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TECHNICAL REPORT

EBTNA UTILITY GENE TEST

Genetic testing for tetralogy of Fallot

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Abstract

Tetralogy of Fallot (ToF) combines congenital cardiac defects including ventricular septal defect, pulmonary stenosis, an overriding aorta and right ventricular hypertrophy. Clinical manifestation of this defect depends on the direction and volume of shunting of blood through the ventricular septal defect and the associated right ventricular and pulmonary artery pressures. ToF accounts for 3-5% of congenital heart defects or 0.28 cases every 1000 live births. ToF has autosomal dominant inheritance. 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.

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Tetralogy of Fallot

(Other synonyms: Fallot's syndrome, Fallot's tetralogy)

General information about the disease

Tetralogy of Fallot (ToF) is combination of congenital heart defects including ventricular septal defect, an over-riding of the aorta, right ventricular outflow obstruction due to pulmonary stenosis, and right ventricular hypertrophy (1). Embryologically, ToF is the direct result of antero-cephalad deviation of the developing ventricular septal outlet, or its fibrous remnant should this septum fail to muscularise (2). The combination of this deviation with abnormal morphology of the septoparietal trabeculations that encircle the subpulmonary outflow tract produce the right ventricular outflow obstruction typical of ToF (3,4).

A classification of ToF is based on possible combinations of infundibular stenosis of varying severity and a ventricular defect of varying size. Clinical manifestation of this defect depends on the direction and volume of shunting of blood through the ventricular septal defect and the associated right ventricular and pulmonary artery pressures (5).

ToF accounts for 3-5% of all congenital heart defects, or 0.28 cases every 1000 live births. Males and females are affected equally (6). The risk of recurrence in siblings is about 3% if there are no other affected first-degree relatives (7).

Recent advances in diagnostics make it possible to detect ToF prenatally, but where diagnostic tools are not available, infants with ToF may have severe cyanosis, recurrent hypercyanotic spells, squatting, and other consequences of severely reduced pulmonary blood flow in the first weeks and months of life.

Early diagnosis helps plan perinatal management and enables early prostaglandin therapy to maintain ductal patency, thus avoiding life-threatening cyanosis in the early newborn period (7).Diagnosis is based on clinical assessment to identify symptoms,

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echocardiogram, electrocardiogram, chest radiogram, pulse oximetry, echocardiography (septal, colour Doppler and two-dimensional), spin-echo MR imaging, CT, diagnostic catheterization and genetic testing.Differential diagnosis should consider ToF caused by chromosomal disorders.

ToF has autosomal dominant inheritance.

Autosomal dominant non-syndromic ToF (OMIM disease 187500)

- *NKX2*-5 (OMIM gene 600584)
- *GATA4* (OMIM gene 600576)
- *GATA5* (OMIM gene 611496)
- *GATA6* (OMIM gene 601656)
- *ZFPM2* (OMIM gene 603693)
- *GDF1* (OMIM gene 602880)
- *TBX1* (OMIM gene 602054)
- *GJA5* (OMIM gene 121013)

Autosomal dominant syndromic ToF

- Alagille syndrome 1 (ALGS1, OMIM disease 118450) - *JAG1* (OMIM gene 601920)
- DiGeorge syndrome (DGS, OMIM disease 188400) - *TBX1* (OMIM gene 602054)

Other likely genes

- *FOXH1* (OMIM gene 603621) (8), *TBX20* (OMIM gene 606061) (9), *TBX2* (OMIM gene 600747), *TBX5* (OMIM gene 601620) (10), *NKX2-6* (OMIM gene 611770), *HAND2* (OMIM gene 602407) (11).

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

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

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 *GATA4* and *TBX1*.

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:

- 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.

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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%.

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: variations in the aforementioned genes are linked to ToF, 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.

Clinical specificity: is estimated at approximately 99% (12).

Prescription appropriateness

The genetic test is appropriate when:

a) the patient meets the diagnostic criteria for ToF;

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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