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Appropriate Use of Genetic Testing in Congenital Heart Disease Patients

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Abstract
Purpose of Review Congenital heart disease (CHD) remains the most common birth defect, occurring in 1% of all births. Although the exact etiology of CHD is still largely unknown, it is thought to be an interaction of genetic and non-genetic factors. The purposes of this review are to summarize recent advances in CHD genetics and testing and to present a suggested algorithm for appropriate use of genetic testing in patients with CHD.
Recent Findings Advances in genetic testing technology are rapidly expanding the options for screening and are providing further insights into the genetic and molecular background of non-syndromic CHD. As the field advances, the role of the geneticist and genetic counselor will continue to expand as the testing becomes more complex and interpretation of results becomes increasingly challenging.
Summary Coordination of practice between cardiologists and geneticists using a shared clinical structure is essential and will help improve cost utilization and facilitate individualized patient care.

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Introduction

Congenital heart disease (CHD) remains the most common birth defect, with an estimated incidence of approximately 1% of all births [1]. With advances in medical care, overall survival to adulthood in the current era is estimated to exceed 90% [2]. As a result, the population of adults with congenital heart disease (ACHD) is growing rapidly, changing the landscape of CHD care in various aspects. Many patients in this population are reaching reproductive age and are seeking genetic counseling to better understand the etiology of their underlying CHD and to understand the risks to future offspring.

Although exact etiology of CHD is still largely unknown, it is thought to be an interaction of genetic and non-genetic factors. Non-genetic and potentially modifiable known risk factors include parental conditions and environmental exposure including therapeutic or non-therapeutic drugs during periconceptional period and first trimester [3]. Although a number of genetic differences can lead to CHD, one of the potential mechanisms is thought to be due to combinations of multiple gene involvement (the so-called "polygenic model"). The heterogeneous nature of CHD is, in part, a result of incomplete penetrance and variable expressivity of genes as well as epigenetic factors and gene modifiers, contributing to more complex patterns of inheritance of CHD. Therefore, even when specific genes or a group of genetic factors are the cause, it may not be immediately recognized.

Recent advances in genetic testing technology are rapidly expanding the options for screening and are giving further insights into the genetic and molecular background of non-syndromic CHD. In addition, there are some guidelines for

screening patients with CHD for underlying genetic abnormalities [4–6]. While these guidelines provide useful information about screening recommendations, further research is needed on phenotypic expression, clinical outcomes, and long-term survival. Recognizing this need, the National Heart, Lung, and Blood Institute funds the Congenital Heart Genomic Network Consortium to investigate relationships between genetic factors, clinical features, and outcomes in CHD [7]. The first report from this consortium described de novo gene mutations in histone-modifying genes in sporadic cases of severe CHD by studying trios (patient and both parents). These results further implicate hundreds of genes that collectively contribute to 10% of sporadic cases of severe CHD [8•]. Additional research is needed to study underlying mechanisms and to identify additional genetic causes. Despite this rapid advancement in knowledge of genetic causes, there are no recent practice guidelines available for practitioners to optimally guide their approach to genetic testing in patients with CHD. The purposes of this review are to summarize recent advances in CHD genetics and present a suggested algorithm for appropriate use of genetic testing in patients with CHD.

Role of Genetics in Congenital Heart Disease

Genetic causes of CHD can be classified into several broad categories: (1) chromosomal aneuploidy, (2) large chromosomal deletions or duplications, (3) single gene mutation, and (4) copy number variation. However, only a small percentage of CHD cases have an established etiology, characterized by either genetic abnormalities or environmental factors. Instead, most of sporadic cases of CHD have an unknown etiology. In these cases, environmental factors in addition to a polygenic model, in which combinations of multiple genes altering susceptibility to CHD lesions, are thought to contribute to causality.

Many of causative genetic abnormalities occur de novo, and inherited forms are relatively rare. Genetics of CHD is made more complex due to the impact of incomplete penetrance (not everyone with a genetic change expresses the same phenotype) and variable expressivity (a genetic change can have variable phenotypes along a continuum of disease). Moreover, a specific genetic abnormality can present with variable phenotypes (phenotypic heterogeneity), and patients with same CHD phenotype can have different genetic abnormalities (locus heterogeneity). Approximately 20–30% of CHD are "syndromic" and have non-cardiac involvement (see Table 1), while 70–80% of cardiac anomalies are "non-syndromic or isolated" without clear non-cardiac involvement [9–11]. It is likely, however, to be more of a "spectrum," as there are more genetic abnormalities identified for clinical phenotypes (see Fig. 1).

Table 1 Syndromes associated with CHD [4, 23–26]

Syndrome	Chromosomal or gene abnormality	Incidence of disease	Type of CHD typically associated	Incidence of CHD
Aneuploidies				
Down syndrome	Trisomy 21	1/700	AV septal defect, VSD, PDA, TOF	40–50%
Edward syndrome	Trisomy 18	1/5000	Septal defects (specifically VSD), PDA, polyvalvular disease	90–100%
Patau syndrome	Trisomy 13	1/16,000	ASD, VSD, PDA	80%
Turner syndrome	45, X	1/2500	Coarctation of the aorta, bicuspid aortic valve, partial anomalous pulmonary venous return, coronary anomalies	25–35%
Kleinfelter syndrome	47, XXY	1/500–1/1000	PDA, ASD	50%
Microdeletion syndromes				
22q11.2 deletion syndrome		1/4000–1/7000	Conotruncal malformations (Tetralogy of Fallot, interrupted aortic arch, VSD, truncus arteriosus)	75%
Williams syndrome	7q11.23 deletion	1/7500	Supravalvar aortic stenosis, peripheral pulmonic stenosis	53–85%
Smith-Magenis sydrome	17p11.2 deletion	1/15,000	ASD, VSD, mitral valve prolapse, pulmonary stenosis, pulmonary atresia	<45%
Single gene disorders				
Alagille syndrome	JAG1, NOTCH2	1/30,000–1/50,000	Peripheral pulmonary artery stenosis	85–94%
Ellis-van Creveld syndrome	EVC, EVC2	1/60,000–1/200,000	ASD, single atrium	70%
Holt-Oram syndrome	TBX5	1/100,000	Septal defects and/or conduction defects	75%
RASopathies (Noonan syndrome and related disorders)	BRAF, CBL, HRAS, KRAS, MAP2K1, MAP2K2, NRAS, PTPN11, RAF1, RIT1, SHOC2, SOS1, SOS2	1/3500	Pulmonary valve stenosis, hypertrophic cardiomyopathy	50–80%
CHARGE syndrome	CHD7	1/8500–1/10,000	Conotruncal malformations, AV septal defect, aortic arch anomalies	75–85%

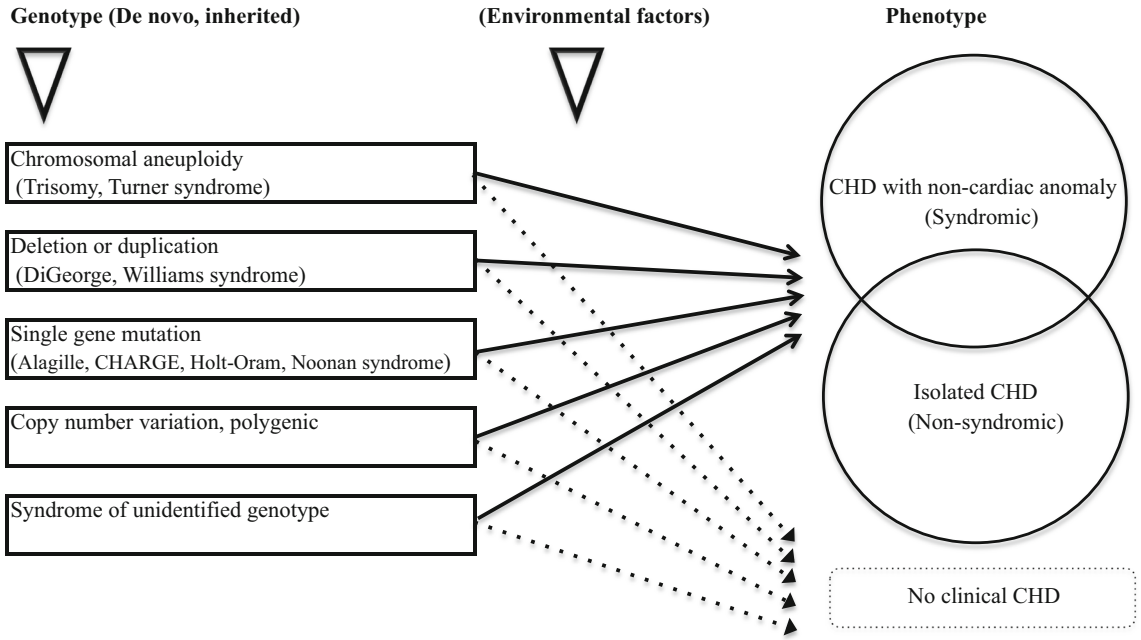

Fig. 1 Conceptual diagram showing causes of CHD based on genotype and phenotype. *Arrows with solid line* clinical CHD. *Arrows with broken line* no clinical CHD. *CHD* congenital heart disease

Future advances in genetic testing will likely reveal previously unrecognized abnormalities, which may manifest later in life.

Available Genetic Testing

Genetic testing can be divided into three main types: (1) identification of chromosomal abnormalities by karyotype looking for the presence or absence of entire chromosomes (i.e., Down syndrome), large-size translocations (chromosomal material not in the correct place) and large deletions or duplications; (2) identification of subtle chromosomal aneuploidy by fluorescent in situ hybridization (FISH) or cytogenic microarray (CMA), which identifies microdeletions or duplications (small pieces missing or extra) that are not visible on karyotype (e.g., 22q11.2 deletion syndrome or William syndrome), also referred to as copy number variations or CNVs; and (3) single gene testing by single gene sequencing or by using "next generation sequencing" techniques, including whole exome sequencing (WES, which examines the coding region of known genes) or whole genome sequencing (WGS, which sequences nearly all the genome), FBN1 mutations in Marfan syndrome being an example of the latter. Clearly, advances in genetic testing technology have expanded options greatly and are progressing with improved speed and resolution. Selection of the best test can be complicated, and choosing incorrectly can result in not identifying the patient's underlying disorder in addition to wasting time and resources.

The higher resolution tests such as CMA, WGS, and WES can detect subtle abnormalities including population or family single nucleotide polymorphisms (SNPs) and repetitions or small losses of genetic material (CNVs). These findings are not always pathogenic, but may be familial; therefore, appropriate interpretation is important. Moreover, adequate understanding of the potential results from any of these tests is important for interpretation.

Abnormalities have been detected by all these methods in individuals with CHDs. There is an increased frequency of CNVs in patients with CHD [12]. Use of WES or WGS is revealing more genetic variability in CHD patients. In some cases, these variations have identified a known diagnostic genetic change; and in other cases, they have provided insights about the significance of certain genetic variations and have accelerated identification of novel pathologic gene mutations. With development of "next generation sequencing," many more CHD causative gene mutations have been identified; and this technique is providing increasing discernment of CHD molecular mechanisms and association with other non-cardiac comorbidities [13]. This growing comprehension correlates with the reports by Homsy and colleagues that patients with CHD, neurodevelopmental disabilities, and extra-cardiac congenital anomalies had a higher percentage of de novo mutations, which damage their proteins that are highly expressed in developing heart and brain. Additionally, mutations in the same genes were shared at high rates

between patients with neurodevelopmental disabilities and CHD [14••].

In the past, due to limited technology, the study of genetic causes of CHD focused on syndromic CHD. More recent research has shifted the focus from identifying single genes and chromosomal differences in syndromic CHD to the discovery of genes in non-syndromic CHD, especially those with an apparent genetic cause. Several of these more recent discoveries have identified novel regulatory genes, genes for sarcomeric proteins, and membrane receptor genes. An example of regulatory gene causes includes mutations in NKX2-5, which encodes a transcription factor and is associated with septal defects, conotruncal anomaly, and hypoplastic left heart syndrome. GATA4 and TBX20 are other known genes encoding transcription factors associated with septal defects.

Because the possible underlying genetic causes are numerous, each testing method has strengths and weaknesses, and the field is progressing rapidly. Prior to considering genetic testing, a discussion with a professional who is aware of the strengths and pitfalls is an absolute necessity. Clinical geneticists and genetic counselors have expertise in this testing and can help determine the most appropriate testing through their specific training in gathering a family history and assessing the patient phenotype. They have received specialized training and are specifically skilled in discussing the possible implications and consequences of genetic testing results (both positive and negative) for the patient and their entire family. They can assist in choosing the most appropriate testing method and help to interpret the sometimes complex results.

In the absence of practice guidelines on choosing the types of genetic testing in CHD, both underutilization and overutilization occurs [15, 16]. Tests should be appropriately chosen based upon their differing strengths and weaknesses, as well as respecting their financial impact. Clearly, a multidisciplinary approach in collaboration with the genetics team will provide the best patient care when genetic testing is being considered. Local genetic professionals can be identified with contact information using the American College of Medical Genetics website (www.acmg.net) or the National Society for Genetic Counselors (www.nsgc.org). ESM 1: Appendix Table 1 has a list of additional resources available concerning testing and genetic disorders. ESM 1: Appendix Table 2 lists the testing currently available and a brief description of their strengths and weaknesses. ESM 1: Appendix Table 3 lists a comprehensive, but not complete list of genetic changes that have been described in CHD.

The Value of Genetic Testing

Establishing the diagnosis with genetic testing can help direct the screening of both cardiac and non-cardiac complications, as well as guide prognosis. Specific gene mutations or changes have the potential to dictate the best therapeutic option for patients, and such examples are expected to increase in the future. In general, patients with syndromes have poorer long-term survival compared with patients without these syndromes [17].

Determining the genetic cause of a specific individual's CHD is important from the psychosocial perspective, as evaluation of other family members may be indicated and identification of the genetic cause could potentially offer earlier diagnosis and treatment. A recent single-center study, which screened first-degree relatives of hypoplastic left heart syndrome probands, showed 11% of these previously unsuspected relatives had a cardiovascular malformation [18].

Furthermore, the use of genetic testing is expected to increase in the ACHD population, with increasing numbers of patients entering reproductive age. The recurrence risk in offspring who have an affected mother or father is approximately 3–8%, with a higher rate in maternal CHD [19–21]. The risk of CHD in siblings of an affected individual, when neither parent is affected, is approximately 1–6% and higher if multiple siblings are affected [11]. In the past, we were unable to identify all the potential genetic causes for CHD that are now understood. Thus, there may be benefit from genetic evaluation and subsequent testing in patients who had negative genetic testing as a child, as the techniques and knowledge base have significantly advanced over the last two decades. As technology advances, so does the ability to test for a broader variety of genetic mutation types with improved sensitivity. Genetic testing can help to estimate recurrence pattern and recurrence risk in future offspring, and this is expected to continue to improve.

Suggested Approach to Genetic Evaluation in Patients with CHD

Several guidelines have been published suggesting which patients should be screened for underlying genetic defects. In 2007, the American Heart Association (AHA) published a scientific statement recommending cytogenetic testing in CHD in (1) patients with recognizable chromosomal syndromes; (2) patients with dysmorphic features, growth retardation, developmental delay/intellectual disability, or multiple congenital anomalies; (3) patients with parents who had multiple miscarriages and/or siblings with birth defects; or (4) patients with major cardiac or visceral organ malformations by prenatal ultrasound and/or fetal echocardiography [4]. This statement also provided algorithms for providers to assess for non-cardiac abnormalities based on the initial cardiac defects. In 2010, the American College of Medical Genetics and Genomics published practice guidelines on the use of postnatal CMA testing. Testing is recommended in individuals with multiple congenital anomalies, whether or not a well-

delineated syndrome is present, those with apparently non-syndromic developmental delay or intellectual disability or those with autism spectrum disorder [6]. More recently, the Heart Rhythm Society published a consensus statement on genetic evaluation of channelopathies and cardiomyopathies [5]. In addition, genetic evaluation should be offered in patients with CHD lesions associated with high prevalence of 22q11 deletion syndrome and/or DiGeorge syndrome, including Tetralogy of Fallot, interrupted aortic arch, truncus arteriosus, ventricular septal defect with aortic arch anomaly, isolated aortic arch anomaly, or discontinuous branch pulmonary arteries [4]. The guideline on management of adults with CHD recommended offering genetic counseling to all patients who have congenital syndromes [22].

Genetic testing can also be considered in the following cases:

1. Patients with family history of one or more CHD in first-degree relatives
2. Patients with growth delay, developmental delay, or learning disorders
3. Patients with left-sided obstructive lesions
4. Patients with CHD or having a first-degree relative with CHD who are of reproductive age and interested in genetic evaluation.

An algorithm for genetic evaluation and counseling in patients with isolated or non-syndromic CHD is presented in Fig. 2.

Conclusions and Future Directions

In summary, there have been many recent advances in understanding CHD genetics and the development of new tools for genetic testing. We present a suggested algorithm for the appropriate use of genetic testing and counseling in patients with CHD, including details of some initial testing modalities. Tools to centralize information on known genotype and phenotype associations in CHD such as Online Mendelian Inheritance of Man (OMIM) or GeneTests (genetests.org) are great resources and can help practitioners to determine indications for genetic evaluation and further care.

Certainly, developing practice guidelines for genetic screening can be particularly challenging in this field,

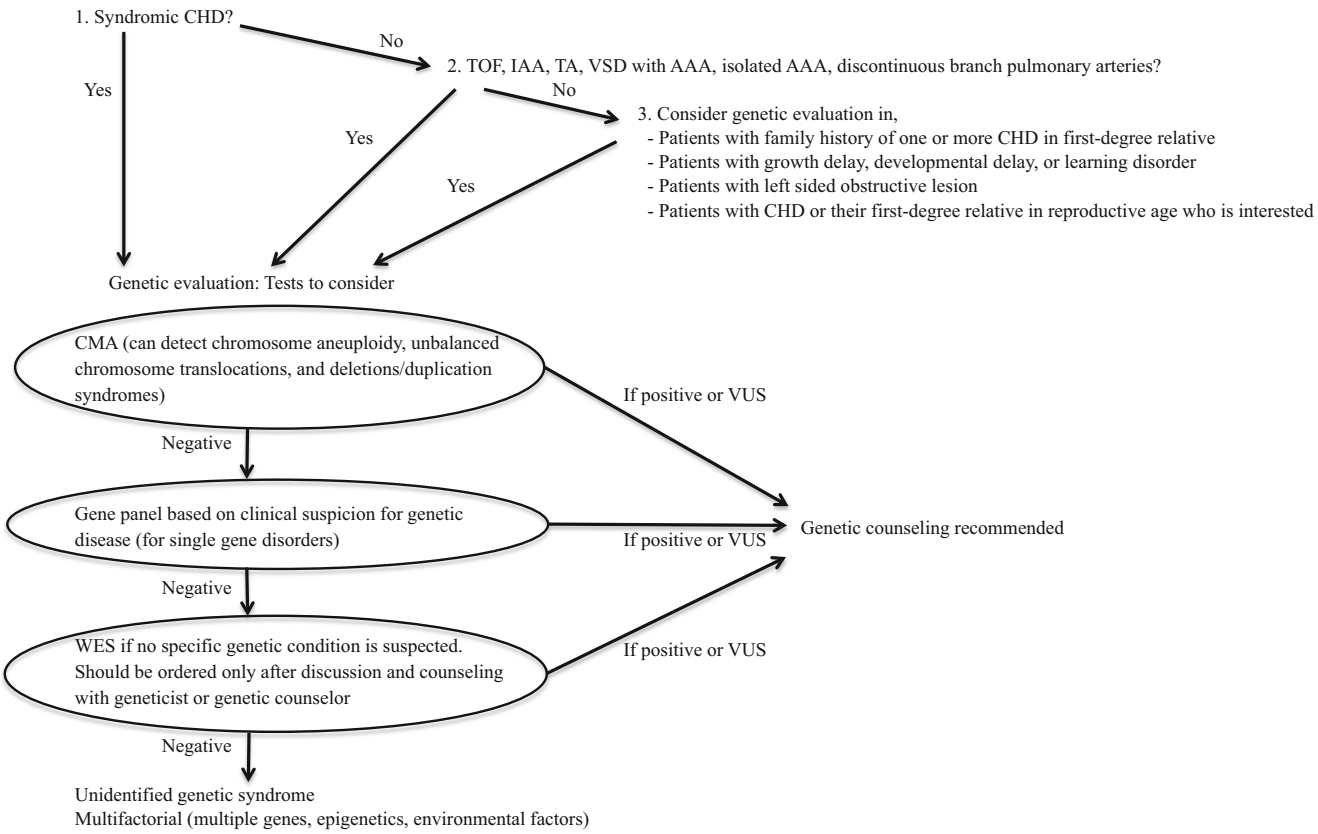

Fig. 2 Proposed algorithm of genetic evaluation and counseling for patients with Congenital heart disease (CHD). *AAA* aortic arch anomaly, *CMA* chromosome microarray, *IAA* interrupted aortic arch, *TA* truncus arteriosus, *TOF* Tetralogy of Fallot, *VSD* ventricular septal defect, *VUS* variant of uncertain significance, *WES* whole-exome sequencing

as new research findings are discovered almost daily. While there are a number of screening guidelines detailing which patients with CHD should be tested, consultation with a geneticist should be first undertaken to ensure a complete evaluation. The role of the geneticist and genetic counselor in CHD genetics will continue to expand as the testing becomes more complex, interpretation of results becomes increasingly challenging, and counseling on the implication of the testing on long-term outcomes is better understood. Coordination of practice between cardiologists and geneticists using a shared clinical structure will help prevent diagnostic odysseys, improve cost utilization, and facilitate individualized patient care. This coordination becomes even more important as CHD patients reach reproductive age and require further pre-conceptual genetic counseling. The growing ACHD population continues to change the landscape of care in CHD and provides key insights into the long-term outcomes of genetic defects in CHD.

Compliance with Ethical Standards

Conflict of Interest Seiji Ito, Kimberly A. Chapman, Monisha Kisling, and Anitha S. John declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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