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TECHNICAL REPORT
EBTNA UTILITY GENE TEST

Genetic testing for Ebstein anomaly

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Abstract

Ebstein anomaly (EA) is a rare congenital tricuspid valve malformation, characterized by downward displacement of the septal leaflet and an atrialized right ventricle. About 80% of cases of EA are non-syndromic; in the other 20%, the anomaly is associated with a chromosomal or Mendelian syndrome. The prevalence of EA is estimated at about 1 per 20,000 live births, and accounts for less than 1% of all congenital heart defects. EA has autosomal dominant inheritance. Likely causative genes are: *NKX2-5*, *MYH7* and *TPM1*. 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, potential risk assessment and access to clinical trials.

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General information about the disease

Ebstein anomaly (EA) is a rare congenital tricuspid valve malformation, characterized by downward displacement of the septal leaflet and an atrialized right ventricle with great variability in clinical, anatomical, echocardiographic and electrocardiographic features, all of which influence prognosis. It may manifest with severe symptoms at birth or may remain asymptomatic until adulthood. It is sometimes associated with septal defects, transposition of large vessels, preexcitation syndromes, or left ventricular non-compaction (1, 2). Isolated forms of EA are non-syndromic, but 20% also have extracardiac defects associated with chromosomal and Mendelian genetic disorders, such as Apert, Noonan, CHARGE, Holt–Oram, Cornelia de Lange, and Kabuki syndromes and VACTERL association (3).

Morphological characteristics of EA include: adherence of the septal and posterior leaflets to the underlying myocardium; downward (apical) displacement of the functional annulus; dilation of the "atrialized" portion of the right ventricle, with various degrees of hypertrophy and thinning of the wall; redundancy, fenestrations and tethering of the anterior leaflet; dilation of the right atrioventricular junction (true tricuspid annulus) (4,5).

Patients with EA have conduction system abnormalities, which are at least partly due to compression of the atrioventricular node by the septal malformation, accessory pathways, and abnormalities of the right bundle branch. Right bundle branch block is frequent in humans with EA and this block has also been noted in animals with EA (6-8).

The prevalence of EA is estimated at about 1 in 20,000 live births, with equal occurrence in males and females. It accounts for less than 1% of all congenital heart defects (9,10). Advances in diagnostics now make it possible to detect EA in the fetal or neonatal period. Prognosis is poor: mortality is 29% at 1 year, 34% at 10 years, and 56% at 30 years (11, 12).

The diagnostic work-up includes clinical assessment to identify symptoms, echocardiogram, electrocardiogram, chest radiogram, integration of septal and color Doppler with two-dimensional echocardiography, spin-echo MR imaging, CT, diagnostic catheterization and genetic testing.

Differential diagnosis should consider EA caused by Down syndrome and other chromosomal disorders.

Non-syndromic EA has autosomal dominant inheritance with variable expressivity and incomplete penetrance.

Candidate genes for EA (OMIM disease 224700)

- *NKX2-5* (OMIM gene 600584) (10)
- *MYH7* (OMIM gene 160760) (10)
- *TPM1* (OMIM gene 191010) (13)

In humans, mutations in the *NKX2-5* gene provided the first evidence that genetic factors are etiologically crucial in non-syndromic congenital heart diseases (14). The most common phenotypes are secundum atrial septal defect and atrioventricular conduction disturbance, but cases of EA are also reported (15, 16).

MYH7 mutations are predominantly found in EA associated with left ventricular non-compaction cardiomyopathy (LVNC) (17); a convincing hypothesis regarding this association is that LVNC is the result of altered cell regulation during wall formation (18) and EA may be caused by an arrest in directional growth during this process (19, 20).

TPM1 encodes α-tropomyosin, which is involved in myocardial contraction, as well as in stabilization of non-muscle cytoskeletal actin filaments. Nijak et al. reported an LVNC-EA family with a *TPM1* mutation, and established an association between EA and *TPM1*-related LVNC (21).

Pathogenic variants may include missense and nonsense mutations and small 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 2 accredited medical genetic laboratories in the EU, and in the GTR database, offered by 11 accredited medical genetic laboratories in the US.

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

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_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%.

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: variations in the aforementioned genes are linked to EA, but may be individual variations (identified in one or a few families) and total epidemiological data is therefore not available.

Clinical sensitivity will be estimated based on internal cases. (22)

Clinical specificity: data not available.

Prescription appropriateness

The genetic test is appropriate when:

a) the patient meets the diagnostic criteria for EA;

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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