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The EuroBiotech Journal

TECHNICAL REPORT
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Genetic testing for vascular Ehlers-Danlos syndrome and other variants with fragility of the middle arteries

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Abstract

Ehlers-Danlos syndrome (EDS) is an umbrella term for various inherited connective tissue disorders associated with mutations in genes involved in extracellular matrix formation. "The 2017 International Classification of Ehlers-Danlos Syndromes and related disorders" identifies 13 clinical types with mutations in 19 distinct genes. The present module focuses on forms with major vascular involvement: vascular EDS (vEDS) caused by heterozygous mutations in *COL3A1,* "vascular-like" EDS (vlEDS) caused by recurrent mutations in *COL1A1,* classical EDS with vascular fragility associated with heterozygous mutations in *COL5A1*, and kyphoscoliotic EDS associated with recessive variations in *PLOD1* and *FKBP14*. The overall prevalence of EDS is estimated between 1/10,000 and 1/25,000 and vEDS accounts for about 5 to 10% of all EDS cases. This Utility Gene Test was prepared on the basis of an analysis of the literature and existing diagnostic protocols. Molecular testing is useful for diagnosis confirmation, as well as differential diagnosis, appropriate genetic counselling and access to clinical trials.

Keywords: Kyphoscoliotic Ehlers-Danlos syndrome, vascular Ehlers-Danlos syndrome, *COL1A1*, *COL3A1, COL5A1, FKBP14, PLOD1*, EBTNA UTILITY GENE TEST

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DOI: 10.2478/ebtj-2018-0034

Vascular Ehlers-Danlos syndrome and other variants with fragility of the middle arteries

(Other synonyms: classical Ehlers-Danlos syndrome with vascular fragility, vascular-like Ehlers-Danlos syndrome, kyphoscoliotic Ehlers-Danlos syndrome)

General information about the disease

Ehlers-Danlos syndrome (EDS) is of a group of inherited connective tissue disorders caused by mutations in genes involved in extracellular matrix formation. The mutations cause a loss of structural integrity in different organ systems. Different types of EDS exist, distinguishable on the basis of the gene(s) involved, clinical manifestations and prognosis. The present module considers forms of EDS with vascular involvement. Among the various forms of EDS, vascular EDS (vEDS; OMIM 130050) is the most common among variants with a major risk of middle artery ruptures and fragility (e.g. spontaneous ruptures) of hollow organs. In most patients vEDS is defined by typical facial features (e.g. large eyes, small chin, sunken cheeks, thin nose and lips, lobeless ears), acrogeria, translucent skin with evident subcutaneous vessels on the trunk and lower back, easy bruising and severe arterial, digestive and uterine complications. Complications are vascular and include arterial and venous abnormalities with arterial rupture (1-3). vEDS is always caused by heterozygous mutations in the *COL3A1* gene.

Prevalence of EDS as a whole is estimated between 1/10,000 and 1/25,000, without significant ethnic variations. The "2017 International Classification of Ehlers-Danlos Syndromes and related disorders" identifies 13 clinical subtypes (4), among which

vEDS probably accounts for about 5 to 10% of cases (5). The combination of two major criteria such as arterial rupture, intestinal rupture or uterine rupture during pregnancy and positive family history is strong diagnostic evidence of vEDS.

In cases who meet the clinical diagnostic criteria, genetic testing should be done to identify variations in *COL3A1* in order to confirm the diagnosis (2, 4). Mutations in *COL5A1* are normally associated with classical EDS (OMIM disease 130000) that is characterized by hyperextensible skin and joint laxity. However, some reports describe cases of classical EDS due to mutations in *COL5A1* and potentially deadly middle artery fragility (6, 7). Rare families with classical EDS and vascular fragility are described with the recurrent mutations in COL1A1 (4). Vascular rupture has also been described in kyphoscoliotic EDS (OMIM disease 225400) caused by homozygous or compound heterozygous mutations in the *PLOD1* and *FKBP14* genes (8).

Differential diagnosis of vEDS should consider other forms of EDS, isolated arterial aneurysm, Loeys-Dietz syndromes, autosomal dominant polycystic kidney disease, Marfan syndrome, pseudoxanthoma elasticum and hereditary hemorrhagic telangiectasia (9).

vEDS regularly associates with mutations in *COL3A1* (OMIM gene 120180). Heterozygous mutations in *COL1A1* (OMIM gene 120150) and *COL5A1* (OMIM gene 120215) may occasionally associate with similar phenotypes of middle artery ruptures/dissections/aneurysms. Recessive variants in *PLOD1* (OMIM gene 153454) and *FKBP14* cause the rarer kyphoscoliotic type.

Pathogenic variants may include missense, nonsense, splicing, small insertions, small deletions, small indels, gross insertions, gross deletions and complex rearrangements.

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 23 accredited medical genetic laboratories in the EU, and in the GTR database, offered by 2 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 (9).

Test strategy

In clear-cut phenotypes, targeted Sanger sequencing of *COL3A1* may be considered a first tier approach for confirmation of the diagnosis. More commonly, a multi-gene next generation sequencing panel is used for the detection of nucleotide variations in coding exons and flanking introns of all the above genes.

Potentially causative variants and regions with low coverage are Sanger-sequenced. Sanger sequencing is also used for family segregation studies.

Multiplex Ligation Probe Amplification (MLPA) is used to detect duplications and deletions in the *COL3A1* gene.

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 correla-

tion or other genetically-based diseases.

Risk for progeny

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

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%.

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: 95% for vEDS (10).

Clinical specificity: nearly 100% (10).

Prescription appropriateness

The genetic test is appropriate when:

a) the patient meets the diagnostic criteria for EDS with vascular involvement;

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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