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REVIEW

EBTNA UTILITY GENE TEST

From vascular biology to vascular medicine

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Abstract

Cardiovascular disorders include various conditions characterized by morphological and functional defects of the heart and vascular system. Molecular biology techniques (in particular DNA sequencing) have recently offered new insights into the etiology of cardiovascular defects, revealing their association with germline as well as somatic mutations.

Genetic tests are evaluated on the basis of their analytical and clinical validity, clinical utility, and ethical, legal and social implications. Next generation sequencing is so far the best approach for molecular diagnosis of congenital heart defects and vascular anomalies, the genetic and phenotypic heterogeneity of which makes them difficult to diagnose. Understanding the molecular causes of congenital heart defects and vascular anomalies has permitted clinical trials of drugs targeting affected genes and pathways.

The articles in this Special Issue aim to provide guidance for those concerned with diagnosis and research in the field of cardiovascular defects. The approach to genetic testing is discussed.

Keywords: Next generation sequencing, cardiovascular disorders, congenital heart defects, vascular anomalies, genetic testing, EBTNA UTILITY GENE TEST

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Introduction

Cardiovascular defects encompass various disorders characterized by morphological defects of the heart and vascular system (veins, arteries, capillaries and lymphatic vessels). This extremely large and heterogeneous group of disorders is studied by cardiologists, angiologists and cardiovascular surgeons at macroscopic level, and by pathologists at microscopic level. Molecular biology has recently offered new insights into the etiology of cardiovascular defects. In particular, molecular biology techniques have revealed that many cardiovascular defects are not only associated with germline mutations (affecting all cells of the body and potentially transmissible to offspring) but also with somatic mutations (specific to affected tissue and not transmissible to offspring) (1, 2). In some cases, a somatic mutation in affected tissue and a germline variant are necessary for the defect to manifest (second-hit mechanism) (3, 4).

Molecular biology has enabled researchers to classify cardiovascular defects on the basis of their molecular etiology. Understanding the molecular causes of these disorders has permitted clinical trials of drugs targeting affected genes or pathways. In general, potentially therapeutic molecules are first tested *in vitro*, then in animal models, and finally in human subjects (5).

New technologies such as next generation sequencing (NGS) make it possible to sequence many genes in a single operation. This article aims to provide guidance for anyone concerned with diagnosis and research of cardiovascular defects, and discusses a correct approach to genetic testing.

Genetic cardiovascular defects can be classified as follows:

- Congenital heart defects (CHDs)
  - atrial septal defect
  - ventricular septal defect
  - atrioventricular septal defect
  - Ebstein anomaly
  - pulmonary stenosis
  - aortic valve stenosis
  - bicuspid aortic valve
  - tetralogy of Fallot

- Vascular anomalies (VAs)
  - coarctation of aorta
  - arteriovenous malformations
  - capillary malformations
  - hemangioma
  - large-caliber vessel aneurysms
  - lymphatic malformations
  - Mendelian stroke (hemorrhagic or ischemic)
  - cerebral cavernous malformations

- Syndromic heart and/or vascular malformations
  - RASopathies
  - Marfan and Marfan-like syndromes
  - vascular Ehlers-Danlos syndrome
  - lymphedema-distichiasis syndrome
  - Hennekam syndrome
  - Emberger syndrome

Genetic cardiovascular malformations have a genetic component that can be identified by appropriate genetic tests. The variety of genetic tests has increased over the years. Ways of evaluating genetic tests have recently been developed.

Genetic tests are evaluated on the basis of their analytical validity, clinical validity, clinical utility, and ethical, legal and social implications. The evaluation model process is known as ACCE (analytical validity, clinical validity, clinical utility, ethical, legal and social implications) and includes collecting, evaluating, interpreting and reporting data on DNA testing for disorders with a genetic component (6). Analytical validity is the accuracy with which a particular genetic characteristic is identified in a given laboratory test. Clinical validity is the accuracy with which a genetic test identifies clinical status. It is assessed on the basis of the criteria used to select subjects to be tested, possible clinical outcomes, and the comparability of cases and controls (7, 8). Clinical utility refers to the risks and benefits resulting from test use. It is evaluated on the basis of whether it reduces the morbidity or mortality of persons tested, provides information relevant to their health, and assists in reproductive decision-making (9).

Ethical, legal, social and psychosocial implications for affected individuals, their families, and the population are also included in the risk/benefit balance of genetic testing. The primary aim of genetic testing should be reduction of morbidity, mortality and disability of patients (10).

Guidelines regarding the clinical utility of genetic testing for some of the above disorders can be found in GeneReviews (11-21). GeneReviews is an international point-of-care resource for clinicians. It provides clinically relevant and medically actionable information for inherited conditions in a standardized journal-style format, covering diagnosis, management, and genetic counseling for patients and their families. Each chapter in GeneReviews is written by one or more experts on the specific condition or disease and undergoes rigorous editing and peer review before being published online. GeneReviews contains chapters focused on a single gene or phenotype (~95%) and overviews summarizing genetic causes of common conditions (such as deafness and hearing loss, Alzheimer disease) (~5%). Each GeneReviews chapter is updated every two to four years by the author(s) in a formal and comprehensive process curated by the GeneReviews editors. Additional revisions may occur more frequently, as needed, to reflect significant changes in clinically relevant information (22).

Genetic counseling

The likelihood that a genetic test be informative depends on the information exchanged during genetic counseling, which should include molecular biology, mode of inheritance, recurrence risk, genetic testing, and research initiatives (23). Counselors should explain the utility of the genetic test: advantages, disadvantages and risk/benefit ratio. Considering patients' perceptions of genetic diseases and services during genetic counseling facilitates their understanding of the information provided and satisfaction with the counseling experience (24). Studies on the perception of genetics among adults in the general population found that although they lacked genetic knowledge, they recognized the potential benefits and limitations of genetic testing (25). Benefits of testing include increasing control over one's life (26), preventing disease (26) and obtaining information for future generations (24, 27). Limitations of testing include emotional distress about results, fear of discrimination, test credibility, treatment expense, and confidentiality breaches (28). Older adults wanted professional support when sharing results and indicated they would disclose results to other potentially affected family members only if there were a possible treatment for a certain disease (24). They were concerned that communicating results to family members might cause psychological distress or actual physical illness (24, 27). Genetic information can be retrieved from different databases (Online Mendelian Inheritance in Man (OMIM, https://www.omim.org/), Genetic Testing Registry (GTR, https://www.ncbi.nlm.nih.gov/gtr/) and/or Orphanet (http://www.orpha.net/consor/cgi-bin/index.php?lng=IT) by counselors and provided to patients during a counseling session. The genetic test should take place only after obtaining informed consent and information about clinical features, other tests performed, and pedigree. Counseling is necessary before and after genetic testing (29). Finally, patients should be informed about clinical trials and therapies (if any), the risk of recurrence and the possibility of testing other family members (29).

Techniques for identifying the molecular basis of cardiac and vascular anomalies

The quality and utility of genetic tests depend on their reliability, validity, sensitivity, specificity and on their positive and negative predictive value (30). Chromosomes and genes are analyzed for the diagnosis of cardiac and vascular anomalies. Cytogenetic tests, such as karyotyping and array CGH, give geneticists information about chromosome number and morphology and about the possible presence of large genomic rearrangements (duplications, deletions, insertions and translocations). Since next generation sequencing (NGS) technologies enable simultaneous analysis of many genes from several patients, genetic screening based on NGS has enhanced diagnostic sensitivity (31, 32). It is recommended, for example, for diseases involving vascular and cardiac anomalies that have different modes of inheritance, variable penetrance, variable expressivity and genetic as well as phenotypic heterogeneity (31, 32).

When patients do not have a clear diagnosis or when the sequencing of all known associated genes gives negative results, a second possibility is to sequence the whole exome (33, 34).

Impact of genetic testing on clinical practice

Molecular genetic testing is particularly important in patients with CHDs or VAs, disorders characterized by extreme genetic and phenotypic heterogeneity and not always inherited from an affected subject. Since they are often sporadic (due to *de novo* germline or somatic mutations), it may be difficult to reach a definite diagnosis and to assess the risk for progeny from clinical examination alone. For example, hereditary hemorrhagic telangiectasia (HHT) can be caused by mutations in *SMAD4*. Mutations in this gene are usually associated with juvenile polyposis (JP) and HHT (35), but patients with *SMAD4* mutations and only one of the two manifestations have been reported (36). In addition, HHT has features that overlap with other disorders, such as ataxia-telangiectasia caused by mutations in *ATM*, capillary malformation-arteriovenous malformation caused by mutations in *RASA1*, hereditary benign telangiectasia with unknown genetic causes and pulmonary arteriovenous malformations, in which patients often show HHT (18).

Genetic tests also have prognostic value for patients and their relatives. For example, patients can present with arteriovenous malformations affecting different parts of the body. These malformations may also be caused by mutations in *PTEN*, which are associated with a higher risk of developing colon cancer (32). These patients can therefore be monitored to detect any cancer cells as early as possible (32) and relatives can be screened for undiagnosed disease.

An accurate molecular diagnosis can be important for pharmacological therapies. Sirolimus (an mTOR inhibitor) can be used in patients with vascular anomalies and PI3K/AKT/mTor impairment, refractory to standard care (ClinicalTrials.gov Identifier: NCT02638389). Patients can be recruited for a phase III multicentric trial on the efficacy and safety of Sirolimus. Thalidomide is another drug scheduled for trials in patients with recurrent small intestinal bleeding due to gastrointestinal vascular malformations (ClinicalTrials.gov Identifier: NCT02707484). Although this drug was reviled in the 1960s for its teratogenic effects, it has been reassessed for treatment of cancer and leprosy (37, 38). Insights into its mechanism of action have also revealed its utility for treating vascular disease (37, 38). Since thalidomide bypasses the TGF-beta pathway, it may be used in patients with mutations in genes involved in that pathway (37, 38).

Conclusions

New technologies, such as next generation sequencing, have allowed researchers and clinicians to understand the molecular basis of many disorders involving vascular and cardiac anomalies (1, 2). This knowledge is fundamental for correctly and fully informing patients about their illness (including information such as type of inheritance and risk of recurrence) and for correct follow-up (29). If the etiology of the disease is known, such as the impairment of a specific molecular pathway, patients can be enrolled in clinical trials that test drugs specifically targeting that pathway (29).

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