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TECHNICAL REPORT
EBTNA UTILITY GENE TEST

Genetic testing for atrioventricular septal defect

Yeltay Rakhmanov[1], Paolo Enrico Maltese[1*], Stefano Paolacci[2], Francesca Fanelli[2], Tommaso Beccari[3], Munis Dundar[4] and Matteo Bertelli[1,2]

Abstract

Atrioventricular septal defect (AVSD) is a congenital heart defect characterized by a shared atrioventricular junction coexisting with deficient atrioventricular septation. The main morphological characteristic of AVSD is a common atrioventricular canal. The prevalence of AVSD is estimated at 0.31/1000 live births and is higher among subjects with *PTPN11* mutations. ASD may have autosomal dominant or autosomal recessive inheritance. This Utility Gene Test was prepared 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: Atrioventricular septal defect, pulmonary vascular function heart malformations heart failure, EBTNA UTILITY GENE TEST

[1]MAGI's Lab, Rovereto, Italy

[2]MAGI Euregio, Bolzano, Italy

[3]Department of Pharmaceutical Sciences, University of Perugia, Perugia, Italy

[4]Department of Medical Genetics, Erciyes University Medical School, Kayseri, Turkey

*Corresponding author: P. E. Maltese
E-mail: paolo.maltese@assomagi.org

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Atrioventricular septal defect

(Other synonyms: Atrioventricular canal defect, endocardial cushion defect)

General information about the disease

Atrioventricular septal defect (AVSD) is a group of congenital heart malformations characterized by common atrioventricular junction coexisting with deficient atrioventricular septation. If there are separate atrioventricular valve orifices, despite the common junction, it is called *ostium primium* atrial septal defect. In complete AVSD the valve itself is also shared. The main morphological feature of AVSD is therefore a common atrioventricular junction (1). The defect is classified as complete if a single atrioventricular valve and an atrial septal defect (*ostium primum*) coexist with a posterior ventricular septal defect. The defect is classified as partial if there are two separate right and left atrioventricular valves with a cleft mitral valve and an atrial septal but not ventricular defect (2-7). The clinical features depend on the morphology of the defect. In babies with complete AVSD and a considerable interventricular defect, heart failure may occur in the first few months due to decompensation of pulmonary vascular function. If regurgitation is intense with a common atrioventricular valve, heart failure may occur sooner. Patients with AVSD may have the following clinical symptoms: mild cyanosis, congestive heart failure, right ventricular impulse, increased pulmonic component of second heart sound, variable ejection systolic murmur, apical mid-diastolic murmur (in large left to right shunt), pansystolic murmur (with atrioventricular valve regurgitation) (1, 8).

Prevalence of AVSD is estimated at 0.31/1000 live births (9). Digilio et al. reported a higher prevalence in subjects with *PTPN11* mutations, but this association was not significant, possibly due to low statistical power (10).

The diagnostic work-up should include clinical assessment to identify symptoms, echocardiogram, electrocardiogram, chest radiogram, spin-echo MR imaging, CT, diagnostic catheterisation and genetic testing.

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

Inheritance of AVSD is autosomal dominant.

Non-syndromic AVSD

- AVSD1 (OMIM disease 606215) - susceptibility locus mapped to chromosome 1p31-p21
- AVSD3 (OMIM disease 600309) - *GJA1* (OMIM gene 121014);
- AVSD4 (OMIM disease 614430) - *GATA4* (OMIM gene 600576);
- AVSD5 (OMIM disease 614474) - *GATA6* (OMIM gene 601656);
- Congenital heart defects, multiple types, 4 (CHTD4, OMIM disease 615779) - *NR2F2* (OMIM gene 107773).

Syndromic AVSD

- AVSD2, partial, with heterotaxy syndrome (OMIM disease 606217) - *CRELD1* (OMIM gene 607170);
- Noonan Syndrome 1 (NS1, OMIM disease 163950) - *PTPN11* (OMIM gene 176876).

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

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 10 accredited medical genetic laboratories in the EU, and in the GTR database, offered by 9 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

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 region 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 *CRELD1 and GATA4* genes.

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 genes and diseases.

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 AVSD, but may be individual variations (identified in one or few families) and total epidemiological data is therefore not available. Clinical sensitivity will be estimated on the basis of internal cases (11).

Clinical specificity: data not available

Prescription appropriateness

The genetic test is appropriate when:

a) the patient meets the diagnostic criteria for AVSD;

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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