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

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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

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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, ACVRL1 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</td>
<td>N=152 (73%)</td>
<td>104/152 (68%)</td>
</tr>
<tr>
<td>No causative variant identified,</td>
<td>N=56 (27%)</td>
<td>33/56 (59%)</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):

<table>
<thead>
<tr>
<th></th>
<th>Causes of HHT variant identified</th>
<th>N=152 (73%)</th>
<th>148/152 (97%)</th>
<th>4*/152 (3%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No causative variant identified, N=56 (27%)</td>
<td>47/56 (84%)</td>
<td>9/56 (16%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 loo</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 loo</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 CURAÇAO CRITERIA AND HHT “GENE 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 (epistaxes, 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 [Plauchu 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 1. The Curaçao Criteria

<table>
<thead>
<tr>
<th>The HHT diagnosis is</th>
<th>if 3 criteria are present.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definite</td>
<td>if 2 criteria are present, and</td>
</tr>
<tr>
<td>Possible or suspected</td>
<td>if fewer than 2 criteria are present</td>
</tr>
<tr>
<td>Unlikely</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Criteria</th>
<th>spontaneous, recurrent nose bleeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Epistaxis</td>
<td>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>2. Telangiectases</td>
<td></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>3. Visceral lesions</td>
<td>a first degree relative with HHT according to these criteria</td>
</tr>
<tr>
<td></td>
<td>(with or without bleeding)</td>
</tr>
<tr>
<td></td>
<td>- Gastrointestinal telangiectasia</td>
</tr>
<tr>
<td></td>
<td>- Spinal AVM</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.

Table 1

<table>
<thead>
<tr>
<th>Curação Criteria</th>
<th>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 otolaryngologist 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, ACVR1 or SMAD4 pathogenic or likely pathogenic DNA variant</td>
</tr>
</tbody>
</table>

Comment:

C) Comment on current “Gene-Negative” Cases [3]

The gene negative cases reported in the current manuscript (the majority of whom had with 3 or more Curação criteria) fall into 3 groups:

- Patients with a variant of uncertain significance (VUS) in the ACVR1, 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 ACVR1, ENG or SMAD4 genes where the VUS has not been reported, as per current National guidelines.[6,7]
- Patients with no known variant in the ACVR1, ENG or SMAD4 genes where classical HHT is suspected. Ongoing evaluation is in progress.

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

D) References:

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