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TECHNICAL REPORT
EBTNA UTILITY GENE TEST

Genetic testing for hereditary hemorrhagic telangiectasia

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Abstract

Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant vascular dysplasia characterized by telangiectases and arteriovenous malformations. These lesions cause bleeding, particularly in the nose, gastrointestinal tract and brain. HHT has incomplete penetrance, variable expressivity and genetic heterogeneity. *De novo* mutations associated with the onset of sporadic HHT have been reported. This Utility Gene Test was prepared on the basis of an analysis of the literature and existing diagnostic protocols. It is useful for confirming diagnosis, as well as for differential diagnosis, couple risk assessment and access to clinical trials.

Keywords: Hereditary hemorrhagic telangiectasia, *ACVRL1*, *ENG*, *GDF2*, *SMAD4*, EBTNA UTILITY GENE TEST

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Hereditary hemorrhagic telangiectasia

(Other synonyms: Telangiectasia, hereditary hemorrhagic, type 1; telangiectasia, hereditary hemorrhagic of Rendu, Osler and Weber; telangiectasia, hereditary hemorrhagic, type 2; juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome)

General information about the disease

Hereditary haemorrhagic telangiectasia (HHT) can be subdivided in hereditary hemorrhagic telangiectasia, type 1 (OMIM disease 187300), and type 2 (OMIM disease 600376), juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome (OMIM disease 175050). All these conditions are characterized by recurrent epistaxis, cutaneous telangiectasia, and visceral arteriovenous malformations (AVMs) that affect the lungs, gastrointestinal tract, liver, brain and other internal organs (1).

The population prevalence rate varies according to different authors. Porteus et al. (2) documented a minimum prevalence of 1 in 40000. Bideau et al. (3) reported a higher prevalence of HHT cases (1/2300) in an isolated French valley. HHT has autosomal dominant inheritance with incomplete penetrance, and sporadic cases due to *de novo* mutation have also been described (4). By the age of 20 years, 50% of individuals have developed some signs of HHT (5).

HHT appears with nosebleeds, and telangiectases on the lips, hands and oral mucosa. Telangiectases in the nasal and gastrointestinal mucosa, and brain arteriovenous malformations generally present with bleeding (6).

Clinical diagnosis of HHT is established when three out of four Curaçao's diagnostic criteria are met. These criteria are: 1) recurrent spontaneous mild to severe epistaxis; 2) multiple mucocutaneous telangiectases; 3) AVMs or telangiectases in internal organs, such as lungs, brain, liver, intestines, stomach and spinal cord; 4) more than one affected family member (7).

The differential diagnosis should consider von Willebrand disease, ataxia-telangiectasia, calcinosis, Raynaud phenomenon, sclerodactyly, telangiectasia syndrome, capillary malformation-arteriovenous malformation and hereditary benign telangiectasia.

Identification of a heterozygous pathogenic variant in *ACVRL1* (OMIM gene 601284), *ENG* (OMIM gene 131195), *GDF2* (OMIM gene 605120), or *SMAD4* (OMIM gene 600993) establishes the diagnosis (8).

All these genes are involved in the transforming growth factor beta/bone morphogenetic protein (TGF-beta/BMP) pathway. *ACVRL1* encodes a type I cell-surface receptor for the TGF-beta superfamily of ligands, *ENG* encodes the glycoprotein most expressed in the vascular endothelium and is necessary for the assembly of the TGF-beta receptor complex, *GDF2* encodes a secreted ligand of the TGF-beta superfamily of proteins, *SMAD4* encodes a member of the Smad family of signal transduction proteins. In response to TGF-beta signalling, small proteins are activated by serine/threonine receptor kinases through phosphorylation (9).

Pathogenic variants may include missense, nonsense, splicing, small insertions, small deletions, small indels, gross deletions, duplications.

Aims of the test

- To determine the gene defect responsible for the disease;
- To confirm clinical diagnosis;
- To assess the recurrence risk and perform genetic counselling for at-risk/affected individuals.

Test characteristics

Specialist centers/ Published Guidelines

The test is listed in the Orphanet database and is offered by 24 accredited medical genetic laboratories in the EU, and in the GTR database, offered by 18 accredited medical genetic laboratories in the US.

Guidelines for clinical use of the test are described in Genetics Home Reference (ghr.nlm.nih.gov) and Gene Reviews (6).

Test strategy

A multi-gene next generation sequencing panel is used for the detection of nucleotide variations in coding exons and flanking introns of the above genes. Potentially causative variants and region with low coverage are Sanger-sequenced. Sanger sequencing is also used for family segregation studies.

Multiplex Ligation Probe Amplification (MLPA) is used to detect insertions and deletions in *ACVRL1* and *ENG* .

To perform molecular diagnosis, a single sample of biological material is normally sufficient. This may be 1 ml peripheral blood in a sterile tube with 0.5 ml K_3EDTA or 1 ml saliva in a sterile tube with 0.5 ml ethanol 95%. Sampling rarely has to be repeated.

Gene-disease associations and the interpretation of genetic variants are rapidly developing fields. It is therefore possible that the genes mentioned in this note may change as new scientific data is acquired. It is also possible that genetic variants today defined as of "unknown or uncertain significance" may acquire clinical importance.

Genetic test results

Positive

Identification of pathogenic variants in the above genes confirms the clinical diagnosis and is an indication for family studies.

A pathogenic variant is known to be causative for a given genetic disorder based on previous reports, or predicted to be causative based on loss of protein function or expected significant damage to proteins or protein/protein interactions. In this way it is possible to obtain a molecular diagnosis in new/other subjects, establish the risk of recurrence in family members and plan preventive and/or therapeutic measures.

Inconclusive

Detection of a variant of unknown or uncertain significance (*VUS*): a new variation without any evident pathogenic significance or a known variation with insufficient evidence (or with conflicting evidence) to indicate it is *likely benign* or *likely pathogenic* for a given genetic disorder. In these cases, it is advisable to extend testing to the patient's relatives to assess variant segregation and clarify its contribution. In some cases, it could be necessary to perform further examinations/tests or to do a clinical reassessment of pathological signs.

Negative

The absence of variations in the genomic regions investigated does not exclude a clinical diagnosis but suggests the possibility of:

- alterations that cannot be identified by sequencing, such as large rearrangements that cause loss (deletion) or gain (duplication) of extended gene fragments;
- sequence variations in gene regions not investigated by this test, such as regulatory regions (5' and 3' UTR) and deep intronic regions;
- variations in other genes not investigated by the present test.

Unexpected

Unexpected results may emerge from the test, for example information regarding consanguinity, absence of family correlation or other genetically-based diseases.

Risk for progeny

In autosomal dominant transmission, the probability that an affected carrier transmit the variant to his/her children is 50% in any pregnancy, irrespective of the sex of the child conceived.

Limits of the test

The test is limited by current scientific knowledge regarding the gene and disease.

Analytical sensitivity (proportion of positive tests when the genotype is truly present) **and specificity** (proportion of negative tests when the genotype is not present)

NGS Analytical sensitivity >99.99%, with a minimum coverage of 10X; Analytical specificity 99.99%.

SANGER Analytical sensitivity >99.99%; Analytical specificity 99.99%.

MLPA Analytical sensitivity >99.99%; Analytical specificity 99.99%.

Clinical sensitivity (proportion of positive tests if the disease is present) **and clinical specificity** (proportion of negative tests if the disease is not present)

Clinical sensitivity: it is reported that the mutation detection rate for *ENG* and *ACVRL1* ranged from 85% if the ordering physician specifically reported the patient to have all four Curaçao diagnostic criteria (Epistaxis, Telangiectases, AVM, Family History) (8).

Clinical specificity: Data not available

Prescription appropriateness

The genetic test is appropriate when:

a) the patient meets the diagnostic criteria for HHT;

b) the sensitivity of the test is greater than or equal to that of tests described in the literature.

Clinical utility

Clinical management	Utility
Confirmation of clinical diagnosis	Yes
Differential diagnosis	Yes
Couple risk assessment	Yes
Availability of clinical trials can be checked on-line at https://clinicaltrials.gov/	

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