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TECHNICAL REPORT

EBTNA UTILITY GENE TEST

Genetic testing for vascular anomalies

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Abstract

Vascular anomalies (VAs) have phenotypic variability within the same entity, overlapping clinical features between different conditions, allelic and locus heterogeneity and the same disorder can be inherited in different ways. Most VAs are sporadic (paradominant inheritance or *de novo* somatic or germline mutations), but hereditary forms (autosomal dominant or recessive) have been described. This Utility Gene Test was developed on the basis of an analysis of the literature and existing diagnostic protocols. The genetic test is useful for confirming diagnosis, as well as for differential diagnosis, couple risk assessment and access to clinical trials.

Keywords: Vascular anomalies, germline mutations, somatic mutations, EBTNA UTILITY GENE TEST

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Vascular anomalies

(Other synonyms: Vascular anomalies are a group of diseases; see phenotypic variants)

General information about the disease

Vascular anomalies (VAs) combine an extremely heterogeneous group of congenital abnormalities of the vascular system. VAs include vascular tumours, such as hemangioma, and malformations of veins, arteries, capillaries and the lymphatic system. Anomalies may occur during embryogenesis. They may be morphological, structural and/or functional defects affecting different types and calibers of vessels in any anatomical area (1). When more than one type of vessel is affected, the term *mixed anomaly* is used. Vascular anomalies can also occur in the context of syndromes (2). Disorders characterized by VAs have phenotypic variability within the same entity, overlapping clinical features between different conditions, allelic and locus heterogeneity, and the same disorder can be inherited in different ways. Although most vascular anomalies are sporadic (paradominant inheritance or *de novo* somatic mutations), there are well-described syndromic and hereditary forms of VAs (autosomal dominant or recessive). VAs have high clinical variability: indeed they may manifest as monofocal or multifocal lesions and they may be congenital or appear/develop later (3, 4).

Prevalence is unknown.

Diagnostic work-up may include clinical history, clinical examination, vascular echo-Doppler and vascular magnetic resonance imaging.

Vascular anomalies can be classified on the basis of the vessels affected:

Vascular tumours

- Capillary infantile hemangioma (OMIM disease 602089) can be sporadic or have autosomal dominant inheritance. It can be caused either by somatic mutations in *KDR*,

FLT4 (OMIM gene 136352), *DUSP5* (OMIM gene 603069), *GNAQ* (OMIM gene 600998), *GNA11* (OMIM gene 39313) or *GNA14* (OMIM gene 604397) or by germline mutations in *ANTXR1* (OMIM gene 606410) or *KDR* (OMIM gene 191306) (5-8).

- Verrucous venous malformation (OMIM disease not available) is sporadic and is caused by somatic mutations in *MAP3K3* (OMIM gene 602539) (9).
- Pyogenic granuloma (OMIM disease not available) is sporadic and is caused by somatic mutations in *BRAF*, *NRAS* or *KRAS* (10)

Venous malformations

- Multiple cutaneous and mucosal venous malformations (VMCM, OMIM disease 600195) can be caused either by mutations in *TEK* (OMIM gene 600221) with dominant or paradominant inheritance, or by sporadic somatic mutations in *PIK3CA* (OMIM gene 171834) (11, 12).
- Glomuvenous malformations (GVM, OMIM disease 138000) can have dominant or paradominant inheritance and are caused by mutations in *GLMN* (OMIM gene 601749) (13).
- Cerebral cavernous malformations type 1 (CCM1, OMIM disease 116860), type 2 (CCM2, OMIM disease 603284) and type 3 (CCM3, OMIM disease 603285) have dominant or paradominant inheritance and are caused by mutations in *KRIT1* (OMIM gene 604214), *CCM2* (OMIM gene 607929) and *PDCD10* (OMIM gene 609118), respectively (14-16).
- Blue rubber bleb nevus syndrome (OMIM disease 12200) is sporadic and is caused by somatic mutations in *TEK* (17).

Capillary malformations

- Congenital capillary malformations (CMC, OMIM disease 163000) are sporadic and are caused by somatic mutations in *GNAQ* or *GNA11* (18, 19).
- Parkes Weber syndrome (PKWS, OMIM disease 608355) has dominant or paradominant inheritance and is caused by mutations in *RASA1* (OMIM gene 139150) (20).
- Sturge-Weber syndrome (SWS, OMIM 185300) is sporadic and is caused by somatic mutations in *GNAQ* (21).
- Capillary malformations-arteriovenous malformations (CMAVM, OMIM 608354) can be sporadic or have dominant inheritance and are caused either by somatic mutations in *RASA1* or by germline mutations in *RASA1* or *EPHB4* (OMIM gene 600011) (20, 22).
- Hereditary hemorrhagic telangiectasia type 1 (HHT1, OMIM disease 187300) and type 2 (HHT2, OMIM disease 600376) have dominant or paradominant inheritance and are caused by mutations in *ENG* (OMIM gene 131195) and *ACVRL1* (OMIM gene 601284), respectively. Hereditary hemorrhagic telangiectasia type 5 (HHT5, OMIM disease 615506) has autosomal dominant inheritance. It is caused by mutations in *GDF2* (OMIM gene 605120). Pathogenic mutations in *RASA1* have been reported in patients with a form of dominant hereditary hemorrhagic telangiectasia (23, 24).
- Juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome (JPHT, OMIM disease 175050) has autosomal dominant inheritance. It is caused by mutations in *SMAD4* (OMIM gene 600993) (25).

Syndromic arteriovenous anomalies

- Klippel-Trénauny-Weber syndrome (OMIM disease 149000) and congenital lipomatous overgrowth, vascular malformations and epidermal nevi syndrome (CLOVES, OMIM disease 612918) are sporadic and are caused by somatic mutations in *PIK3CA* (26).
- Multiple enchondromatosis, Maffucci type (OMIM disease 614569) is sporadic and is caused by somatic mutations in *IDH1* (OMIM gene 147700) and *IDH2* (OMIM gene 147650) (27).
- Proteus syndrome (OMIM disease 176920) is sporadic and is caused by somatic mutations in *AKT1* (OMIM gene 164730) (28).
- Loeys-Dietz syndrome type 1 (OMIM disease 609192), type 2 (OMIM disease 600168), type 3 (OMIM disease 613795) and type 4 (OMIM disease 614816) have autosomal dominant inheritance. They are caused by mutations in *TGFBR1* (OMIM gene 606145), *TGFBR2* (OMIM gene 190182), *SMAD3* (OMIM gene 603109) and *TGFB2* (OMIM gene 190220), respectively (29-31).
- Ehlers-Danlos syndrome, vascular type (EDSVASC, OMIM disease 130050) has autosomal dominant inheritance. It is caused by mutations in *COL3A1* (OMIM gene 120180) (32).
- Arterial tortuosity syndrome (ATS, OMIM disease 208050) has autosomal recessive inheritance. It is caused by mutations in *SLC2A10* (OMIM gene 606145) (33).
- Cowden syndrome type 1 (CWS1, OMIM disease 158350), type 5 (CWS5, OMIM disease 615108) and type 6 (CWS6, OMIM disease 615109) have autosomal dominant inheritance. They are caused by mutations in *PTEN* (OMIM gene 601728), *PIK3CA*, and *AKT1*, respectively (34, 35).
- Marfan syndrome (MFS, OMIM disease 154700) has autosomal dominant inheritance. It is caused by mutations in *FBN1* (OMIM gene 134797) (36).
- Pseudoxanthoma elasticum (PXE, OMIM disease 264800) has autosomal recessive inheritance. It is caused by mutations in *ABCC6* (OMIM gene 603234) and *ENPP1* (OMIM gene 173335) (37).
- Microcephaly-capillary malformation syndrome; (MIC-CAP, OMIM disease 614261) has autosomal recessive inheritance. It is caused by mutations in *STAMBP* (OMIM gene 606247) (38).

Recently, somatic mutations in *MAP2K1* (OMIM gene 176872) were found in patients with sporadic extracranial arteriovenous malformations (39), and germline mutations in *ELMO2* (OMIM gene 606421) were found in patients with intraosseous vascular malformations (it is unclear if this malformations affect capillary or veins) (40).

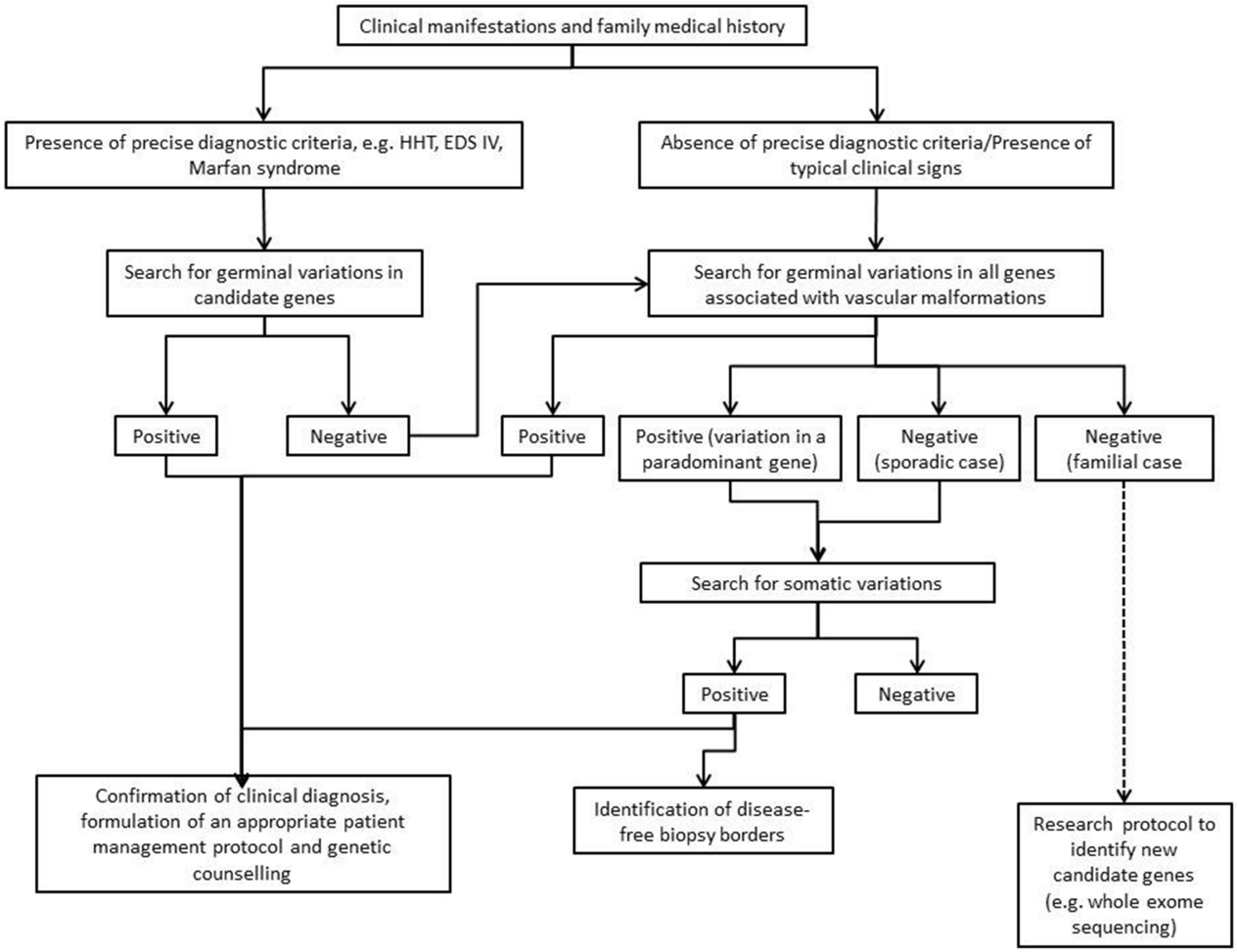

Figure 1. Flow chart of genetic testing for vascular anomalies.

Other likely genes

SOX17 (OMIM gene 610928), *TMEM100* (OMIM gene616334), *NOTCH4* (OMIM gene 164951), *BMP4* (OMIM gene 112262), *BMPR1A* (OMIM gene 601299), *SMAD5* (OMIM gene 603110), *NOTCH3* (OMIM gene 600276), *ACTN4* (OMIM gene 604638), *BMP10* (OMIM gene 608748), *CHST11* (OMIM gene 610128), *CHTOP* (OMIM gene 614206), *DLL4* (OMIM gene 605185), *MGP* (OMIM gene 154870), *MYO9A* (OMIM gene 604875), *NOTCH1* (OMIM gene 190198), *PRRC2B* (OMIM gene not available), *RBPJ* (OMIM gene 147183), *SH3PXD2A* (OMIM gene not available), *SLC20A2* (OMIM gene 158378), *SLC25A20* (OMIM gene 613698), *FOXF1* (OMIM gene 601089), *BMPR2* (OMIM gene 600799).

Pathogenic variants may include missense, nonsense, splicing, small insertions and deletions, small indels, gross insertions, duplications 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 27 accredited medical genetic laboratories in the EU, and in the GTR database, offered by 4 accredited medical genetic laboratories in the US.

Guidelines for clinical use of the test are described in disease-specific chapters of Genetics Home Reference (ghr.nlm.nih.gov) and Gene Reviews (41).

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.

The efficiency of targeted NGS is precious for VAs because of their complex inheritance pattern and genetic and phenotypic heterogeneity. DNA extracted from blood (or saliva) should always be analyzed in tandem with DNA extracted from affected tissues (**Fig. 1**). In fact, performed in this way, the test

makes it possible to identify variants specific for the affected tissue and to determine whether the variant is inherited from the parents or occurred as a sporadic somatic event. In a single experiment, it is possible to identify germline pathogenic variants and/or somatic pathogenic variants (an allelic imbalance of ≥6% is the cut-off for a positive test). The results obtained from targeted NGS are analyzed using an in-house bioinformatic tool that compares results obtained from the germinal lineage (blood or saliva specimens) and affected tissue. For variant selection, a cut-off value (related to biopsy and blood results) is used, and if the variant frequency is higher than the cut-off value it is considered for further analysis. The cut-off depends on tissue quality, extraction method, biocomputing software and other parameters. 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 *ABCC6*, *ACVRL1*, *CCM2*, *COL3A1*, *ENG*, *FBN1*, *KRIT1*, *PDCD10*, *PTEN*, *RASA1*, *SLC2A10*, *TGFBR1* and *TGFB2*.

Sporadic cases with negative test outcome or positive results in genes with paradominant inheritance (*ANTXR1*, *CCM2*, *ENG*, *GLMN*, *KDR*, *KRIT1*, *PDCD10*, *PTEN*, *RASA1* and *TEK*) should be tested for somatic variations. Potentially causative variants need to be verified by further means (e.g. cloning + Sanger sequencing, Sanger sequencing, minisequencing).

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.

A frozen intra-lesional biopsy specimen, in addition to blood or saliva, is necessary to test for somatic variations.

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 protein 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 following possibilities

- 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 genomic regions not investigated by the 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%.

De novo somatic variations cannot be inherited or transmitted.

In paradominant inheritance, only the germline genetic variant is transmitted in an autosomal dominant fashion and the probability that carriers transmit the germline pathogenic variant to their 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 genes and diseases.

Currently, there is no evidence of a genotype-phenotype correlation between mosaicism level and the severity of clinical manifestation.

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%.

MINISEQUENCING 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)

The variations in the aforementioned genes depend closely on the specific disorder. Clinical sensitivity and specificity, based on current genetic knowledge and internal case studies, can be estimated at 20-30% and 99.78%, respectively (42).

No epidemiological data is available for private variants (specific to one or very few families). In such cases, clinical sensitivity is estimated on the basis of internal case studies (42).

Prescription appropriateness

The genetic test is appropriate when:

a) the patient meets the diagnostic criteria for Vas (43);

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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