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Curaçao diagnostic criteria for hereditary hemorrhagic telangiectasia is highly predictive of a pathogenic variant in *ENG* or *ACVRL1* (HHT1 and HHT2)

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Purpose: Determine the variant detection rate for *ENG*, *ACVRL1*, and *SMAD4* in individuals who meet consensus (Curaçao) criteria for the clinical diagnosis of hereditary hemorrhagic telangiectasia.

Methods: Review of HHT center database for individuals with three or more HHT diagnostic criteria, in whom molecular genetic analysis for *ENG*, *ACVRL1*, and *SMAD4* had been performed.

Results: A variant known or suspected to be causal was detected in *ENG* in 67/152 (44.1%; 95% confidence interval [CI], 36.0–52.4%), *ACVRL1* in 79/152 (52.0%; 95% CI, 43.7–60.1%), and *SMAD4* in 2/152 (1.3%; 95% CI, 0.2–4.7%) family probands with definite HHT. Only 4/152 (2.6%; 95% CI, 0.7–6.6%) family probands did not have a variant in one of these genes.

Conclusion: Previous reports of the variant detection rate for *ENG* and *ACVRL1* in HHT patients have come from laboratories, which receive samples from clinicians with a wide range of expertise in recognizing clinical manifestations of HHT. These studies suggest a significantly lower detection rate (~75–85%) than we have found in patients who meet strictly applied consensus criteria (96.1%). Analysis of *SMAD4* adds an additional detection rate of 1.3%. HHT as defined by the Curaçao criteria is highly predictive of a causative variant in either *ENG* or *ACVRL1*.

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INTRODUCTION

Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant vascular dysplasia that occurs in at least 1 in 10,000 individuals.[1] It is characterized by small vascular lesions (telangiectases) of the oral cavity, lips, fingers, and mucosa of the nasal cavity and upper intestinal tract, as well as larger, fast flow vascular lesions (arteriovenous malformations [AVMs]) in the lungs, liver, and brain. Telangiectases and/or AVMs can occur elsewhere, but are not common or considered diagnostic of HHT.

HHT displays age-related penetrance and the average age for development and/or symptomatic presentation of telangiectases and AVMs is very organ specific. For example, recurrent nosebleeds (epistaxis) caused by bleeding telangiectases in the nasal mucosa is the most common symptom and eventually develops in more than 95%, but only 50% of diagnosed individuals report having nosebleeds by age 10.[2] Oral/dermal telangiectases are typically not noted until the third decade of life.[3,4] Thus, observable manifestations can be absent or subtle into adulthood. Yet, cerebral AVMs are usually congenital, with intracranial hemorrhage secondary to these lesions reported in infants and children with HHT.[5] Since the diagnosis of HHT often cannot be made on clinical grounds in the first few decades of life, molecular diagnosis is key to implementation of medical management, which is recommended to begin in the first six months of life.[6]

HHT is a genetically heterogeneous disorder caused by pathogenic variants in multiple genes in the transforming growth factor beta (TGF-β) signaling pathway. Endoglin (*ENG*) and activin A receptor type II–like 1 (*ACVRL1/ALK1*) cause HHT1 (OMIM 187300), and HHT2 (OMIM 600376), respectively.[7,8] Pathogenic variants in *SMAD4* cause a combined juvenile polyposis/HHT (JP/HHT) syndrome (OMIM 175050).[9] Pathogenic variants in these three genes lead to an underproduction of their respective proteins resulting in excessive, abnormal angiogenesis.[10] HHT loci at chromosome 5q31[11] and 7p14,[12] identified by linkage analysis in one or two families respectively, have been designated as HHT3 and HHT4; however, over a decade later the genes remain unknown. Variants in *GDF2* were identified in three individuals with clinical findings suspicious for HHT, but not meeting diagnostic criteria.[13] In fact, more than two decades since the identification

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of *ACVRL1* and *ENG* as genes associated with HHT, no new genes for HHT have been discovered.

Pathogenic variants in *ENG* and *ACVRL1* account for roughly equal percentages of the disorder[14] and are widely accepted, and often quoted, as causing a combined 75–85% of HHT based on multiple series reported in the mid-2000s.[15–19] *SMAD4* is reported to cause 1–2% of HHT.[20] However, it is of note that these reports were based on series of cases submitted to diagnostic laboratories for clinical suspicion of HHT. Laboratories, including ours, receive samples from clinicians with a wide range of expertise in recognizing the clinical manifestations of HHT, as well as in understanding the consensus clinical diagnostic (Curaçao) criteria (Table 1).[6,21]

Methodology used in these mid-2000s series[15–19] typically involved sequencing of exons and intron/exon borders and analysis for large deletions/duplications of *ENG* and *ACVRL1*. Our group has subsequently sequenced noncoding regions of these genes in cohorts selected from samples submitted to our laboratory for suspicion of HHT but with no pathogenic variant detected in the coding regions. Pathogenic variants outside the typically interrogated coding sequence were identified, particularly in the 5'UTR region of *ENG* and deep in intron 9 of *ACVRL1*.[22,23] But these noncoding region variants explained a minority of all suspected HHT cases submitted to our clinical laboratory in which a pathogenic coding region variant of *ENG* or *ACVRL1* had not been found.

Our group forms the core of the University of Utah HHT Center of Excellence, which has provided clinical diagnosis of HHT since 1995, and molecular genetic diagnosis in our Laboratory (ARUP Laboratories) since 2004. It has been our impression that the detection rate of causal variants in patients confirmed to have HHT based on family history, medical history, and physical examination at our specialty clinic is significantly higher than for all samples received into our laboratory from patients reported to have HHT. The purpose of this study was to assess the detection rate for a causal variant in *ENG*, *ACVRL1*, or *SMAD4* for patients diagnosed with HHT based on a detailed, systematic clinical evaluation for manifestations of HHT and the strict application of the Curaçao diagnostic criteria.

MATERIALS AND METHODS

Methods consisted of review of the University of Utah HHT Center of Excellence patient database for individuals with three or more diagnostic criteria for HHT according to Curaçao criteria, and sequencing of exons and intron/exon borders for *ENG*, *ACVRL1*, deletion/duplication analysis of these genes if no suspicious variant was found by sequencing, then *SMAD4* sequencing and deletion/duplication when no suspicious variant was detected in *ENG* or *ACVRL1*. Since 2011 sequencing of the 5'UTR region of *ENG* has also been included in our laboratory's clinical testing protocol.[22] One hundred fifty-two family probands (for genetic testing) were identified who met these criteria.

Clinical evaluation for all patients included history of nosebleed onset, as well as frequency, duration, and intensity; presentation of internal organ AVMs symptoms; careful examination for telangiectases at characteristic sites; and a targeted multigeneration pedigree. Screening for internal organ AVMs was routine for all those considered suspicious for, or confirmed with, HHT based on family history, medical history, and physical examination. The determination of affected status (three or more clinical criteria) for each individual was based on their complete evaluation, including internal organ screening. Molecular genetic testing of the *ENG*, *ACVRL1*, and *SMAD4* genes has been recommended in all family probands with either suspected or definite HHT since clinical testing for these genes became available in the mid-2000s. In <20% of our clinic patients for whom genetic testing is recommended, it is not accomplished, most often due to lack of insurance coverage. This study was approved by the Utah Institutional Review Board (IRB 00039582).

RESULTS

In patients who met Curaçao criteria, 96.1% (146/152; 95% confidence interval [CI], 91.6–98.5%) had a causal or likely causal variant in *ENG* or *ACVRL1*; an additional 1.3% (2/152; 95% CI, 0.2–4.7%) in *SMAD4* (Fig. 1). Only 2.6% (4/152; 95% CI, 0.7–6.6%) did not have a variant in one of these genes.

Of the 148 variants detected in these three genes, 141 individuals had a pathogenic or likely pathogenic variant. One hundred one of the pathogenic/likely pathogenic variants were found in a single proband and 14 were reoccurring (Table S1). The remaining seven variants were classified as variants of uncertain significance (VUS). A list of the VUS and evidence for pathogenicity is provided in Table 2. No other pathogenic variant or variant suspicious for being pathogenic was identified in any case.

Table 1 Consensus (Curaçao) clinical diagnostic criteria for hereditary hemorrhagic telangiectasia (HHT).

Curaçao diagnostic criteria
1. Epistaxis—spontaneous, **recurrent**
2. Telangiectases—multiple at **characteristic sites** (lips, oral cavity, fingers, nose)
3. Visceral lesions—**such as** gastrointestinal telangiectasia; pulmonary, cerebral, hepatic, spinal arteriovenous malformation
4. Family history—first degree relative with HHT **according to these criteria**
Definite if 3 or more criteria are present

Bold: emphasis of points important for accurate application of criteria.

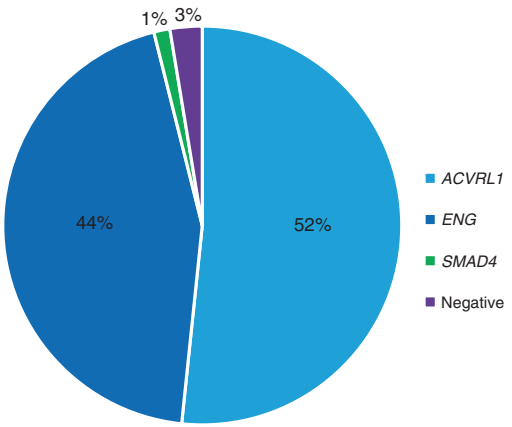

Fig. 1 Molecular genetic test results in 152 probands who met Curaçao criteria for hereditary hemorrhagic telangiectasia (HHT).

DISCUSSION

Patients with a definite clinical diagnosis of HHT based on targeted physical examination, medical history, and family history had a causative variant in *ENG*, *ACVRL1*, or *SMAD4* by sequencing of coding regions, intron/exon borders, and large deletion/duplication analysis approximately 97% of the time. This reaffirms the value of the Curaçao criteria in the clinical diagnosis of HHT, and also suggests that these known genes account for the vast majority of cases.

The 4 of 152 cases with no pathogenic or suspicious variant detected could represent patients with true HHT, with unidentified variants in a known gene or in an alternative unknown gene. It is of note that two of these four cases had additional molecular interrogation on a research basis; one had whole-genome sequencing and the other a custom genome sequencing test limited to the noncoding regions of known HHT/HHT overlapping genes. In neither case was a suspicious variant identified. For the other two cases, either the sample and/or consent was not available for additional testing on a research basis.

The alternative is that these 4/152 individuals were false positives for HHT by the application of Curaçao criteria. One had epistaxis, telangiectases, and a mother reportedly diagnosed with HHT but not examined by our team. Two had epistaxis, telangiectases, a single pulmonary AVM big enough to have been treated, but no family history except one daughter with epistaxis in one. The fourth has mild nosebleeds and relatively few telangiectases for age; but a sister who met diagnostic criteria (epistaxis, telangiectases, pulmonary AVM) and son with epistaxis and history of intracranial hemorrhage reportedly secondary to a cerebral AVM.

Overall, the high variant detection rate of ~97% for these three genes in this study suggests that many cases of presumed HHT found to be negative in previous laboratory based studies likely represent misapplication of the Curaçao criteria. In our laboratory, clinicians ordering genetic testing for HHT are contacted for various reasons to clarify clinical information provided on forms required with sample submission. Over time,

Table 2 Evidence for variants of uncertain significance (VUS) in this study.

Variant	gnomAD	Functional domain	PolyPhen-2 SIFT	Nucleotide conservation	Amino acid conservation	Reference
ACVRL1 [a]c.500C>G p.(Ser167Cys)	Absent	-	Probably damaging Benign	Highly conserved (phyloP: 4.89)	Highly conserved, up to zebrafish (considering 9 species)	17
ACVRL1 c.812C>A p.(Thr271Lys)	Absent	Protein kinase domain	Probably damaging Damaging	Highly conserved (phyloP: 6.02)	Highly conserved, up to zebrafish (considering 9 species)	17
ACVRL1 c.1023C>G p.(Asn341Lys)	Absent	Protein kinase domain	Probably damaging Damaging	Weakly conserved (phyloP: 0.29)	Highly conserved, up to zebrafish (considering 9 species)	14
ACVRL1 c.1361_1375del p.(Arg454_Asp458del)	Absent	Protein kinase domain	NA	NA	NA	This study
ACVRL1 c.1438C>T p.(Leu480Phe)	Absent	Protein kinase domain	Probably damaging Damaging	Highly conserved (phyloP: 5.37)	Highly conserved, up to zebrafish (considering 9 species)	26
ENG c.875T>A p.(Leu292His)	Absent	-	Probably damaging Damaging	Weakly conserved (phyloP: 0.53)	Moderately conserved (considering 12 species)	This study
ENG c.1109T>C p.(Leu370Pro)	Absent	Zona pellucida domain	Probably damaging Damaging	Moderately conserved (phyloP: 2.22)	Weakly conserved (considering 12 species)	19

[a]Splicing algorithms (Alamut v.2.11.0) predict that this variant creates a cryptic splice donor site and may alter splicing.
Transcripts used in analysis: NM_000020.2 (*ACVRL1*) and NM_001114753.2 (*ENG*). Predicted functional domains obtained from InterPro domain database.[27] Missense prediction algorithms used: PolyPhen-2 (v2.2.2r398)[28] and SIFT (v.1.0.3).[29] PhyloP scores obtained from the University of California–Santa Cruz (UCSC) 46-species alignment,[30] and amino acid conservation calculation obtained from Alamut (v.2.11.0) using a curated orthologue alignment (Interactive Biosoftware).

the high frequency of misapplication of the Curaçao criteria, or lack of knowledge about the criteria, by physicians without familiarity with this rare vascular disorder has become apparent. For example, it is typical for red lesions on the trunk of the body most likely to be nonvascular pigmented lesions, to be noted as telangiectases. Or that epistaxis reported remotely in childhood, but not continuing into adulthood, is considered a diagnostic criteria. Or that an AVM of the extremity is considered suggestive of HHT. On one hand, a lower bar for clinical suspicion of HHT for purposes of ordering molecular genetic testing means that fewer cases of HHT will be missed. However, it has led to an underestimate of the variant detection rate in the known HHT genes in cases that meet clinical diagnostic criteria.

This underestimation of the clinical sensitivity of genetic testing for HHT has likely contributed to the underuse of molecular genetic testing in HHT families. This is concerning because the establishment of a causative variant in an HHT family allows for molecular diagnosis in at-risk family members. This is of particular importance in this disorder because it is not possible to rule out the diagnosis of HHT on clinical grounds in childhood; yet medical surveillance in the first six months of life is recommended according to consensus medical management guidelines. In particular, it is recommended that asymptomatic children of a parent with HHT be considered to have possible HHT, unless excluded by genetic testing. In addition, it is recommended that clinical screening for children with possible or definite HHT include magnetic resonance image (MRI) in the first six months of life to rule out a cerebral AVM.[6] This examination is not trivial because it requires sedation at this age. It is thus medically indicated to rule out HHT by molecular genetic analysis in the first few months of life in those unaffected babies born to a patient with HHT, sparing them costly and unnecessary medical surveillance that should otherwise commence. Furthermore, a pathogenic variant in either *ENG* or *ACVRL1* provides reassurance that the additional risks for gastrointestinal polyps and malignancy associated with a variant in *SMAD4* is not a concern in a given family.

It should be noted that not all the sequence variants detected in the family probands in this study can be classified as pathogenic/likely pathogenic by current American College of Medical Genetics and Genomics (ACMG) guidelines.[24] Missense variants are the most common variant type in both the *ENG* and *ACVRL1* genes, and are difficult to classify as pathogenic by ACMG guidelines without functional studies. However, in our experience, most missense variants initially classified as VUS due to insufficient evidence of causation are actually pathogenic based on computational predictions and subsequent family cosegregation studies.[25] Available evidence, provided in Table **2**, suggests that these detected variants formally classified as VUS are more likely pathogenic than benign. Seven of seven of these variants were absent from gnomAD, indicating they are not common polymorphisms.

Despite the difficulty of interpreting noncoding region variants in general, we have recently shown the potential value of including a CT-rich hotspot region of *ACVRL1* intron 9 and the 5'UTR of *ENG* in a molecular diagnostic testing algorithm for HHT.[22,23] It is difficult to know how much this addition of noncoding region analysis will improve clinical sensitivity because the cohorts in which noncoding regions of HHT genes have been interrogated came from samples submitted to our laboratory with the previously mentioned limitation regarding inconsistency in applying HHT clinical diagnostic criteria. However, one case in this current series, with the pathogenic *ENG* c.−127C>T variant, would have been unexplained except for the addition of the *ENG* 5'UTR to our laboratory's clinical testing algorithm a few years ago. On the other hand, more than two decades after the report of the *ACVRL1* and *ENG* genes, no additional genes for HHT have been discovered.

In conclusion, for patients who have HHT according to the Curaçao diagnostic criteria, the detection rate is ~97% for a causative/likely causative variant in an exon or intron/exon border of *ENG*, *ACVRL1*, or *SMAD4*. This is much higher than previously reported in studies done using laboratory databases in which cases without definite HHT were included. This is encouraging because patients with this specific vascular malformation disorder are at risk for the serious consequences of AVMs in multiple internal organs, which can largely be prevented with early diagnosis and medical screening. Relatedly, as-yet undiscovered genes likely contribute little if any to the causation of HHT; and variants in noncoding regions of the known genes may explain the small percentage of HHT cases in which no pathogenic variant is currently identified.

SUPPLEMENTARY INFORMATION

The online version of this article (https://doi.org/10.1038/s41436-020-0775-8) contains supplementary material, which is available to authorized users.

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DISCLOSURE

The authors declare no conflicts of interest.

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