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EBTNA UTILITY GENE TEST

Genetic testing for large-caliber vessel aneurysms

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Abstract

Large-caliber vessels are those with a diameter of 10 mm or more. Most aneurysms remain asymptomatic until they expand or rupture. Aortic aneurysms are of special interest for physicians and scientists because of their prevalence. Aortic aneurysms and dissections account for 1-2% of all deaths in western countries. Expansion and rupture of vascular aneurysms show a strong correlation with hyperlipidemia, hypertension, smoking, sex and age. Heritability estimates have been as high as 70%. This Utility Gene Test was developed 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: Large-caliber vessel aneurysms, EBTNA UTILITY GENE TEST

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General information about the disease

Large-caliber vessels have a diameter of at least 10 mm. Aneurysms in the following vessels are included in this group: ascending aorta, aortic arch, descending thoracic aorta (sub-diaphragmatic aorta, infra-diaphragmatic aorta), suprarenal abdominal aorta, infra-renal aorta, brachycephalic artery, subclavian artery, common carotid artery, common iliac artery, axillary vein, retro-aortic innominate vein, superior vena cava, common femoral vein, external iliac vein, common iliac vein, inferior vena cava, pulmonary arteries and pulmonary veins. Due to their clinical importance, the etiology and genetic causes of aneurysms of large-caliber arteries have been well studied, whereas there is little literature on vein aneurysms due to an absence of specific symptoms, unless they are complicated by their various manifestations, one being deep vein thrombosis (1).

More than 80% of cases of pulmonary venous aneurysm are congenital and half or more are associated with hereditary hemorrhagic telangiectasia (2). Pulmonary artery dilation is a minor cardiovascular criterion in the diagnosis of Marfan syndrome (3).

Aortic aneurysms include thoracic aortic aneurysm (AAT) and abdominal aortic aneurysm (AAA) and account for 1-2% of all deaths in western countries. Heritability estimates have been as high as 70% (4-9).

The incidence of AAT is ≈10.4 per 100 000 person-years.

Abdominal aortic aneurysms are strongly linked to hyperlipidemia, hypertension, smoking, sex and age. The incidence of AAAs is about 8% among men over the age of 60 years (10).

Little is known about the pathogenesis of aortic aneurysms, however chronic inflammatory processes have a significant effect on the development of this defect. Upregulation of cytokine pathways associated with increased matrix turnover by matrix metalloproteinases (MMPs) has been observed (10,11). In this regard, genetic influences on the regulation of inflammatory responses plays crucial role (12).

Prevalence is unknown.

Vascular aneurysms remain undiagnosed until they expand or rupture, when they

can give rise to local compression, including early satiety, nausea, vomiting, urinary symptoms, vein thrombosis due to venous compression, abdominal pain, groin pain and embolic phenomena affecting the toes (13).

Diagnosis is based on routine physical examination, palpation, arterial blood pressure, chest radiogram, pulse oximetry, Doppler and two-dimensional echocardiography, spin-echo MR imaging, CT and genetic testing.

The condition can be part of an inherited syndrome or can manifest as an isolated trait, whether sporadic or affecting more than one family member.

Autosomal dominant non-syndromic large-caliber vessel aneurysms

- Thoracic aortic aneurysm 4 (AAT4, OMIM disease 132900) - *MYH11* (OMIM gene 160745);
- Thoracic aortic aneurysm 6 (AAT6, OMIM disease 611788) - *ACTA2* (OMIM gene 102620);
- Thoracic aortic aneurysm 7 (AAT7, OMIM disease 613780) - *MYLK* (OMIM gene 600922);
- Thoracic aortic aneurysm 8 (AAT8, OMIM disease 615436) - *PRKG1* (OMIM gene 176894);
- Thoracic aortic aneurysm 9 (AAT9, OMIM disease 616166) - *MFAP5* (OMIM gene 601103);
- Thoracic aortic aneurysm 10 (AAT10, OMIM disease 617168) - *LOX* (OMIM gene 153455);
- Thoracic aortic aneurysm 11 (AAT11, OMIM disease 617349) - *FOXE3* (OMIM gene 601094);
- Aortic valve disease 1 (AOVD1, OMIM disease109730) - *NOTCH1* (OMIM gene 190198).

Autosomal dominant syndromic large-caliber vessel aneurysms

- Ehlers-Danlos syndrome, vascular type (EDSVASC, OMIM disease 130050) – *COL3A1* (OMIM gene 120180);
- Loeys-Dietz syndrome 1 (LDS1, OMIM disease 609192) - *TGFBR1* (OMIM gene 190181);
- Loeys-Dietz syndrome 2 (LDS2, OMIM disease 610168) - *TGFBR2* (OMIM gene 190182);
- Loeys-Dietz syndrome 3 (LDS3, OMIM disease 613795) - *SMAD3* (OMIM gene 603109);
- Loeys-Dietz syndrome 4 (LDS4, OMIM disease 614816) - *TGFB2* (OMIM gene 190220);
- Loeys-Dietz syndrome 5 (LDS5, OMIM disease 615582) - *TGFB3* (OMIM gene 190230);
- Marfan Syndrome (MFS, OMIM disease 154700) - *FBN1* (OMIM gene 134797);
- Contractural arachnodactyly, congenital (CCA, OMIM disease 121050) - *FBN2* (OMIM gene 612570);
- Cutis laxa, autosomal dominant (OMIM disease 123700) - *ELN* (OMIM gene 130160);
- Shprintzen-Goldberg craniosynostosis syndrome with aortic aneurysm (SGS, OMIM disease 182212) – *SKI* (OMIM gene 164780) (14).

Autosomal recessive syndromic large-caliber vessel aneurysms

- Cutis laxa, autosomal recessive, type IB (ARCL1B, OMIM disease 614437) - *EFEMP2* (OMIM gene 604633);
- Arterial tortuosity syndrome (ATS, OMIM disease 208050) - *SLC2A10* (OMIM gene 606145).

X-linked syndromic large-caliber vessel aneurysms

- Meester-Loeys syndrome (MRLS, OMIM disease 300989) – *BGN* (OMIM gene 301870);
- Heterotopia, periventricular (OMIM disease 300049) – *FLNA* (OMIM gene 300017).

Likely candidate genes for non-syndromic and syndromic large vessel aneurysm are: *MAT2A* (15), *SMAD2* (16), *COL5A1* and *COL5A2* (17).

Pathogenic variants may include missense, nonsense, splicing, small insertions, deletions, indels and rarely gross deletions and/or 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

Guidelines for clinical use of the test are described in Genetics Home Reference (ghr.nlm.nih.gov) and Gene Reviews (18).

Test strategy

Clinically distinguishable syndromes can be analyzed by sequencing only those genes known to be associated with that specific disease using Sanger or Next Generation Sequencing (NGS); if the results are negative, or more generally if clinical signs are ambiguous for diagnosis, a multi-gene NGS panel is used to detect 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.

Multiplex Ligation Probe Amplification (MLPA) is used to detect duplications and deletions in *COL3A1, FBN1, SLC2A10* and *FLNA*.

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

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%.

In X-linked transmission, affected males transmit the pathogenic variant to their daughters and the probability that a female carrier transmit the pathogenic variant to her offspring is 50% in any pregnancy irrespective of the sex of the child conceived. Females who inherit the pathogenic variant are carriers and usually unaffected. Males who inherit the pathogenic variant are affected.

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: 20-25% for familial non syndromic AAT [18,19]. In syndromic forms, clinical sensitivity depends on the specific clinical criteria.

Clinical specificity is estimated at approximately 99% (19, 20).

Prescription appropriateness

The genetic test is appropriate when:

a) the patient meets the diagnostic criteria for Large-caliber vessel aneurysms;

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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