

Genotype – phenotype relationship in Hereditary Hemorrhagic Telangiectasia

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Abstract

Background: Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disorder characterized by vascular malformations in multiple organ systems, resulting in mucocutaneous telangiectases and arteriovenous malformations predominantly in the lungs (PAVM), brain (CAVM) and liver (HAVM). Mutations in the ENG gene and ALK-1 gene lead to HHT1 and HHT2 respectively. In this study a genotype-phenotype analysis has been performed.

Methods: A uniformly and well-classified large group of HHT patients and their family members were screened for the 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.

Results: 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, in HHT1 a PAVM has a higher prevalence in women compared to men. In HHT1 and HHT2 HAVM has a higher frequency in women.

Conclusions: 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 counseling can be given more accurate when the family mutation is known.

Introduction

Hereditary hemorrhagic telangiectasia (HHT) or Rendu-Osler-Weber disease is an autosomal dominant disorder characterized by vascular malformations in multiple organ systems.

Estimates of the frequency of the disease vary widely, the prevalence in the Netherlands is estimated 1:10,000, in the Dutch Antilles the prevalence is at least 1:1,330 [1].

The clinical symptoms of HHT are caused by direct arteriovenous connections without an intervening capillary bed. The resulting mucocutaneous telangiectases can occur anywhere, but particularly occur in the oral cavity (lips, tongue), in the nose, in the conjunctivae and on the fingertips. Telangiectases in the nasal mucosa can result in epistaxis, usually the first and most common symptom, present in more than 90% of patients with HHT [2,3,4].

Larger arteriovenous malformations are mostly located in the lung (pulmonary arteriovenous malformations, PAVM), brain (cerebral arteriovenous malformations, CAVM) and liver (hepatic arteriovenous malformations, HAVM) [3,4,5,6,7]. PAVMs are estimated to develop in 15-35% of the patients [3,8], resulting in a right to left shunt. PAVMs can cause hypoxemia, 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 the patients), but are probably under-recognized [6,10,11,12]. Although they are often silent, they can cause headache, seizures, ischemia and bleeding [6,10]. The bleeding risks ranges from less than 1% to 1.5-2% per annum per patient [10,13].

The frequency of hepatic involvement in HHT varies considerably, mainly because HAVM has not been regularly screened for in HHT patients. The frequency is estimated to be up to 32% [8,14,15,16,17]. Liver involvement predominantly concerns shunts between hepatic artery and hepatic veins. HAVMs are often asymptomatic, but may lead to a high cardiac output with heart failure and lead to portal hypertension and biliary disease.

Gastrointestinal bleeding is usually present at an older age, due to telangiectases in the gastrointestinal tract, which can cause a severe anemia. The estimated prevalence is 15-45% [4,6,8,18].

It should be emphasized that there is a considerable inter- en intra familial 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 mechanism in both forms of HHT [8,19,20].

In 2004, patients with clinical features of both HHT and juvenile polyposis were shown to carry mutations in the MADH4 gene [22]. At this point, 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 characterization 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% 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 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 reported 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, data were not cross checked with a review of the medical records. The participants were from the United Kingdom and the USA, areas with different screening protocols and population backgrounds. In 83 participants they found a PAVM significantly more often in HHT1 (35%) compared to HHT2 (0%).

Abdalla et al. [8] reported the analysis of ALK-1 patients (281) documented in the literature. They found PAVM in only 5% of the patients, CAVM in 2%, HAVM in 13% and gastrointestinal 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 mutations (HHT1) and ALK-1 mutations (HHT2). This is the first study to use clinical data obtained from one national HHT center covering a circumscribed region in North Western Europe with equal access to health care 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 in the St. Antonius hospital, specialized 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 the probands as well as their family members. At the time of this analysis, the database consisted of 1291 persons 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 gastrointestinal 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 of the proband was isolated and the exons 1-14 of the ENG gene and exons 1-10 of the ALK-1 gene and their flanking intronic sequences were amplified using PCR (polymerase chain reaction). 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 present 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 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 counseling and DNA analysis which was performed in 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? consists of probands and their relatives of 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 visceral manifestations

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]. In patients with normal results, a PAVM was considered excluded. Patients with a suspected PAVM were offered subsequently a conventional angiography or 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 detected but confirmation through subsequent angiography or CT analysis was not performed, the PAVM was classified as doubtful.

Until 2001, the 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 done with CT or MRI only when, after counseling, requested by the patient or because of symptoms. The screening for HAVM was not done routinely. Ultrasonography, CT or MRI was done in case of elevated alkaline phosphatase level or gamma glutamyl transpeptidase, the presence of a murmur over the liver, heart failure or abdominal pain. Between 1996 and 2003 screening for a HAVM was also performed before embolization of a PAVM, in order to avoid embolization complications [28].

The search for gastrointestinal involvement was only done when an unexplained iron deficiency anemia was detected or in case of overt bleeding, by means of a regular diagnostic endoscopy or videocapsule endoscopy. Only when multiple gastrointestinal telangiectases were detected, GI involvement was considered confirmed.

The proportion of subjects in each group with visceral manifestations was calculated.

The statistical analysis was performed using 2 x 2 table analysis with the chi-square test. To compensate for multiple testing, the p-value for individual tests were multiplied by the number of comparisons made (Bonferroni correction, p_c).

Results

A total of 1,291 (558 men, 733 women) persons were included in the database, containing patients and their family members screened for HHT symptoms. Of these, 1,130 persons were older than 16 years at the time of the screening. Four persons were excluded from the analysis because MADH4 mutations were detected as a cause of HHT and juvenile polyposis. The 1,126 persons 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 diagnosed clinically or genetically with HHT (table 1).

Telangiectases detected on physical examination or in the nose (rhinoscopy) were present in 98.4% of the patients, 97.2% of the 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 is a preponderance of women in the database, in affected family members as well as in the unaffected family members. There is no significant difference in gender ratio between the three groups.

Table 1

	HHT1	HHT2	HHT?
Probands and family			
No of subjects (m/f ratio)	735 (0.74)	216 (0.77)	175 (0.68)
Mean age (SD)	44.8 yr (17.2)	46.2 yr (16.6)	47.8 yr (18.1)
- HHT present (m/f ratio)	380 (0.74)	128 (0.71)	76 (0.58)
Mean age (SD)	48,4 yr (18.2)	51,2 yr (16.2)	53,7 yr (15.5)
- HHT possible (m/f ratio)	84 (0.83)	25 (1.27)	60 (0.67)
Mean age (SD)	40.0 yr (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)	41.2 yr (14.7)	39.1 yr (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
Males	161 (42,4%)	53 (41,4%)	28 (36,8%)
Females	219 (57,6%)	75 (58,6%)	48 (63,1%)
Mean age (SD)	48,4 yr (18.2)	51,2 yr (16.2)	53,7 yr (15.5)
Mean age M (SD)	47,9 (18,8)	53,5 (13,6)	51,9 (13,6)
Mean age F (SD)	48,8 (17,7)	49,7 (17,7)	54,8 (16,5)

Proportion of males (M) and females (F) for HHT1, HHT2 and HHT?, with mean ages (SD). Depicted are the family members with a clinical and or genetic certain diagnosis, ascertained from the database. The 'HHT present' group consists of patients with a clinically and or genetically confirmed diagnosis. Patients are labeled possible when two of the four criteria are present. Excluded are patients with HHT in combination with juvenile polyposis. (M/F ratio) indicates the proportion of males, (SD) gives the standard deviation

PAVM

The frequency of visceral manifestations found in HHT1, HHT2 and HHT? are depicted in table 2 and table 3.

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).

151 Males with HHT1 were screened for PAVM, of which 8 had a doubtful result. Of the remaining 143 males, 58 males had a PAVM (40.6%). Of the 208 females screened for a PAVM, 8 had an uncertain result. 109 Out of the remaining 200 females had a PAVM (54.5%). Therefore, PAVM occurred not only significantly more often in HHT1 compared to

HHT2 ($p = 6 \times 10^{-16}$), but occurred also more often in HHT1 women compared to 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, 11 had a doubtful result. A PAVM was detected in 27 out of the remaining 60 patients (45%), in 10 out of 20 males and in 17 out of 40 females.

Table 2

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

Prevalence of visceral manifestations in HHT1, HHT2 and HHT?. Patients with a doubtful result are not included in the analysis. Denominators vary between categories since not all patients underwent all examinations for all visceral organs. The statistical analysis is performed comparing HHT1 and HHT2. P values are depicted, the p values after correction for multiple testing are between brackets.

Table 3

HHT1	males	females	p value
PAVM	58/143 (40.6%)	109/200 (54.5%)	$p = 0.011$ ($p_c = 0.054$)
CAVM	14/109 (12.8%)	24/151 (15.9%)	$p = 0.49$ (ns)
PAVM + CAVM	6/105 (5.7%)	16/148 (10.8%)	$p = 0.16$ (ns)
HAVM	1/56 (1.8%)	10/88 (11.4%)	$p = 0.035$ ($p_c = 0.174$)
GI telangiectases	23/33 (69.7%)	33/45 (73.3%)	ns (ns)

HHT2	males	females	p value
PAVM	2/50 (4%)	4/64 (6.3%)	$p = 0.593$ (ns)
CAVM	0/28 (0%)	1/48 (2.1%)	$p = 0.442$ (ns)
PAVM + CAVM	0/27 (0%)	0/40 (0%)	ns (ns)
HAVM	2/12 (16.7%)	11/20 (55%)	$p = 0.033$ ($p_c = 0.162$)
GI telangiectases	11/16 (68.8%)	8/13 (61.5%)	ns (ns)

Prevalence of visceral manifestations in HHT1 and HHT2, for males and females. Patients with a doubtful result are not included in the analysis. Denominators vary since not all patients underwent examination for all visceral organs. The statistical analysis is performed comparing HHT1 and HHT2. P values are depicted, the p values after correction for multiple testing are between brackets.

CAVM

A total of 268 HHT1 patients were investigated for a CAVM, in 8 patients (4 males and 4 females) the presence of a CAVM could not be definitely determined. The frequency of

CAVM in HHT1 was 14.6% (38/260). Out of 76 HHT2 patients (28 males/48 females) screened for CAVM none had a doubtful result and only one female had a CAVM (1.3%). In HHT1 of the 109 males 14 had a CAVM (12.8%) compared to 24/151 of the females (15.9%). In the HHT? group 2 patients had a dubious result. Of the remaining 42 patients (16 males / 26 females) 4 had a CAVM (9.5%).

The combination of PAVM and CAVM in the same patient was found only in HHT1. Included were patients, who were screened for both manifestations, i.e. had screening performed for PAVM and CAVM and 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 males and 16 females) out of 253 patients (8.7%). Of the 231 patients without the combination, 120 had only PAVM, 13 had only CAVM and 98 had no PAVM or CAVM. In the HHT2 patients the combination CAVM plus PAVM was not detected in any of the 67 patients. Six of the 67 patients had a PAVM, one a CAVM and 60 no manifestation. In the HHT? group the combination was detected in one out of 38 patients (2.6%).

HAVM

In HHT1 162 patients (61 males / 101 females) were screened for HAVM, of which 18 had a doubtful result. Of the remaining 144 patients (56 males/88 females) one male and 10 females were diagnosed with a HAVM. In HHT2 the liver was examined in 38 patients of whom six had a doubtful result. A HAVM was detected in 13 patients (2 males / 11 females) out of the remaining 32 patients (12 males / 20 females). Significantly more HAVMs were detected in HHT2 (40.6% versus 7.6%, $p = 0.0004$). Furthermore, in both groups HAVMs are present more often in women than in men. However this difference was not significant after Bonferroni correction.

In the HHT? group HAVM had a frequency of 21.2% (7/33).

Gastrointestinal localization of HHT (telangiectases) was investigated in 78 HHT1 patients, in 56 (23 males/ 33 females) multiple telangiectases were detected. 29 HHT2 patients underwent screening of the GI tract and in 19 GI telangiectases were found (11 males, 8 females).

In HHT? the intestines were investigated in 16 patients, of which 11 were diagnosed with HHT of the bowels.

In an attempt to correct for possible referral bias, we performed second analysis, in which we excluded the proband of each of the families, the 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 females have a HAVM. An increased frequency of CAVM was again observed in HHT1 compared to HHT2, but this trend was not significant after Bonferroni correction, nor was the difference in PAVM in HHT1 between men and women significant.

Table 4

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

HHT1	males	females	p value
PAVM	50/132 (37.9%)	83/168 (49.4%)	p = 0.046 (p _c = 0.23)
CAVM	13/101 (12.9%)	18/124 (14.5%)	p = 0.72 (ns)
CAVM + PAVM	5/97 (5.2%)	11/121 (9.1%)	p = 0.35 (ns)
HAVM	0/50 (0%)	9/69 (13.0%)	p = 0.008 (p _c = 0.04)
GI telangiectasia	20/29 (69%)	24/33 (72.7%)	ns (ns)

HHT2	males	females	p value
PAVM	1/39 (2.6%)	2/49 (4.1%)	p = 0.70 (ns)
CAVM	0/20 (0%)	1/30 (3.3%)	p = 0.41 (ns)
CAVM + PAVM	0/19	0/26	ns (ns)
HAVM	2/9 (22.2%)	6/12 (50%)	p = 0.19 (ns)
GI telangiectasia	8/11 (72.7%)	4/7 (57.1%)	Ns (ns)

Prevalence of visceral manifestations in HHT1, HHT2 and HHT? after exclusion of the proband of each family (first patient referred and ascertained) in order correct for possible referral bias. Patients in whom the presence of the visceral manifestation was doubtful are also excluded. Denominators vary between groups since not all patients underwent examinations of all visceral organs. The statistical analysis is performed comparing HHT1 and HHT2 in the upper table and comparing male and female in the lower two tables. P values are given, the p values after correction for multiple testing are given between brackets.

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 center. In this study we compare 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. In HHT1 51.7% of the family members were diagnosed with HHT, in 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 the 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 to HHT2.

In the HHT1 and HHT2 group the family members with possible diagnosis are 11.4% and 11.5% respectively. The mean ages of these groups are 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 is a significant female preponderance. The female preponderance is uniformly present in the database of 1291 persons (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 is also found among unaffected family members and among the groups that were screened but with an uncertain diagnostic result. Only in the HHT2 “possible affected” group there is a male preponderance, but this is a small group. This finding may reflect the notion of families as well as physicians that women have more often an AVM than men. Therefore women in a family are more aware of HHT or stimulated to have screening performed. Another explanation might be that a different attitude towards health care exists between men and women. This was suggested to be the cause for female preponderance in for example colon cancer families [29]. The fact that there is a female preponderance in the affected as well as in the unaffected cohort can also raise the question whether there is a difference in genetic fitness between males and females. When there is a disadvantage for male fetuses in the early embryonic period, more females 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. (2003) reported PAVM in 34.7% of HHT1 patients and no PAVM in HHT2 patients. The combined data published by Abdalla et al. 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. Since 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 the 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 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 find significant different frequencies for HHT1 and HHT2; due 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].

The combination PAVM and CAVM in the same patient is found only in the HHT1 cohort, in 8.7% of the 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 like 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 a bias is the fact that in HHT1 relatively more asymptomatic patients have been screened because of the high number of embolizations. When all patients with a PAVM were excluded, only 2.4% (1/41) of the HHT1 patients had a HAVM compared to 40.7% (11/27) of the HHT2 patients, which is still significantly different ($p_c = 2.5 \times 10^{-4}$).

Telangiectases of the gastrointestinal 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 anemia or overt gastrointestinal bleeding were examined. Therefore, true prevalences are hard to estimate.

The group named HHT? exists of patients from families with an unknown genotype, because either DNA was unavailable (66 patients from 37 families) or no mutation could be detected (10 patients from 7 families). In these 7 families subsequent MLPA analysis revealed no large rearrangements, making HHT1 or HHT2 in these families unlikely. In the 10 patients 3 out of 8 patients had a PAVM, 0 out of 6 a CAVM, 0 out of 7 a HAVM and 3 out of 3 gastrointestinal manifestations. We assume that most of the remaining HHT? patients have either HHT1 or HHT2. However, we can not exclude the possibility of one or more other 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 indeed a mix of both HHT1 and HHT2 in HHT.

Differences between males and females

To our knowledge systematic phenotype analysis in relation to gender has not been performed before. 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 females compared to men in HHT1 and more HAVMs were found in females in both HHT1 and HHT2. The differences were not significant after correction for multiple testing, but the prevalence of manifestations shows obvious differences between men and women. Since women have more frequently a PAVM and consequently more women underwent embolizations, more asymptomatic women will have been screened for HAVM. Correction for patients with a PAVM resulted in very small groups; in HHT1 no males and 1/22 females (4.5%) had a HAVM, in HHT2 2/10 males (20%) and 9/17 females (53%).

Explanations for these gender related differences are still diffuse, like environmental factors, modifier genes or hormonal differences. Also 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. In for example 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 gender differences and the intrafamilial variability may provide an interesting clue for the search for (gender) 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' phenotype, we performed a second analysis excluding the probands (Table 4). 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, the statistical significance was lost after correction for multiple testing. The different frequency in PAVMs, CAVMs and HAVMs between HHT1 males and HHT1 females showed only slight changes compared with the analysis of the whole group. Despite this correction, there may still be a referral bias left, due 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, less family members of probands with the less severe phenotype had screening performed.

Genetic Counseling

These data show that a significant phenotypic difference exists between HHT1 and HHT2. Genetic counseling 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. In HHT1 the chance of having a PAVM above the age of 16 years is 45-50%. The risk of having a CAVM is 13-15%. In HHT2 PAVM is present in 3-5% and CAVM in 1-2%.

Risk estimates for HAVM and gastrointestinal involvement are hard 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%, in HHT1 between 2.5% and 8%.

It is our opinion that the differences between males and females 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 emphasized that there can be considerable intra- 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 can not be given as the factors determining the clinical expressions (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 expressions show 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 a proper clinical diagnosis. This, despite the fact that the finding of HAVM usually has little therapeutic consequences.

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