Pulmonary arteriovenous malformations may be the only clinical criterion present in genetically confirmed hereditary haemorrhagic telangiectasia

Emily Anderson 1,2, Lakshya Sharma 3, Ali Alsafi 4, Claire L Shovlin 1,3

ABSTRACT

Pulmonary arteriovenous malformations (PAVMs) result in preventable complications demanding specialty care. Underlying hereditary haemorrhagic telangiectasia (HHT) can be identified by genetic testing, if the diagnosis is considered. Retrospectively reviewing 152 unrelated adults with genetically confirmed HHT due to ACVRL1, ENG or SMAD4, we found that only 104/152 (68%) met a clinical diagnosis of HHT with three Curaçao criteria. The genetic diagnostic rate was similar for patients with three (104/137, 76%) or one to two (48/71, 68%; p=0.25) criteria. Of 83 unrelated probands with PAVM(s) and genetically-confirmed HHT, 20/83 (24%) had few, if any, features of HHT. Enhanced clinical suspicion, as well as HHT genetic testing, is recommended if one or more PAVMs are present.

INTRODUCTION

Pulmonary arteriovenous malformations (PAVMs) are capillary-free vascular communications between pulmonary arteries and pulmonary veins, resulting in an anatomical intrapulmonary right-to-left shunt.1 PAVM prevalence was estimated by general population screening thoracic CT imaging at 38 per 100 000 individuals in Japan (95% CI 18 to 76), corresponding to 1/2631 (95% CI 1/1315 to 1/5555).2 Although this seems high for respiratory clinical practice, the majority of PAVMs are asymptomatic and undiagnosed,1 despite continuous right-to-left shunting, which impairs gas exchange and places individuals at high risk of neurological complications from paradoxical emboli.1,3 Neurological risks are present whether or not the individual has symptoms from the PAVM(s).1 Details of appropriate management of patients with PAVMs are outlined in recent British Thoracic Society1 and Cardiovascular and Interventional Radiological Society of Europe4 statements.

Clinicians and patients might be aware that PAVMs can be a familial condition, most commonly due to hereditary haemorrhagic telangiectasia (HHT). HHT is a multisystemic vascular dysplasia, inherited as an autosomal dominant trait, and important to diagnose early because screening and interventions have led to reduced morbidity and mortality.4–6 If HHT is diagnosed in an individual with PAVMs, broadly speaking, each of their first-degree relatives (child, sibling and parent) has a 50% chance of having HHT, and at least 50% of HHT-affected relatives are expected to have PAVMs.1,3,5–7 Establishing an HHT diagnosis in the family is therefore critical for optimal management of the greatest number of individuals.

A definite clinical diagnosis of HHT requires three of four Curaçao criteria, namely, recurrent spontaneous nosebleeds, mucocutaneous telangiectasia at characteristic sites, visceral involvement (such as gastrointestinal telangiectasia or pulmonary, hepatic, cerebral or spinal arteriovenous malformations) and a positive family history.6 8 Many consider that if HHT is present, these clinical features will be clearly evident and do not appreciate the importance of suspecting the diagnosis and offering HHT management to patients with one or two criteria as recommended6 8 (online supplemental data 1). This issue was exemplified by the April 2020 release of the National Health Service (NHS) National Genomic Test Directory (online supplemental data 1). For a patient who is the first of their family considered for HHT (the proband), HHT gene testing eligibility was contingent on them already having three Curaçao criteria, that is, a definite clinical diagnosis of HHT. Thus, patients with PAVMs were only eligible if they had two further Curaçao criteria from nosebleeds, telangiectasia and a family history of HHT.

RETROSPETIVE REVIEW

To address if under-recognition of HHT could compromise personal management or predictive testing for relatives, we retrospectively reviewed data on the 208 unrelated adults genetically tested for HHT through NHS clinical diagnostic testing via gene panels or 100,000 Genomes Project whole-genome sequencing in two UK centres (Liverpool, 2007–2019, and Imperial, 2015–2019). Many had clear-cut HHT, for instance, 14 of 27 (52%) Liverpool patients and 123 of 181 (68%) Imperial patients had a definite diagnosis of HHT, meeting three Curaçao Criteria.3 Of the 208 patients, 124 were unrelated adults with PAVMs managed at the PAVM reference centre at Hammersmith Hospital.

The genes most frequently harbouring HHT-causal variants are ENG, ACVRL1 and SMAD4, when patients are heterozygotes with one wild type (normal) allele and one loss-of-function variant allele.7 Of the 208 unrelated adult probands undergoing genetic testing for suspected HHT across the two centres, at the time of analysis, 152 (73%) had a causative variant identified through NHS clinical diagnostic testing. Only 104 (68%) of 152 patients with genetically confirmed HHT due to ENG, ACVRL1 or SMAD4 met three criteria for a definite clinical diagnosis of HHT. As detailed in figure 1, 48/152 (32%) genetically diagnosed patients with
HHT met only one or two criteria, most commonly a PAVM, though many had a family history of nosebleeds over several generations without a diagnosis of a relative with HHT (figure 2). Furthermore, the rate of a positive HHT genetic test in the 137 patients already meeting a clinical diagnosis of HHT with three Curaçao criteria (104/137, 76.0%) was no higher than that in the 71 patients with fewer than three Curaçao Criteria (48/71, 68%, \( p = 0.25 \); figure 1).

Focussing on the cohort of 124 unrelated adults with PAVMs who were the first in their family to have a gene test, 83 (67%) were found to be heterozygous for a likely pathogenic or pathogenic variant in ENG, ACVRL1 or SMAD4, that is, confirmed to have HHT. However, HHT was not always clinically apparent: while 63/83 (76%) of patients with PAVMs and genetically confirmed HHT met three or more Curaçao criteria, 20/83 (24%) displayed little evidence of HHT, including patients with single PAVMs. None of these 20 met the family history HHT criterion; only 3/20 (15%) had classical HHT telangiectasia, and although some reported occasional or regular nosebleeds, PAVMs could be the only HHT feature across two genetically confirmed generations.

**CONCLUSION**

One-third of adult patients with genetically confirmed HHT and one-quarter of adult patients with PAVMs and genetically confirmed HHT did not meet clinical diagnostic criteria for HHT. Gene testing is an efficient route to establish the diagnosis of HHT, whether or not HHT is clinically apparent, and is now an important component of care for patients with one or more PAVMs.

**IMPLICATIONS**

Based on these data, and supported by the British Thoracic Society, other specialist societies, patient groups and expert clinicians, the NHS Genomic Test Directory was amended to allow HHT gene testing for patients with one or more PAVMs (online supplemental data 1). Proposing an HHT genetic test is important for respiratory and radiological clinicians treating a patient with PAVMs. Additionally, up to 20% of PAVMs referred for embolisation are incidental findings on imaging (Alsafi A and Shovlin CL, unpublished data 2022), and it is important that the reporting radiologist raises the high likelihood of its association with HHT, which may otherwise go unrecognised. Referring clinicians will receive gene test reports that may identify one of multiple HHT causal DNA variants. Currently, ClinVar lists 2804 different variants across the three major HHT genes, with 814 different variants classified as pathogenic or likely pathogenic (figure 3). The presence of one such variant defines the individual as having HHT, whether or not they or their family have clinical features. Importantly, however, unless the genetic variant has already been identified for the family, the converse is not true, and a negative gene test does not exclude HHT. These false-negative tests may reflect the presence of an unreported DNA variant that did not meet sufficient molecular criteria to be reported as likely pathogenic or an undetected DNA variant in an uncaptured region of the genome. Thus, for a patient with one or more PAVMs without a positive genetic test for HHT, screening first-degree relatives once in adult life for PAVMs remains good practice, as in the British Thoracic Society Clinical statement.

Anderson E, et al. Thorax 2022;0:1–3. doi:10.1136/thoraxjnl-2021-218332

---

**Figure 1** Genotype–phenotype analyses in 208 genotyped patients with HHT. Proportion of genotyped patients with (black) and without (pale yellow) causal HHT variant identification, if (A) meeting three Curaçao criteria for ‘definite HHT’; (B) meeting one to two Curaçao criteria. The Curaçao criteria are spontaneous, recurrent nosebleeds; multiple telangiectasia at characteristic sites (lips, oral cavity, fingers and nose); visceral lesions such as gastrointestinal telangiectasia (with or without bleeding); pulmonary AVM, hepatic AVM, cerebral AVM, spinal AVM and a first-degree relative with HHT according to these criteria. AVM, arteriovenous malformation; HHT, hereditary haemorrhagic telangiectasia.

**Figure 2** Genotype–phenotype analyses in subgroups of genotyped patients with HHT who did not meet the threshold for a clinical diagnosis of HHT. Proportion of genotyped patients with (black) and without (pale yellow) causal HHT variant identification, if meeting the stated patterns. 1CC from spontaneous, recurrent nosebleeds; multiple telangiectasia at characteristic sites (lips, oral cavity, fingers and nose); visceral lesions such as gastrointestinal telangiectasia (with or without bleeding) or HHT-site AVMs (pulmonary AVM, hepatic AVM, cerebral AVM and spinal AVM); and a first-degree relative with HHT according to these criteria. 1CC: severe nosebleeds requiring blood transfusions. 1CC: 1 Curaçao criteria; AVM, arteriovenous malformation; HHT, hereditary haemorrhagic telangiectasia.

**Figure 3** Causal HHT variants on ClinVar. The distribution of 814 unique likely pathogenic and pathogenic variants in ENG, ACVRL1 and SMAD4 listed in ClinVar. Loss-of-function variants meeting very strong evidence of pathogenicity include frameshifts (green) due to an out-of-frame insertion or deletion (indels); nonsense (stop gain) substitutions generating a premature stop codon and splice site variants affecting the exon–flanking consensus (+1 and +2) splice sites. Missense variants (purple) where a single amino acid is substituted represent a higher proportion of pathogenic and likely pathogenic variants in ACVRL1 than ENG or SMAD4 (109/264 (41%) compared with 85/550 (15.5%) for ENG/SMAD4 (p<0.0001, Fisher’s exact test). HHT, hereditary haemorrhagic telangiectasia.
Acknowledgements We thank our colleagues in the Genomic Medicine, HHT and PAVM services at Imperial College Healthcare National Health Service (NHS) Trust and the Liverpool Centre for Genomic Medicine. We also thank the supporters of our January 2021 National Genomic Test Directory Amendment proposal: the British Thoracic Society, the British Rhinological Society, ENT-UK, the British Society for Gastroenterology Liver Section, the British Association for the Study of the Liver, the NHS Hereditary Haemorrhagic Telangiectasia Rare Disease Collaborative Network, the Telangiectasia Self Help Group, HHT UK and the 40 consultants with HHT expertise who supported the amendment. This research was made possible through access to the data and findings generated by the 100,000 Genomes Project. The 100,000 Genomes Project is managed by Genomics England Limited (a wholly owned company of the Department of Health and Social Care). The 100,000 Genomes Project is funded by the National Institute for Health Research and NHS England. The Wellcome Trust, Cancer Research UK and the Medical Research Council have also funded research infrastructure. The 100,000 Genomes Project uses data provided by patients and collected by the National Health Service as part of their care and support. The views expressed are those of the authors and not necessarily those of funders, the NHS, the NIHR, or the Department of Health and Social Care.

Contributors EA was responsible for conception and design, acquisition analysis and interpretation of Liverpool data; performed data analysis; and contributed to manuscript writing and revision. LS examined ClinVar data, generated figure 3 and contributed to manuscript revision. AA was responsible for PAVM patient review, treatment and radiological management insights. CLS was responsible for planning, conception and design, acquisition analysis, and interpretation of Imperial data; supervised LS; performed data analysis; wrote the manuscript; generated figures 1 and 2; and contributed to manuscript revision. All authors approved the final manuscript.

Funding Funding was received from the National Institute of Health Research Biomedical Research Centre Scheme (Imperial BRC) and Imperial College Faculty of Medicine BSc project funds (to CLS for LS). The views expressed are those of the authors and not necessarily those of funders, the NHS, the National Institute for Health Research or the Department of Health and Social Care.

Competing interests The authors have no financial competing interests to declare. CLS chairs the Genomics England Respiratory GeCIP; the North Thames Genomic Medicine Service Alliance R&D Committee, the NHS Hereditary Haemorrhagic Telangiectasia Rare Disease Collaborative Network and the British Thoracic Society Pulmonary AVM Clinical Statement Group; sits on the Cure HHT Global Research and Medical Advisory Board, the ClinGen Hereditary Hemorrhagic Telangiectasia Variant Curation Expert Panel; and chaired the European Reference Network for Rare Multisystemic Vascular Diseases HHT Working Group 2016–2020.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/.

ORCID iDs
Emily Anderson http://orcid.org/0000-0002-0202-4028
Lakshya Sharma http://orcid.org/0000-0002-3509-6140
Ali Alsafi http://orcid.org/0000-0001-6688-8637
Claire L Shovlin http://orcid.org/0000-0001-9007-7775

REFERENCES
Genetically confirmed hereditary haemorrhagic telangiectasia commonly displays few manifestations - pulmonary arteriovenous malformations may be the sole clinical diagnostic criterion

Emily Anderson, Lakshya Sharma, Ali Alsafi and Claire L Shovlin

DATA SUPPLEMENT

1. Executive Summary of Amendment Proposal to National Genomic Test Directory

2. Current NHS National Genomic Test Directory Entry for Hereditary Haemorrhagic Telangiectasia

3. Notes on the Curaçao Criteria and HHT “Gene Negative” Cases
   A) 2000: Original Manuscript Extracts
   B) 2021: European Reference Network (VASCERN) 2021 Frameworks Manuscript Extracts
   C) Notes on the Curaçao Criteria and HHT “Gene Negative” Cases
   D) References
Proposed Changes to the National Genomic Test Directory
R186 - Hereditary Haemorrhagic Telangiectasia

Claire L. Shovlin, Imperial College and Imperial Healthcare NHS Trust:
Professor of Practice (Clinical and Molecular Medicine), and Honorary Consultant Physician (Adult)
Lead for Imperial HHT Services 1999; Chair of VASCERN HHT 2016-2020, and Chair of Respiratory GeCIP

Dr Emily Anderson, Liverpool Centre for Genomic Medicine
ST6 Registrar in Clinical Genetics, previous Specialty Trainee in Paediatrics (MRCPCH)
Coordinator of Liverpool HHT Network

EXECUTIVE SUMMARY

KEY MESSAGES:

- To enable similar improvements in outcomes and survival to continental Europe, genomic testing should be offered to all patients with a strong clinical suspicion of HHT. This is particularly relevant for probands in mainstream medicine with pulmonary and hepatic AVMs.

- Confirming an HHT genomic diagnosis helps resolve diagnostic uncertainty, provides prognostic information for the patient, and enables patients to access appropriate screening and therapies.

- Having a confirmed genomic diagnosis allows cascade testing for the family: Predictive testing for family members identifies those at risk of HHT-related complications, providing a window of opportunity to diagnose and treat asymptomatic AVMs before the patient develops significant morbidity e.g. brain abscess, stroke, haemorrhage or maternal death in pregnancy.

- Predictive testing can also identify those who can be reassured and discharged, without undergoing unnecessary investigation for AVMs with cost, radiation and other burdens.

Current inclusion criteria are overly restrictive, preventing many families with HHT from being able to access genomic testing. This is evidenced by data from both Liverpool and London.

There is additional new data on relevant causal genes.

We propose 4 evidence-based amendments:
1. Adjustments to the existing family history criterion
2. Modifying eligibility criteria regarding AVMs, particularly pulmonary and cerebral AVMs
3. Adding a criterion for very severe, life-threatening disease in the proband
4. The addition of two further genes (GDF2 and RASA1) to the HHT panel, as phenotypes associated with variants in these genes have significant overlap with HHT.

If these proposed amendments are accepted:

- Case detection would increase from 68.4% to 97.3%
- 75.9% of tests would be predicted to identify a clinically relevant variant

Overall, the proposed changes will:
- Increase suitability for use in both Clinical Genetics and Mainstream Specialist Services;
- Modestly increase the number of patients eligible for testing, but
- Retain a high diagnostic yield.

The proposal is supported by the Telangiectasia Self Help Group (TSHG); NHS Hereditary Haemorrhagic Telangiectasia RDCN; the British Association for the Study of the Liver (BASL); the British Society for Gastroenterology; the British Thoracic Society; ENT-UK; the British Rhinological Society, and 40 named consultants with HHT expertise from 8 specialities and 23 NHS institutions spanning all seven NHS GLHs.
SUPPORTING DETAILS

The autosomal dominant disorder hereditary haemorrhagic telangiectasia (HHT) commonly causes abnormal vessels in the lung, liver, brain and spine, as well as evident epistaxis and telangiectasia. AVMs are usually silent but can cause catastrophic complications, such as brain abscess, stroke or maternal death in pregnancy, yet many of these complications can be prevented.[1] Life expectancy is improved by appropriate screening and management [2,3], but is lower in the UK than Europe [2-4].

The validated Curaçao Criteria (CC) provide a definite clinical HHT diagnosis if 3 of the 4 are present: nosebleeds, characteristic telangiectasia, visceral AVMs, and first degree relative meeting 3CC [5].

The most common genes harbouring HHT-causative variants are ENG, ACVR1 and SMAD4 (with SMAD4 also necessitating bowel surveillance from childhood due to gastrointestinal polyposis). Overlapping phenotypes have recently been described caused by pathogenic variants in further genes including GDF2, RASA1, and EPHB4 [6,7]. Furthermore, low-level mosaicism has recently been described as a mechanism of disease, and shown to be detectable by 100,000 Genomes WGS pipelines [8].

THE ROLE OF GENOMIC TESTING IN HHT

Cascade testing in a family – once the genomic variant is known – allows for screening and pre-clinical intervention [9], instead of waiting for presentation with a preventable complication. Genomic testing may also modify management due to emerging genotype-specific data [10]. However, cascade testing is only possible if genomic testing has already been offered within the family.

- When the genomic variant in the family is known, screening investigations and management are targeted to individuals confirmed to have the familial variant.
- In the absence of a confirmed variant, relatives at 50% risk may have screening / management entailing radiation exposure, patient inconvenience, use of resources and NHS costs.

CURRENT TEST DIRECTORY CRITERIA EVALUATIONS:

In order to be eligible for molecular testing, at least 3 CC need to be met, i.e. to have a definite clinical diagnosis of HHT. This sets an inappropriately high threshold, as evidenced by a retrospective review of more than 200 adult patients undergoing diagnostic testing across two centres (Liverpool and London), demonstrating that around one third of patients with HHT where a causative variant was identified would not have been eligible for testing (Table 1).

<table>
<thead>
<tr>
<th>208 adult probands gene tested for suspected HHT</th>
<th>Meets current criteria</th>
<th>Does not meet current criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Causative HHT variant identified, N=152 (73%)</td>
<td>104/152 (68%)</td>
<td>48/152 (32%)</td>
</tr>
<tr>
<td>Causative variant identified, N=56 (27%)</td>
<td>33/56 (59%)</td>
<td>23/56 (41%)</td>
</tr>
</tbody>
</table>

**Table 1:** Combined unpublished data from two centres (Liverpool Centre for Genomic Medicine: 27 patients; 2007-2019; Imperial VASCERN HHT London centre: 181 patients; 2015-2018). Of these 208 adult patients with suspected HHT who underwent diagnostic testing, one* presented with polyposis and would have been eligible for the inherited cancer panel which includes SMAD4.

Reasons why the audited probands with causative variants did not meet the current criteria included:

- Patients presenting with pulmonary AVMs (more than one almost always due to HHT), even if AVMs were also at other sites, will not satisfy the current criteria unless reporting further features of HHT.
  - Illustrative example from London: grandmother with multiple pulmonary AVMs and her daughter each have pathogenic ENG variant but neither has nosebleeds or telangiectasia.
- The phenotype of HHT shows age-related penetrance. This provides particular diagnostic difficulty in children and young people, who are much less likely to fulfill the current testing criteria than adults.
  - Illustrative example from Liverpool: child with nosebleeds and AVMs (cerebral and pulmonary) has pathogenic ENG variant but no telangiectasia or family history.
- The family history criterion in the current iteration of the criteria is very restrictive. Many individuals who did not meet the criteria reported a family history of severe epistaxis, AVMs, cerebral haemorrhage, cerebral abscess etc, but no one in the family had a confirmed diagnosis of HHT.
  - Illustrative FH examples- 7 with nosebleeds over 3 generations; 5 over 4 generations.
PROPOSED AMENDMENTS

(1) The proposed amendments to expand the eligibility criteria, would

(A) Ensure test access to the vast majority of patients with suspected HHT (Table 2):

| Causative HHT variant identified | N=152 (73%) | 148/152 (97%) | 4*/152 (3%) |
| No causative variant identified, | N=56 (27%)  | 47/56 (84%)   | 9/56 (16%) |

Table 2: Combined unpublished data from the two centres- see Table 1 for further details

(B) Only modestly increase the total number of tests, effectively counteracting the decrease in number of tests resulting from implementation of the current overly-stringent criteria:

- In the Liverpool/London audit in Tables 1 and 2, numbers would increase by 43% (137 to 195).
- In a separate audit of all 241 unrelated probands reviewed for known or suspected HHT in London in 2019, test numbers would increase by only 25% (84 to 105), since 127 (53%) had already undergone gene testing personally or via an affected relative, and 84 (35%) met the current inclusion criteria.

(C) Leave the pick-up rate of a pathogenic or likely pathogenic variant constant: 104/137 (75.9%) vs 148/195 (75.9%).

(D) Crucially, improve the diagnostic rate from 68.4% (104/152) to 97.3% (148/152, Table 2).

(2) The proposed amendments to expand the current panel for HHT:

Currently ENG, ACVR1L1, SMAD4 and EPHB4 are included on the panel. Variants in GDF2 and RASA1 have been identified in families with an initial clinical diagnosis of HHT based on the phenotype, where molecular testing had previously been uninformative [5-7]. We also suggest second line WGS for selected “gene-negative” cases following phenotypic review, in order to capture alternate molecular diagnoses.

REFERENCES

1) Orphanet Definition of HHT 2019, www.orpha.net/consor/www/cgi-bin/OC_Exp.php?lng=EN&Expert=774

We thank our colleagues for their support, and thank Connor Davieson and Lakshya Sharma for reviewing grammar and syntax.
Supplemental Item 2
National Genomic Test Directory for HHT, published 4th October 2021

**R186 Hereditary haemorrhagic telangiectasia**

**Testing Criteria**
Test where any THREE of the following criteria are met:

1. Epistaxis: spontaneous, recurrent nose bleeds
2. Telangiectases: multiple, at characteristic sites (lips, oral cavity, fingers, nose)
3. Visceral lesions such as gastrointestinal telangiectasia (with or without bleeding), pulmonary arteriovenous malformation (AVM), hepatic AVM, cerebral AVMs, spinal AVM
4. Family history: a first degree relative with HHT according to these criteria (as above) or an autosomal dominant family history of nosebleeds or first degree relative with cerebral AVM / cerebral haemorrhage / pulmonary or hepatic AVM.

Alternatively, test where any ONE of the following criteria are met:

A) Personal history of at least one pulmonary AVM*
B) Personal history of two or more AVMs at one or more characteristic sites (pulmonary*, cerebral, hepatic or spinal)
C) Personal history of at least one AVM and severe epistaxis or characteristic telangiectasia or family history
D) Personal history of telangiectasia, and refractory or severe epistaxis (e.g. requiring recurrent transfusions) *

*Pulmonary AVM only if confirmed by cross sectional imaging (usually thoracic CT scan), and/or later therapeutic angiography/surgery. Do not diagnose if only supported by a positive right-to-left shunt study ("bubble echo") or chest x-ray.

**To Note: if there is no antecedent family history implying a "first in family" case more likely to be mosaic.**

Referrals for testing will be triaged by the Genomic Laboratory; testing should be targeted at those where a genetic or genomic diagnosis will guide management for the proband or family.

**Where in Pathway**
At presentation

**Requesting Specialties**
- Clinical Genetics
- Dermatology
- Gastroenterology
- Neurology
- Respiratory Medicine

**Specialist Service Group**
- Respiratory

**Associated Tests**
Please note all the tests below will be undertaken for R186 Clinical Indication requests

<table>
<thead>
<tr>
<th>Code</th>
<th>Name</th>
<th>Optional Family Structure</th>
<th>Scope(s)</th>
<th>Target Type</th>
<th>Target Name</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>R186.1</td>
<td>Hereditary haemorrhagic telangiectasia Small panel</td>
<td>Singleton</td>
<td>Small variants</td>
<td>Panel of genes or loc</td>
<td>Hereditary haemorrhagic telangiectasia (123)</td>
<td>Small panel</td>
</tr>
<tr>
<td>R186.2</td>
<td>Hereditary haemorrhagic telangiectasia</td>
<td>Singleton</td>
<td>Exon level CNVs</td>
<td>Panel of genes or loc</td>
<td>Hereditary haemorrhagic telangiectasia (123)</td>
<td>Exon level CNV detection by MLPA or equivalent</td>
</tr>
</tbody>
</table>

For more information see https://www.england.nhs.uk/publication/national-genomic-test-directories/
SUPPLEMENTAL ITEM 3: NOTES ON THE CURAÇÃO CRITERIA AND HHT “GONE NEGATIVE” CASES

A) 2000 Original Manuscript Extracts:
DIAGNOSTIC CRITERIA FOR HEREDITARY HEMORRHAGIC TELANGIECTASIA (RENDU-OsLER-WEBER SYNDROME).

ABSTRACT:
(HHT) is easily recognized in individuals displaying the classical triad of epistaxis, telangiectasia, and a suitable family history, but the disease is more difficult to diagnosis in many patients. Serious consequences may result if visceral arteriovenous malformations, particularly in the pulmonary circulation, are unrecognized and left untreated. In spite of the identification of two of the disease-causing genes (endoglin and ALK-1), only a clinical diagnosis of HHT can be provided for the majority of individuals. On behalf of the Scientific Advisory Board of the HHT Foundation International, Inc., we present consensus clinical diagnostic criteria. The four criteria (epistaxis, telangiectasia, visceral lesions and an appropriate family history) are carefully delineated. The HHT diagnosis is definite if three criteria are present. A diagnosis of HHT cannot be established in patients with only two criteria, but should be recorded as possible or suspected to maintain a high index of clinical suspicion. If fewer than two criteria are present, HHT is unlikely, although children of affected individuals should be considered at risk in view of age-related penetration in this disorder. These criteria may be refined as molecular diagnostic tests become available in the next few years.

KEY TEXT EXTRACTS:
- “...the significance of subtle disease manifestations is often overlooked. Conversely, within HHT families, the HHT medical community has been concerned about overdiagnosis, given that in this situation an individual may be diagnosed as affected on the basis of epistaxis alone (when epistaxis is common in the general population), or an incorrect interpretation of cutaneous vascular lesions, leading to problems in clinical management and hampering research efforts.”
- “...Overall, these diagnostic criteria are more stringent than those employed previously [Plauhu et al., 1989]. However, use of the labels “possible” or “suspected” HHT if only two criteria are satisfied maintains a high clinical profile for HHT.”
- Table: See opposite

TABLE I. The Curação Criteria*

<table>
<thead>
<tr>
<th>The HHT diagnosis</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definite</td>
<td>1. Epistaxis: spontaneous, recurrent nose bleeds</td>
</tr>
<tr>
<td></td>
<td>2. Telangiectases: multiple, at characteristic sites:</td>
</tr>
<tr>
<td></td>
<td>- lips</td>
</tr>
<tr>
<td></td>
<td>- oral cavity</td>
</tr>
<tr>
<td></td>
<td>- fingers</td>
</tr>
<tr>
<td></td>
<td>- nose</td>
</tr>
<tr>
<td>Possible or suspected</td>
<td>3. Visceral lesions: such as</td>
</tr>
<tr>
<td></td>
<td>- Gastrointestinal telangiectasia (with or without bleeding)</td>
</tr>
<tr>
<td></td>
<td>- Pulmonary AVM</td>
</tr>
<tr>
<td></td>
<td>- Hepatic AVM</td>
</tr>
<tr>
<td></td>
<td>- Cerebral AVMs</td>
</tr>
<tr>
<td></td>
<td>- Spinal AVM</td>
</tr>
<tr>
<td>Unlikely</td>
<td>4. Family history: a first degree relative with HHT according to these criteria</td>
</tr>
</tbody>
</table>

*All offspring of an individual with HHT are at risk of having the disease since HHT may not manifest until late in life. If there is any concern regarding the presence of physical signs, an experienced physician should be consulted. Coagulation disorders should be excluded. The presence of visceral abnormalities in children should prompt a particularly careful check of other family members. These criteria are likely to be further refined as molecular diagnostic tests become available in the next few years.
B) European Reference Network (VASCERN) 2021 Frameworks Manuscript Extracts:

Table 1
The Curacao Criteria. The HHT diagnosis is definite if 3 of the 4 criteria are present, possible or suspected if 2 criteria are present, and unlikely if fewer than 2 criteria are present. A pathogenic (null) sequence variant in ENG, ACVR1L or SMAD4 also defines definite clinical HHT according to current understanding. A negative HHT gene test does not exclude HHT unless the gene variant causing HHT has been identified in another affected family member.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Curacao Criteria Definition</th>
<th>Distinguishable from non HHT?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Epistaxis</td>
<td>Spontaneous, recurrent nose bleeds</td>
<td>Not possible by history: range from nil/minor to extreme. For patients with recurrent epistaxis, nasal examination by an otorhinolaryngologist can identify other causes for epistaxis and exclude HHT.</td>
</tr>
<tr>
<td>2. Telangiectases</td>
<td>Multiple, at characteristic sites • Lips • Oral cavity • Fingers • Nose</td>
<td>Yes - only some telangiectasia at characteristic sites are diagnostic, e.g. Finger pads not nailfold or dorsum of hand. Examples are shown below. Note that arms, chest and legs are not characteristic sites: telangiectasia at these sites are not a diagnostic criterion.</td>
</tr>
<tr>
<td>3. Visceral lesions</td>
<td>Such as gastrointestinal telangiectasia (with or without bleeding); pulmonary AVM; hepatic AVM; cerebral AVM; spinal AVM</td>
<td>Multiple AVMs at a particular site are more likely to be due to HHT than a single AVM</td>
</tr>
<tr>
<td>4. Family history</td>
<td>A first degree relative with HHT according to these criteria</td>
<td>Yes, by an ENG, ACVR1L or SMAD4 pathogenic or likely pathogenic DNA variant</td>
</tr>
</tbody>
</table>

Comment:

D) References:
2. Shovlin et al. The European Rare Disease Network for HHT Frameworks for management of hereditary haemorrhagic telangiectasia in general and speciality care. Eur J Med Genet. 2021 Nov 1;65(1):104370. Full authors listed in (B)
3. Anderson et al 2022 (current manuscript)

C) Comment on current “Gene-Negative” Cases [3]
The gene negative cases reported in the current manuscript (the majority of whom had 3 or more Curacao criteria) fall into 3 groups:

- Patients with a variant of uncertain significance (VUS) in the ACVR1L, ENG or SMAD4 genes known to the team where functional studies and re-evaluation of pathogenicity status is ongoing [4], for example [5] which was of the first GDF2 family assignment in the cohort.
- Patients with a VUS in the ACVR1L, ENG or SMAD4 genes where the VUS has not been reported, as per current NHS practice.[6,7]
- Patients with no known variant in the ACVR1L, ENG or SMAD4 genes where classical HHT is suspected. Ongoing evaluating is in progress.

The gene negative cases reported in the current manuscript do not include any patients with other vasculopathies are suspected, or known (EPHB4).