained anaemia or overt GI bleeding were examined. Therefore, the true prevalences are hard to estimate.

The group named HHT? comprised patients from families with an unknown genotype, either because DNA was unavailable (66 patients from 37 families) or no mutation could be detected (10 patients from 7 families). In these seven families, subsequent MLPA analysis revealed no large rearrangements, making HHT1 or HHT2 in these families unlikely. In the 10 patients, 3 of 8 patients had a PAVM, 0 of 6 a CAVM, 0 of 7 an HAVM, and 3 of 3 GI manifestations. We assume that most of the remaining HHT? patients have either HHT1 or HHT2. However, we cannot exclude the possibility of one or more alternative genes for HHT with a much lower frequency in this population. The relatively high prevalence of PAVM and CAVM in HHT? suggests a larger proportion of HHT1 in the HHT? panel. On the other hand, the presence of HAVMs is higher than would be expected in HHT1, suggesting that there is indeed a mix of both HHT1 and HHT2 in HHT?

Differences between men and women

To our knowledge, systematic phenotype analysis in relation to sex has not been previously performed. There are publications suggesting that women are more prone to develop visceral manifestations, but significant differences have not been reported. We found a higher prevalence of PAVMs in women compared with men for HHT1, and more HAVMs were found in women for both HHT1 and HHT2. The

Table 5 Prevalence of visceral manifestations in men and women after exclusion of the proband of each family (first patient referred and ascertained) in order to correct for possible referral bias.

	Men	Women	p
HHT1			
PAVM	50/132 (37.9%)	83/168 (49.4%)	0.046 ($p_c=0.23$)
CAVM	13/101 (12.9%)	18/124 (14.5%)	0.72 (NS)
CAVM + PAVM	5/97 (5.2%)	11/121 (9.1%)	0.35 (NS)
HAVM	0/50 (0%)	9/69 (13.0%)	0.008 ($p_c=0.04$)
GI telangiectasia	20/29 (69%)	24/33 (72.7%)	NS (NS)
HHT2			
PAVM	1/39 (2.6%)	2/49 (4.1%)	0.70 (NS)
CAVM	0/20 (0%)	1/30 (3.3%)	0.41 (NS)
CAVM + PAVM	0/19	0/26	NS (NS)
HAVM	2/9 (22.2%)	6/12 (50%)	0.19 (NS)
GI telangiectasia	8/11 (72.7%)	4/7 (57.1%)	NS (NS)

Patients with a doubtful result were not included in the analysis. Denominators varied between categories because not all patients underwent all examinations for all visceral organs. The statistical analysis was performed comparing men and women. The p values are shown, with p values after correction for multiple testing in brackets

differences were not significant after correction for multiple testing, but the prevalence of manifestations shows obvious differences between men and women. As women more frequently have a PAVM, and consequently more women underwent embolisations in our study, more asymptomatic women will have been screened for HAVM. Correction for patients with a PAVM resulted in very small groups; for HHT1 no men and 1/22 women (4.5%) had an HAVM, while for HHT2, there were 2/10 men (20%) and 9/17 women (53%).

Explanations for these sex related differences are still diffuse, such as environmental factors, modifier genes, or hormonal differences. Additionally, within families there is a wide variety of expression of symptoms. The six HHT2 patients with a PAVM did not cluster in a single family but were from six different families. The 167 PAVMs in HHT1 occurred in 51 (of 63) families, with some degree of familial clustering. For example, in one family 7 of the 8 affected family members had a PAVM, while in another family a low prevalence was detected (9 of 28 patients). The observed sex differences and the intrafamilial variability may provide an interesting clue for the search for (sex) related genetic and/or environmental factors interacting with the major gene mutations.

In order to correct for potential referral bias associated with features of the probands' phenotypes, we performed a second analysis excluding the probands (tables 4 and 5). After this correction, the difference between HHT1 and HHT2 for PAVM and HAVM remained statistically significant. The proportion of patients with a CAVM showed a minor change, and the statistical significance was lost after correction for multiple testing. The different frequency in PAVMs, CAVMs, and HAVMs between HHT1 men and HHT1 women showed only slight changes compared with the analysis of the whole group. Despite this correction, there may still be a referral bias left, owing to the effect of the severity of the phenotype in the family of the proband. This seems to be confirmed by the proportion of unaffected family members in HHT1 (36%) and HHT2 (29%). Apparently, fewer family members of probands with the less severe phenotype had screening performed.

Genetic counselling

These data show that a significant phenotypic difference exists between HHT1 and HHT2. Genetic counselling of patients and family members can be given more accurately when the pathogenic gene mutation in the family is known. We intend to use the prevalence found before and after correction for referral bias. For HHT1, the chance of having a PAVM above the age of 16 years is 45–50%, and the risk of having a CAVM is 13–15%. For HHT2, PAVM is present in 3–5% and CAVM in 1–2%. Risk estimates for HAVM and GI involvement are difficult to give because most patients were symptomatic at the time of the screening. For HHT2, the frequency of HAVM appears to be between 38% and 41%, while for HHT1, it is between 2.5% and 8%.

It is our opinion that the differences between men and women should be confirmed by others, before adjusted percentages for sex difference can be used. For the time being, the significant difference between the sexes justifies mentioning that women with HHT1 are more likely to develop a PAVM or HAVM and women with HHT2 are more prone to develop HAVM. It should of course always be emphasised that there can be considerable intrafamilial and interfamilial variability and that the frequencies we calculated are averages and subject to potentially referral and selection bias. Family specific risk values may or may not vary but cannot be given, because the factors determining the clinical expression (genetic, environmental, or both) are still unknown.

Three out of four Curaçao criteria are required for a definite clinical diagnosis of HHT. Our data show that visceral involvement (PAVM and CAVM) is rare in HHT2 and will be of little value in the clinical diagnosis. Assuming similar degrees of clinical variability for the remaining three criteria, there may be a larger proportion of patients with HHT2 that remain undiagnosed than for HHT1. This raises the question as to how to apply the Curaçao criteria in HHT now that we are more aware of the fact that clinical expression shows consistent variability between sexes and is dependent on the type of gene involved. The prevalence of HAVM in HHT2 is high, and routine screening for HAVM with ultrasound Doppler might be indicated in members of HHT2 families as well as in new HHT patients and their relatives, for whom a molecular genetic diagnosis is not yet available, in order to arrive at the correct clinical diagnosis, despite the fact that the finding of HAVM usually has few therapeutic consequences.

Authors' affiliations

T G W Letteboer, B P C Koeleman, D Lindhout, J K Ploos van Amstel, DBG-Department of Medical Genetics, University Medical Center Utrecht, The Netherlands
J J Mager, R J Snijder, C J J Westermann, St Antonius Hospital, Nieuwegein, The Netherlands

Competing interests: there are no competing interests.

Correspondence to: Dr T G W Letteboer, DBG-Department of Medical Genetics, University Medical Center Utrecht, PO Box 85090, 3508 AB Utrecht, The Netherlands; t.g.w.letteboer@umcutrecht.nl

Received 6 June 2005
Revised version received 19 August 2005
Accepted for publication 6 September 2005
Published Online First 9 September 2005

REFERENCES

1 **Westermann CJ**, Rosina AF, De Vries V, de Coteau PA. The prevalence and manifestations of hereditary hemorrhagic telangiectasia in the Afro-Caribbean population of the Netherlands Antilles: a family screening. *Am J Med Genet A* 2003;**116**:324–8.
2 **Haitjema T**, Balder W, Disch FJ, Westermann CJ. Epistaxis in hereditary haemorrhagic telangiectasia. *Rhinology* 1996;**34**:176–8.
3 **Shovlin CL**, Letarte M. Hereditary haemorrhagic telangiectasia and pulmonary arteriovenous malformations: issues in clinical management and review of pathogenic mechanisms. *Thorax* 1999;**54**:714–29.
4 **Berg J**, Porteous M, Reinhardt D, Gallione C, Holloway S, Umasunthar T, Lux A, McKinnon W, Marchuk D, Guttmacher A. Hereditary haemorrhagic telangiectasia: a questionnaire based study to delineate the different phenotypes caused by endoglin and ALK1 mutations. *J Med Genet* 2003;**40**:585–90.
5 **Guttmacher AE**, Marchuk DA, White RI Jr. Hereditary hemorrhagic telangiectasia. *N Engl J Med* 1995;**333**:918–24.
6 **Haitjema T**, Disch F, Overtoom TT, Westermann CJ, Lammers JW. Screening family members of patients with hereditary hemorrhagic telangiectasia. *Am J Med* 1995;**99**:519–24.
7 **Berg JN**, Guttmacher AE, Marchuk DA, Porteous ME. Clinical heterogeneity in hereditary haemorrhagic telangiectasia: are pulmonary arteriovenous malformations more common in families linked to endoglin? *J Med Genet* 1996;**33**:256–7.
8 **Abdalla SA**, Geisthoff UW, Bonneau D, Plauchu H, McDonald J, Kennedy S, Faughnan ME, Letarte M. Visceral manifestations in hereditary haemorrhagic telangiectasia type 2. *J Med Genet* 2003;**40**:494–502.
9 **Khurshid I**, Downie GH. Pulmonary arteriovenous malformation. *Postgrad Med J* 2002;**78**:191–7.
10 **Willemse RB**, Mager JJ, Westermann CJ, Overtoom TT, Mauser H, Wolbers JG. Bleeding risk of cerebrovascular malformations in hereditary hemorrhagic telangiectasia. *J Neurosurg* 2000;**92**:779–84.
11 **Fulbright RK**, Chaloupka JC, Putman CM, Sze GK, Merriam MM, Lee GK, Fayad PB, Awad IA, White RI Jr. MR of hereditary hemorrhagic telangiectasia: prevalence and spectrum of cerebrovascular malformations. *AJNR Am J Neuroradiol* 1998;**19**:477–84.
12 **Moussouttas M**, Fayad P, Rosenblatt M, Hashimoto M, Pollak J, Henderson K, Ma TY, White RI. Pulmonary arteriovenous malformations: cerebral ischemia and neurologic manifestations. *Neurology* 2000;**55**:959–64.

13 **Easey AJ**, Wallace GM, Hughes JM, Jackson JE, Taylor WJ, Shovlin CL. Should asymptomatic patients with hereditary haemorrhagic telangiectasia (HHT) be screened for cerebral vascular malformations? Data from 22,061 years of HHT patient life. *J Neurol Neurosurg Psychiatry* 2003;**74**:743–8.
14 **Garcia-Tsao G**, Korzenik JR, Young L, Henderson KJ, Jain D, Byrd B, Pollak JS, White RI Jr. Liver disease in patients with hereditary hemorrhagic telangiectasia. *N Engl J Med*, 2000;28, **343**:931–6.
15 **Buscarini E**, Buscarini L, Danesino C, Piantanida M, Civardi G, Quaretti P, Rossi S, Di Stasi M, Silva M. Hepatic vascular malformations in hereditary hemorrhagic telangiectasia: Doppler sonographic screening in a large family. *J Hepatol* 1997;**26**:111–18.
16 **Sabba C**, Pasculli G, Cirulli A, Gallitelli M, Virgilio G, Guastamacchia E, Resta F, Palasciano G. Rendu-Osler-Weber disease: experience with 56 patients. *Ann Ital Med Int* 2002;**17**:173–9.
17 **Kuehl HK**, Caselitz M, Hasenkamp S, Wagner S, El-Harith el-HA, Manns MP, Stuhrmann M. Hepatic manifestation is associated with ALK1 in hereditary hemorrhagic telangiectasia: Identification of five novel ALK1 and one novel ENG mutations. *Hum Mutat* 2005;**25**:320.
18 **Kjeldsen AD**, Oxhoj H, Andersen PE, Green A, Vase P. Prevalence of pulmonary arteriovenous malformations (PAVMs) and occurrence of neurological symptoms in patients with hereditary haemorrhagic telangiectasia (HHT). *J Intern Med* 2000;**248**:255–62.
19 **Bourdeau A**, Cymerman U, Paquet ME, Meschino W, McKinnon WC, Guttmacher AE, Becker L, Letarte M. Endoglin expression is reduced in normal vessels but still detectable in arteriovenous malformations of patients with hereditary hemorrhagic telangiectasia type 1. *Am J Pathol* 2000;**156**:911–23.
20 **Van den Driesche S**, Mummery CL, Westermann CJ. Hereditary hemorrhagic telangiectasia: an update on transforming growth factor beta signaling in vasculogenesis and angiogenesis. *Cardiovasc Res* 2003;**58**:20–31.
21 **Abdalla SA**, Cymerman U, Johnson RM, Deber CM, Letarte M. Disease-associated mutations in conserved residues of ALK-1 kinase domain. *Eur J Hum Genet* 2003;**11**:279–87.
22 **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**:852–9.
23 **Cole SG**, Begbie ME, Wallace GM, Shovlin CL. A new locus for hereditary haemorrhagic telangiectasia (HHT3) maps to chromosome 5. *J Med Genet* 2005;**42**:577–82.
24 **Letteboer TG**, Zewald RA, Kamping EJ, de Haas G, Mager JJ, Snijder RJ, Lindhout D, Hennekam FA, Westermann CJ, Ploos van Amstel JK. Hereditary hemorrhagic telangiectasia: ENG and ALK-1 mutations in Dutch patients. *Hum Genet* 2005;**116**:8–16.
25 **Lesca G**, Plauchu H, Coulet F, Lefebvre S, Plessis G, Odent S, Riviere S, Leheup B, Goizet C, Carette MF, Cordier JF, Pinson S, Soubrier F, Calender A, Giraud S. Molecular screening of ALK1/ACVRL1 and ENG genes in hereditary hemorrhagic telangiectasia in France. *Hum Mutat* 2004;**23**:289–99.
26 **Shovlin CL**, Guttmacher AE, Buscarini E, Faughnan ME, Hyland RH, Westermann CJ, Kjeldsen AD, Plauchu H. Diagnostic criteria for hereditary hemorrhagic telangiectasia (Rendu-Osler-Weber syndrome). *Am J Med Genet*, 2000;6, **91**:66–7.
27 **Cotes JE**, Leathart GL. *Lung function: assessment and application in medicine*, 5th ed. Oxford: Blackwell Scientific Publications, 1993:253–5.
28 **Haitjema T**, ten Berg JM, Overtoom TT, Ernst JM, Westermann CJ. Unusual complications after embolization of a pulmonary arteriovenous malformation. *Chest* 1996;**109**:1401–4.
29 **Holloway S**, Porteous M, Cetnarskyj R, Rush R, Appleton S, Gorman D, Campbell H. Referrals of patients to colorectal cancer genetics services in south-east Scotland. *Fam Cancer* 2005;**4**:151–61.
30 **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;1, **169**:994–1000.