



## Genetic testing for Hennekam syndrome

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### Abstract

Hennekam Syndrome (HS) is a combination of congenital lymphatic malformation, lymphangiectasia and other disorders. It is a very rare disorder with autosomal recessive inheritance. We developed the test protocol “Hennekam Syndrome” on the basis of the latest research findings and diagnostic protocols on lymphatic malformation in HS. The genetic test is useful for confirming diagnosis, as well as for differential diagnosis, couple risk assessment and access to clinical trials.

**Keywords:** Primary lymphatic malformations, Hennekam syndrome, EBTNA UTILITY GENE TEST

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### Hennekam syndrome

(Other synonyms: Hennekam lymphangiectasia-lymphedema syndrome; generalized lymphatic dysplasia)

### General information about the disease

Hennekam Syndrome (HS) combines symptoms such as congenital lymphedema and intestinal lymphangiectasia. Symptoms such as facial anomalies, variable intellectual disabilities and occasionally other malformations can also manifest (1).

Lymphedema is evident at birth and often affects the face and lower extremities asymmetrically. Intestinal lymphangiectasia causes visceral lymphatic vessel rupture and accumulation of lymph in the abdomen. Lymphangiectasia may also involve the kidneys, thyroid gland, pleura, pericardium and the skin. Patients with HS may have microcephaly, craniosynostosis, syndactyly, camptodactyly, chylothorax and the following facial features: flattish median line of face and nose bridge, puffy eyelids, hypertelorism, small low-set ears and small mouth with gingival hypertrophy (2). Prognosis reflects the wide variability of symptoms. Thus, the life expectancy of HS patients varies from death in childhood to survival into adulthood. However, early death caused by severe manifestations has been reported in very few cases (3). The syndrome has autosomal recessive inheritance. The prevalence rate is not available but is estimated to be very rare: since its discovery only about 50 cases have been described (4).

Diagnostic measures include: clinical anamnesis, clinical examinations, lymphoscintigraphy, duodenal biopsy.

Differential diagnosis necessary to distinguish from Noonan and Aagaens syndromes.

Hennekam syndrome is caused by homozygous or compound heterozygous mutation of the *CCBE1* gene in approximately 25% of patients (5) and of the *FAT4* gene in ~20% of cases (6). Recently, Brouillard et al. identified bi-allelic missense mutations in *ADAMTS3* in two siblings with Hennekam syndrome (7). Mutations of these genes were observed in nearly 50% of cases; the causes of the other half are still unknown. Mutation of *CCBE1*

has also been found in Aagaenæs (cholestasis-lymphedema) syndrome – generalized lymphedema with chronic cholestasis and recurrent cholangitis (8). Biallelic mutations in the *FAT4* gene can also cause Van Maldergem syndrome type 2 (8). Direct involvement of *CCBE1* in the development of the mammalian lymphatic vascular system has been proven experimentally (9, 10), but there is no direct evidence of involvement of the *FAT4* or *ADAMTS3* genes. How variations in these genes act and result in the HS phenotype is therefore not completely understood.

The condition has autosomal recessive inheritance.

### HS different phenotypic varieties and their associated genes include:

- Hennekam lymphangiectasia-lymphedema syndrome 1 (HKLLS1, OMIM disease 235510) - *CCBE1* (OMIM gene 612753);
- Hennekam lymphangiectasia-lymphedema syndrome 2 (HKLLS2, OMIM disease 616006) - *FAT4* (OMIM gene 612411);
- Hennekam lymphangiectasia-lymphedema syndrome 3 (HKLLS3 OMIM disease not available) - *ADAMTS3* (OMIM gene 605011).

Pathogenic variants may include missense, splicing, small insertions and deletions.

### 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 6 accredited medical genetic laboratories in the EU, and in the GTR database, offered by 3 accredited medical genetic laboratories in the US.

Guidelines for clinical use of the test are described in Genetics Home Reference ([ghr.nlm.nih.gov](http://ghr.nlm.nih.gov)) (2).

#### 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 regions with low coverage are Sanger-sequenced. Sanger sequencing is also used for family segregation studies.

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<sub>3</sub>EDTA 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 recessive mutations, both parents are usually healthy carriers. In this case, the probability of transmitting the disorder to the offspring is 25% in any pregnancy of the couple, irrespective of the sex of the child. An affected individual generates healthy carrier sons and daughters in all cases, except in pregnancies with a healthy carrier partner. In these cases, the risk of an affected son or daughter is 50%.

## 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%.

## 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 is approximately 40-45%, but in many cases these are individual variations (identified in one or few families) (5, 6).

Clinical sensitivity: data not available.

## Prescription appropriateness

The genetic test is appropriate when:

- a) the patient meets the diagnostic criteria for HS;
- 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 <a href="https://clinicaltrials.gov/">https://clinicaltrials.gov/</a>	

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