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REVIEW

Genetic testing in congenital heart disease: A clinical approach

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Abstract

Congenital heart disease (CHD) is the most common type of birth defect. Traditionally, a polygenic model defined by the interaction of multiple genes and environmental factors was hypothesized to account for different forms of CHD. It is now understood that the contribution of genetics to CHD extends beyond a single unified paradigm. For example, monogenic models and chromosomal abnormalities have been associated with various syndromic and non-syndromic forms of CHD. In such instances, genetic investigation and testing may potentially play an important role in clinical care. A family tree with a detailed phenotypic description serves as the initial screening tool to identify potentially inherited defects and to guide further genetic investigation. The selection of a genetic test is contingent upon the particular diagnostic hypothesis generated by clinical examination. Genetic investigation in CHD may carry the potential to improve prognosis by yielding valuable information with regards to personalized medical care, confidence in the clinical diagnosis, and/or targeted patient follow-up. Moreover, genetic assessment may serve as a tool to predict recurrence risk, define the pattern of inheritance within a family, and evaluate the need for further family screening. In some circumstances, prenatal or preimplantation genetic screening could identify fetuses or embryos at high risk for CHD. Although genetics may appear to constitute a highly specialized sector of cardiology, basic knowledge regarding inheritance patterns, recurrence risks, and available screening and diagnostic tools, including their strengths and limitations, could assist the treating physician in providing sound counsel.

Key words: Congenital heart disease; Genetics; Genetic screening; Genetic testing

Core tip: Monogenic models and chromosomal abnormalities have been associated with syndromic and non-

syndromic forms of congenital heart disease (CHD), paving the way for genetic investigation and testing to shoulder an important role in patient management. Herein, we present an overview of the role of genetics in CHD, propose various clinical scenarios in which genetic testing may be appropriate, and discuss practical implications with regards to when and how to order genetic tests. Summary tables are provided regarding the various genes implicated in syndromic and non-syndromic forms of CHD and recurrence risks in siblings and offspring.

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INTRODUCTION

Congenital heart disease (CHD) afflicts 2 to 3 children per 100 live births[1,2]. It is the most common type of birth defect and encompasses a wide range of malformations. The spectrum of severity ranges from insignificant and even self-resolving lesions, such as ventricular septal defects that spontaneously close, to highly complex and multiorgan manifestations that are incompatible with natural survival. While much progress has been made regarding the management of children and adults with CHD, a greater understanding of underlying etiologies could potentially lead to further advances in preventive care and therapeutic strategies[3].

The complexity and heterogeneity of CHD has traditionally been attributed to multifactorial etiologies arising from interactions between multiple genes and environmental factors (so-called "polygenic model")[4]. Early investigations into environmental factors spawned recommendations for maternal multivitamin supplementation containing folic acid to reduce risks of developing CHD[5-7]. Other implicated maternal factors include pregestational diabetes, pollakiuria, febrile illnesses, rubeola, influenza, alcohol consummation, cigarette smoking, and teratogenic pharmacological agents such as thalidomide, warfarin, angiotensin converting enzyme inhibitors, and certain anticonvulsant and anti-inflammatory drugs[8].

Technological advances have permitted the confirmation of clinically suspected monogenetic subtypes of CHD, with dominant or recessive inheritance patterns. However, some forms of CHD could not be explained by a polygenic model[9], with much higher recurrence risks in first-degree relatives than predicted[3,10]. Chromosomal abnormalities have been associated with cardiac defects, particularly in the setting of syndromic phenotypes (*e.g.,* trisomy 21, DiGeorge, and Williams-Beuren syndromes). In so-called multiplex families with several affected members, identified candidate genes have been consistent with monogenetic models with Mendelian inheritance. Furthermore, the rate of CHD increases with consanguinity, as described in Arabic countries[11].

The fact that monogenic and chromosomal abnormality models account for a substantial proportion of CHD enhances the potential value of genetic investigation and testing[12]. Genetics carries the potential to unravel etiological mysteries that underpin CHD, provide pathophysiological insights, assist in risk assessment, inform clinical management, and counsel families regarding future offspring. The focus of this review is on the genetics of structural CHD, as opposed to other disease categories such as inherited channelopathies. We review known implicated genes and chromosomal abnormalities, discussed when and how to perform genetic testing, and shared our perspective regarding clinical applications.

GENETICS IN STRUCTURAL CONGENITAL HEART DISEASES

Approximately 30% of patients diagnosed with CHD have syndromic phenotypes with extracardiac manifestations. The influence of genetics is well established for chromosomal aneuploidies such as Down, Turner, and DiGeorge syndromes. Other syndromes are linked to a mutation or deletion in one gene, such as Noonan, Alagille, and Holt-Oram syndromes[3]. For the 70% of CHD cases that are non-syndromic, new genes with Mendelian inheritance (dominant or recessive) have been identified, particularly in families with several affected members. Table 1 summarizes current knowledge regarding genetic etiology for several forms of CHD with syndromic or non-syndromic phenotypes.

Genes etiologically linked to CHD directly impact embryologic development. For example, defects in genes responsible for the embryonic formation of the atrial septum (*e.g.,* MYH6, TBX20) can result in atrial septal defects (ASD)[13,14]. In addition to their function in embryologic cardiac development, implicated genes may also play a role in heart regulation throughout life[15]. The critical purpose of these genes, which are primarily transcription factors, explains the possibility of dominant heritability. A mutation that modifies the protein function in one of these genes may have a major effect on cardiac development and regulation. Furthermore, interactions between transcription factors explain the diverse consequences associated with individual mutations. For example, NKX2.5 mutations may result in ASD, atrioventricular block, ventricular septal defect (VSD), Ebstein anomaly, and tetrology of fallot (TOF). GATA4, a transcription factor, has been associated with ASD, VSD, and pulmonary stenosis. TBX1 has been implicated in TOF, patent ductus arteriosus, and interrupted aortic arch; and TBX20 in ASD, VSD, valve defects, and impaired chamber growth. In addition to these transcription factors, other genes with varied roles have been implicated in CHD, such as MYH6, which codes for an alpha myosin heavy chain (ASD) and Notch 1, which is implicated in valve formation (bicuspid aortic valve and aortic stenosis)[15,16].

Table 1 Genes described in syndromic and non-syndromic forms of congenital heart disease

Syndromic: Syndrome name Non syndromic: Gene implicated	Phenotype associated with structural heart disease	Syndromic: Chromosomal aneuploidy, microdeletion or gene/locus/inheritance Non syndromic: Locus/inheritance	Ref.
Atrioventricular septal defect (AVSD)			
Down syndrome[1]	MR, facial dysmorphia	Trisomy 21	[71-73]
Edward syndrome	IUGR, facial dysmorphia, clenched fingers	Trisomy 18	[74]
Patau syndrome	Cleft lip and palate, microphthalmia, polydactyly	Trisomy 13	[74]
Holt-Oram syndrome	Preaxial limb defects, absent or dysmorphic thumbs, cardiac conduction disease	TBX5/12q24.1/AD	[75]
Noonan syndrome	Hypertrophic cardiomyopathy, short stature, broad neck, unusual chest shape, facial dysmorphia, developmental delay	PTPN11/12q24/AD, *de novo*; SOS1/2p21/AD, *de novo*; KRAS /12p12.1/AD, *de novo*	[76]
Ellis-van Creveld syndrome	Common atrium, polydactyly, deformity of upper lip, dwarfism with narrow thorax, ASVD partial to complete	EVC and EVC2/4p16/AR	[77]
Locus 1p31-p21	AVSD partial to complete	Gene no yet found/AD	[78]
CRELD1	Partial AVSD, heterotaxy syndrome	3p25/AD	[79-81]
GATA4	Family with ASD, VSD and one member with AVSD	8p23.1/AD, *de novo*	[82]
Atrial septal defect (ASD)			
Holt Oram syndrome	See AVSD above	See above AVSD	[83,84]
Noonan syndrome	See AVSD above	See above AVSD	[85,86]
Ellis-van Creveld syndrome	See AVSD above	See above AVSD	[77]
Cardiofaciocutaneous syndrome	Hypertrophic cardiomyopathy, facial dysmorphia, skin abnormalities: keratosis pilaris, nevi	MAP2K1/15q22.31/AD, *de novo*; MAP2K2/19p13.3/AD, *de novo*; KRAS/7q34/AD, *de novo*; BRAF/12p12.1/AD, *de novo*	[87]
Cri du Chat	Sound of cry similar to cat's cry, facial dysmorphia, MR	CTNND2/5p15.2/*de novo*	[88]
NK2X-5	+/- Atrioventricular block	5q35.1/AD	[89]
GATA4	+/- Pulmonary stenosis	8p23.1/AD, *de novo*	[82,90]
MYH6		14q11.2/AD	[13]
TBX20		7p14.2/AD	[14,91]
Ventricular septal defect (VSD)			
Holt Oram syndrome	See AVSD above	See AVSD above	[92]
Ellis-van Creveld syndrome	See AVSD above	See AVSD above	[77]
Cri du chat	See AVSD above	See AVSD above	[88]
Down syndrome	See AVSD above	See AVSD above	[93,94]
Edward/Patau syndrome	See AVSD above	See AVSD above	[95,96]
DiGeorge syndrome	Facial dysmorphia, speech delay, learning delay, psychiatric disorder, cleft palate, immune deficiency, hypoplastic/aplastic thymus, hypocalcaemia.	Deletion 22q11.21/*de novo*, AD	[97]
NK2X-5	Atrioventricular block	5q35.1/AD	[89]
GATA4		8p23.1/AD, *de novo*	[82,98]
Ebstein anomaly			
Down syndrome	See AVSD above	See AVSD above	[99]
NKX2-5		5q35.1/AD	[100]
Pulmonary stenosis			
Noonan syndrome[1]	See ASVD above	See AVSD above	[101, 102]
Costello syndrome	Hypertrophic cardiomyopathy, MR, loose skin, facial dysmorphia large mouth	HRAS/11p15.5/AD	[103]
Leopard syndrome	Lentigines, short stature, hearing loss, similar to Noonan syndrome	PTPN11/12q24/AD, *de novo*; RAF1/3p25.2/AD, *de novo*; BRAF/7q34/AD, *de novo*	[104]
Alagille syndrome	Pulmonary branch stenosis, bile duct paucity, cholestasis, facial dysmorphia, deep-set eyes, butterfly vertebrae	JAG1/20p12/AD; NOTCH2/1p12/AD; Deletion/20p12/AD	[105, 106]
Cardiofaciocutaneous syndrome	See ASD above	See ASD above	[107]
GATA4	+/- Atrial septal defect	8p23.1/AD, *de novo*	[90, 108]
Aortic valve stenosis			
Turner syndrome[1]	Female, webbed neck, widely spaced nipples, short stature, streaked ovaries	Monosomy X or mosaics (45,X/46,XX)	[109]
Noonan syndrome	See above AVSD	See above AVSD	[76]
NOTCH1		9q34.3/AD	[16, 110]
SMAD6		15q22.31/?	[111]
Supravalvular aortic stenosis			
Williams-Beuren syndrome[1]	Elfin facies, cocktail personality, hypercalcaemia, developmental delay, thyroid disorder, renal and connective tissue abnormalities.	Deletion/7q11.23/*de novo*, AD	[112]
Aortic coarctation			
Turner syndrome[1]	See aortic valve stenosis above	See aortic valve stenosis above	[109]

Down/Edward/Patau syndrome	See AVSD above	See AVSD above	[113]
NOTCH1		9q34.3/AD	[110]
Bicuspid aortic valve			
Turner syndrome	See aortic valve stenosis above	See aortic valve stenosis above	[109]
Anderson syndrome	Long QT syndrome, ventricular arrhythmias, sudden cardiac death, facial dysmorphia, short stature	KCNJ2/17q24.3/AD	[114]
NOTCH1		9q34.3/AD	[16,110]
SMAD6		15q22.31/?	[111]
Tetralogy of Fallot			
DiGeorge syndrome[1]	See VSD above	See VSD above	[97]
Alagille syndrome	See pulmonary stenosis above	See pulmonary stenosis above	[105,106]
Cat-Eye syndrome	Dysmorphic ears, microphthalmia, anal atresia, renal abnormalities, coloboma, cleft palate	Duplication/22q11/*de novo*	[115]
NKX2.5		5q35.1/AD	[100]
GATA4		8p23.1/AD, *de novo*	[116]
NOTCH1		9q34.3/AD	[16]
FOG2		8q23.1/?	[117,118]
Truncus arteriosus			
DiGeorge syndrome	See VSD above	See VSD above	[97]
Hypoplastic left heart syndrome			
NKX2.5		5q35.1/AD	[119]
NOTCH1		9q34.3/AD	[16,110]

[1]The phenotype syndrome most commonly associated with the particular CHD. AVSD: Atrioventricular septal defect; ASD: Atrial septal defect; VSD: Ventricular septal defect; MR: Mental retardation; IUGR: Intrauterine growth retardation; AD: Autosomal dominant; AR: Autosomic recessive; CHD: Congenital heart disease.

WHEN AND HOW TO PERFORM A GENETIC INVESTIGATION?

When to consider genetic testing

The first clinical situation to consider genetic testing in CHD is the presence of a syndromic phenotype. A comprehensive clinical examination is paramount in recognzing extracardiac involvement. Common physical findings include facial dysmorphia (eye, ear, mouth, nose abnormalities), limb dysmorphia (atrophy, length reduction), hand and feet dysmorphia (polydactyly, short fingers, clinodactyly), and other skeletal abnormalities such as scoliosis[17]. Growth delays may be identified by monitoring height and weight and neurological status must be assessed to diagnose mental impairment and learning disabilities. Other organs must be screened to exclude associated gastrointestinal, urologic, and genital defects. Thus, a thorough investigation often involves a multidisciplinary approach including a neurologist, ophthalmologist, otolaryngologist, gastrointestinal specialist, and orthopedic surgeon. Additional paraclinical testing may be guided by the clinical examination: radiography, abdominal ultrasound, cerebral imaging, and laboratory testing (liver and renal function, and others depending on the clinical examination). While investigating newborns in the intensive care unit can be particularly difficult, it is important to identify defects that may benefit from early surgical intervention. Variable expressivity adds a further layer of complexity justifying a broader screening approach. For example, it has been recommended to screen all children with supra-valvular aortic stenosis or pulmonary stenosis for Williams-Beuren syndrome and those with an interrupted aortic arch, truncus arteriosus, TOF, VSD with aortic arch anomaly, isolated aortic arch anomaly, or discontinuous branch pulmonary arteries for DiGeorge syndrome[17]. In general, genetic consultation is recommended when a probable syndromic phenotype is identified.

The second clinical situation to consider genetic testing is in the context of a multiplex family, *i.e.,* a family in which a person diagnosed with CHD has an afflicted first- or second-degree relative. A comprehensive clinical investigation includes a detailed assesment of past medical, surgical, and family histories. A family history can point to a genetically transmitted disease and is important in understanding inheritance patterns (autosomal recessive, dominant, X-linked, and mitochondrial), penetrance, and expressivity of genetic variations. While some have advocated exhaustive family history questionnaires[18], basic themes include screening for cardiac diseases within families, particular phenotypes such as dysmorphias, aborted pregnancies, other birth defects, infertility, and early deaths. Importantly, in some families with CHD, different phenotypes may be expressed such as a bicuspid aortic valve in one family member and hypoplastic left heart syndrome in another. The origin of all four grandparents may provide relevant information, such as the potential for consanguinity. If positive elements are detected, a detailed family tree should be performed that includes each proband's first- and second-degree relatives. The family tree may be further expanded, depending of which side of the family has diseased members. Supportive documents, such as surgical and autopsy reports, should be sought. It is also important to update family pedigrees to include new events over time.

In summary, the phenotypic description associated with the family tree is an essential tool in guiding further genetic investigation. Identification of a clinical feature related to an established syndrome associated with CHD should prompt syndrome-specific investigation. Wider scale screening is recommended on the basis of variable expressivity

for syndromes such as Williams-Beuren and DiGeorge. For non-syndromic CHD, the family tree may orient the clinician towards a genetic etiology and a specific pattern of inheritance. Nevertheless, in the majority of cases, there are no known karyotype abnormalities to investigate. Currently, genetic testing of known cardiac candidate genes is not routinely recommended in the clinical setting. However, genetic testing of multiplex families in the context of research studies may identify novel mutations in known genes or entirely new causal genes.

Choosing a genetic test

An individualized approach to genetic testing begins with the diagnostic hypotheses elicited by a thorough clinical assessment. In general, chromosomal abnormalities represent changes in the structure or number of chromosomes and are diagnosed by cytogenetic methods. The standard metaphase karyotype analysis detects numerical and structural chromosomal aberrations with a resolution of 5 megabases. It is indicated to search for such anomalies as trisomy 21, 18, 13, or monosomy X. Fluorescence in situ hybridization (FISH) is a method to detect deletion or duplication of specific regions of DNA using targeted probes. It provides a higher resolution than karyotype and is the predominant technique used to identify Williams-Beuren, DiGeorge, and Alagille syndromes. Subtelomere FISH analyses, while less commonly used today, provide a high resolution to detect abnormalities in subtelomere (*i.e.,* DNA segments between telomeric caps and chromatin) and telomere (*i.e.,* regions of repetive nucleotide sequences at each end of a chromatid) DNA regions[17]. Subtelomeric anomalies have been reported in patients with a syndromic phenotype associated with facial dysmorphia and mental retardation combined with CHD such as VSD, ASD, pulmonary stenosis, and right sided aortic arch[19,20].

Array-based comparative genomic hybridization (aCGH) is used to detect unbalanced structural and numerical chromosomal abnormalities with a resolution inferior to 5 megabases, such as copy number variants (CNV), *i.e.,* number of copies of a particular gene that deviate from normal (two for autosomes, one X chromosome for males (XY), and two X chromosomes for females (XX). This molecular karyotype provides rapid identification of duplications/deletions, unbalanced translocations, and aneuploidies. This method analyzes the entire genome and compares it to controls, in contrast to FISH techniques that target specific DNA regions. It may be particularly useful when a probable chromosomal syndrome is identified but the karyotype is normal and there is no known specific region to test[21]. Furthermore, this method is of additional value in detecting CNVs such as in screening for DiGeorge syndrome when the karyotype and 22q11 microdeletion analyses by FISH are unrevealing[20]. Cytogenetic testing has been recommended for all children with CHD associated with mental retardation, developmental delay, dysmorphic features, or other organ involvement and for establishing a prenatal diagnosis when CHD is identified by fetal echocardiography[17]. Most CNV studies in CHD report 10%-25% of abnormal findings across the disease spectrum.

Gene mutations represent a second category of genetic abnormalities. Mutations can affect the coding portion of a gene, a case in which interpretation is usually straightforward. They can also affect the non-coding portion of the genome, in which case they are more difficult to interpret. With the advent of NextGeneration sequencing technologies, large gene panels, which specifically target genes that are known or suspected to play a role in cardiac biology, can be more readily screened than previously possible by Sanger sequencing[22,23]. This approach affords a high quality diagnosis. Gene sequencing can be helpful in conditions such Noonan syndrome, Alagille syndrome with a normal FISH analysis, Holt-Oram syndrome, and several other diseases.

Interpretation of a genetic test

When a genetic variation is diagnosed, the clinician must determine its relation to the phenotype. Although genetic variants are identified with increasing frequency by high throughput sequencing, not all variants are pathogenic[22,23]. Determination of pathogenic potential is based on the following three questions: (1) Has this genetic variant already been described in association with the particular phenotype? (2) Is the genetic variant predicted to alter gene function or regulation, gene coding, or the gene splice site, and does it occur in an evolutionarily conserved nucleotide? and (3) Does the genetic variant segregate with the affected family members and not unaffected members or controls? This assessment is not foolproof. For example, genetic variants may be identified in unaffected family members because of variable penetrance and expressivity. Each genetic result must, therefore, be placed in context of the clinical and family evaluation.

Genetic counseling

Genetic counseling is important before and after genetic testing[24]. Prior to testing, the patient or guarantor should be informed of the risks of a negative result arising from the fact that all genes implicated in a given phenotype have not been identified. Second, the pathogenic potential of a genetic variant may be difficult to determine. Third, if a genetic familial disorder is identified, the patient is responsible for informing the family. After genetic testing, counseling is important to review the results, explain the genetic variant, and discuss implications with the patient and family[25].

OBJECTIVES OF GENETIC TESTING IN CLINICAL PRACTICE

Confidence in the diagnosis

Objectives of genetic testing may vary according to the clinical scenario. One objective is to establish confidence in the diagnosis. An accurate diagnosis could allow the clinician to explain causes and mechanisms of disease, provide more precise prognostic information, and elucidate implications for future offspring. Genetic counseling is of

paramount importance in relaying such information[26].

Appropriate management

Non-cardiac organ involvement: An accurate diagnosis could alert the clinician to the possibility of associated non-cardiac organ involvement. Down, Patau, Edward, DiGeorge, Turner, Williams-Beuren, Noonan, and Alagille syndromes all involve extracardiac abnormalities[27].

Craniofacial anomalies have been associated with endocardial cushion defect, truncus arteriosus, and aortic arch anomalies; respiratory disease with endocardial cushion defect and pulmonary valve disease; genitourinary malformations with septal defects, pulmonary valve disease, aortic valve disease, and truncus arteriosus; and situs inversus with heterotaxy and endocardial cushion defect[27].

Establishing a genetic diagnosis could help orient clinical and paraclinical investigations and subspecialty referral for all potential organs involved. Unrecognized and untreated interactions between various organ pathologies could worsen the cardiac prognosis. Identification of a genetic syndrome may also prove useful in the event of an emergency, when a frequent complication associated with a given syndrome occurs. Moreover, recognition of a syndrome provides a more defined guide for follow-up, including surveillance and screening for reported complications.

Other associated cardiac complications: In addition to the genetic origins of CHD, genetic variations can modulate the propensity to develop associated cardiac complications, such as arrhythmias[28] and heart failure[29,30]. Transcription factors play a key role in the formation of cardiac structures and maintenance of cardiac function and, conversely, their dysregulation can have multifaceted manifestations. For example, in the setting of an ASD, those with an NKX2.5 syndrome are more likely to develop atrioventricular block and progressive ventricular dysfunction[28]. Interestingly, patients with NKX2.5 mutations can also develop dilated cardiomyopathy[31]. *TBX5*, a gene implicated in Holt-Oram syndrome (septation defects, atrioventricular node disease, and upper limb defects) also modulates diastolic function[32]. Genes implicated in RASopathy syndromes responsible for Noonan, Leopard, cardiofaciocutaneous, and Costello syndromes are also responsible for cardiac hypertrophy in later development[33]. Thus, the genetic environment could modulate the prognosis of various forms of CHD, help to elucidate risks of developing conduction defects and systolic and diastolic dysfunction, and a provide a basis to adapt follow-up accordingly.

Overlap of CHD with muscular heart disease: Structural CHD and cardiomyopathy may be modulated by the same mutations that give rise to varied phenotypes within the same family. For example, some family members with a TBX20 mutation may have an underlying ASD, VSD, or mitral valve disease or may present exclusively with pulmonary hypertension or cardiomyopathy[14]. Mutations in MYH6 (alpha-cardiac myosin heavy chain) are associated with various forms of CHD but also dilated and hypertrophic cardiomyopathy[34]. Moreover, mutations in MYH7 have been reported in patients with Ebstein anomaly and left ventricular noncompaction[35,36]. Some family members may have CHD whereas others could develop progressive cardiomyopathy or electrophysiologic disorders. Thus, if a mutation is discovered in a family with a discordant phenotype, clinical screening and genetic testing can identify seemingly phenotypically normal individuals who are at risk of developing cardiomyopathy or electrophysiologic manifestations.

Prognosis: In addition to the prognostic implications of genetic factors discussed above, certain gene defects have been associated with post-operative survival and long-term outcomes. For example, endothelin-1 G5665T has been associated with transplant-free survival in patients with single ventricles, primarily hypoplastic left heart syndrome[37]. This variant is linked to increased vascular reactivity and hypertension. Similarly, in a study of genetic variants involved in vascular response and oxidative stress, two major alleles of two single nucleotide polymorphisms (SNPs; *i.e.,* VEGFA rs833069 and SOD2 rs2758331) were associated with worse transplant-free survival in patients with non-syndromic CHD[38]. The higher number of copies of deleterious alleles, the worse the prognosis[38]. Genotype has also been associated with early postoperative outcomes. For example, in patients with TOF, 22q11.2 deletion (DiGeorge syndrome) predicts a longer cardiopulmonary bypass time and a greater length of stay in intensive care[39]. While several explanations have been proposed, potential factors include a higher prevalence of aortopulmonary shunts and respiratory problems prior to surgical repair in patients with 22q11.2 deletion, resulting in longer mechanical ventilatory support. Conceivably, a SNP profile may one day prove to be of value in pre-operative risk assessment.

Therapeutic potential: Ultimately, the holy grail of genetically diagnosing CHD is to provide targeted curative therapy. While such interventions are currently beyond our reach, provocative studies support its potential. For example, a knock-out model of Wnt2 in null mutants results in a phenotype resembling complete atrioventricular septal defect[40,41]. The phenotype could be rescued *in vivo* by pharmacological activation of Wnt signalling.

Genetics and recurrence risk

With a Mendelian pattern of inheritance, recurrence risks are 50% and 25% for autosomally dominant and recessive genes, respectively. However, variable penetrance complicates these predictions, even for syndromic CHD. In the majority of cases with CHD, difficulties in estimating recurrence risks are compounded by the absence of a clear genetic diagnosis[42,43]. Estimates are, therefore, largely based on a detailed family tree and the published literature[18].

In patients with atrial septal defects, the recurrence

Table 2 Recurrence risks for non-syndromic congenital heart disease in first-degree relatives

Type of non-syndromic CHD	Recurrence risk of same CHD in first-degree relatives (%)	Recurrence risk of discordant CHD in first-degree relatives (%)	Recurrence risk of any CHD in first-degree relatives (%)
ASVD	1.10	2.2	3.30
ASD	0.88	2.4	3.28
VSD	0.67	1.9	2.57
ASD and VSD	0.24	2.2	2.44
Conotruncal defect[1]	1.30	2.4	3.70
Right ventricular outflow tract obstruction[2]	1.70	3.0	4.70
Left sided obstructions[3]	0.79	2.4	3.19

The recurrence risks for non-syndromic CHD in first-degree relatives are derived from a Danish cohort study[10,56]. [1]Tetralogy of fallot, truncus arteriosus, interrupted aortic arch, double outlet ventricle, transposition of the arteries; [2]Pulmonary valve stenosis, infundibular or subvalvular stenosis, double chambered right ventricle; [3]Bicuspid aortic valve, aortic coartaction, aortic stenosis, hypoplastic left heart, shone complex. First-degree relatives include parents, siblings and twins; CHD: Congenital heart disease; ASVD: Atrioventricular septal defect; ASD: Atrial septal defect; VSD: Ventricular septal defect.

risk has been estimated to be 3% in first-degree relatives, although a dominant inheritance pattern has been described in some families. A CHD recurrence risk of 1.2% was reported for first-degree relatives with an isolated septal defect[10]. For probands with atrioventricular septal defects, the prevalence of any CHD in a family member appears to be in the order of 12%-15% overall, 1%-2% of parents, 2%-4% of siblings, and 10%-14% of offspring[44-46]. Risk of recurrence is greater if the mother rather than the father has the atrioventricular septal defect (*i.e.,* 14% *vs* 10%). Nevertheless, exact figures remain debated with some studies reporting considerably lower risks[10]. In TOF, the recurrence risk has been estimated to be 2.5%-3% overall, with a phenotype that is often concordant[44,47]. However, the recurrence risk in offspring is higher when the mother is affected[9]. Moreover, some families without a 22q11 deletion syndrome have been suspected of having a recessive inheritance pattern[48]. In complete transposition of the great arteries, a very low recurrence risk has been described with no offspring affected in a British collaborative study, suggesting a sporadic model[9]. Other studies have reported a recurrence risk of 1.8% in siblings[49] and 2.7% in first-degree relatives (siblings and parents)[50], which includes varied forms of CHD such as aortic valve stenosis and double outlet right ventricle[50]. In patients with congenitally corrected transposition of the great arteries, a 5.2% recurrence risk was reported in siblings, with concordant and discordant phenotypes, including complete transposition of the great arteries, suggesting that some genes may be common to both types of transposition[51].

Left-sided obstructive lesions (*e.g.,* aortic coarctation, hypoplastic left heart syndrome, aortic stenosis, bicuspid aortic valve, and hypoplastic aortic arch) may segregate within families, suggesting a common genetic basis[52,53]. Overall recurrence risks have ranged from 1.8% to 3.2% of siblings, 3% of offspring of affected fathers, and 8% to 13% of offspring of affected mothers[52]. However, much higher recurrence risks have been described in certain geographic locations, such as 37% of first-degree relatives in Texas[52]. Moreover, some defects appear to have higher recurrence risks, such as aortic coarctation (13% of siblings)[50], hypoplastic left heart syndrome (31% of siblings)[50], and bicuspid aortic valves (> 10% of siblings)[54,55]. However, considerably lower recurrence risks for left-sided obstructive lesions in first degree relatives have also been reported (*e.g.,* 0.79% with a relative risk of 12.9)[10].

As noted by the examples above, estimating recurrence risks is an imperfect science. Empiric estimates consider the mathematical prediction of recurrence in a polygenic model of inheritance combined with the type of CHD, current knowledge base, and relationship to proband. As a general rule of thumb, recurrence risks are in the order of 1% to 6% for siblings of affected probands with unaffected parents and increase to approximately 10% when two siblings are affected. Recurrence risks in offspring are greater than siblings, higher if the proband is the mother[3], and generally higher for left-sided obstructive lesions (8%-10%). A recent population-based study from Denmark challenges these statistics and provides far lower estimates for first-degree relatives than previously reported, as summarized in Table 2[10]. These disparate results could be explained, in part, by differences in the study designs and methodologies employed, and underscore the difficulties in accurately quantifying recurrence risks. Estimating recurrence risks must consider an in depth analysis of the family history to identify specific patterns of inheritance. If the pedigree is not informative and estimates are based on a polygenic model of inheritance, limitations of empiric estimates should be discussed with the patients, including the possibility of under- or overestimation. The notion of concordant or discordant recurrent phenotypes should also be conveyed. Overall, exact concordance is low for left-sided obstructive defects (26%), intermediate for outflow tract defects (37%), and higher for septal defects (48%)[43]. Conceptually, CHD may be grouped into constellations of malformations such as septal defects, conotroncal anomalies, and left-sided obstructive lesions that share implicated genes, although such a concept is not universally supported[56].

Assessing family members

A strong case has been made for screening first-degree relatives of patients with left-sided obstructive lesions and bicuspid aortic valves. As previously noted, recurrent phenotypes in first-degree relatives are relatively common and frequently discordant such that a bicuspid aortic valve, aortic coarctation, and/or aortic dilation may be identified in asymptomatic family members. Echocardiographic screening has been recommended for first-degree relatives of patients with bicuspid aortic valve or supra-aortic stenosis, since a physical examination alone lacks sensitivity[57]. The rationale for family screening is that early

detection may help avert complications related to aortic dilatation (*e.g.,* 6-fold higher risk of aortic dissection), aortic stenosis, aortic insufficiency, endocarditis, and aortic coarctation (*e.g.,* arterial hypertension). Early detection may lead to lifestyle recommendations (*e.g.,* limit isometric exercises), enhanced monitoring (*e.g.,* for progressive aortic dilatation), or preventive surgery (*e.g.,* prior to aortic dissection). Age at screening remains controversial. It should generally be proposed to adults if not previously performed during childhood.

At present, systematic screening of first-degree relatives is not recommended for other forms of non-syndromic CHD. However, fetal echocardiographic screening is indicated if either parent is afflicted with any form of CHD. It should be performed in a specialized center at 18-20 wk of gestation[58]. Early detection of complex CHD can drastically improve outcomes by planning delivery in a specialized (level 3) tertiary care center with appropriate monitoring and early catheter-based or surgical interventions when indicated[58-60]. Furthermore, prenatal diagnosis may lead to a parental decision to terminate the pregnancy.

Finally, identification of a specific mutation in a multiplex family with CHD may allow for targeted screening of additional family members. While there is no clear-cut indication for genetic screening to identify CHD in family members with structurally normal hearts, there may be a rationale to screen seemingly normal family members for entities that include CHD as one aspect of a multiple constellation phenotype.

Prenatal diagnosis

The impact of a prenatal diagnosis of CHD on the pregnancy termination rate varies by region. For example, reported pregnancy termination rates for severe CHD identified by prenatal screening were 45% in the Netherlands[58], 49% in Boston, MA[61], and 86% in Switzerland[62]. In a study from France, factors associated with pregnancy termination included severity of CHD, gestational age at diagnosis, presence of chromosomal abnormalities, and parental ethnicity[63].

Fetal genetic screening for CHD is also possible, including genome-wide high-resolution SNP arrays to identify CNVs[64] and competitive genomic hybridization to detect submicroscopic chromosomal aberrations[65]. A prenatal diagnostic test can be performed after chorionic villus sampling before 14 wk of gestation. Thus far, such testing has been limited to specific disease entities such as trisomy 21, 18, and 13, cystic fibrosis, and microdeletion syndromes (*e.g.,* DiGeorge). It could also be performed for any severe monogenetic disease if the result could influence the decision to terminate pregnancy[66]. Preimplantation diagnostic testing could be proposed in selected cases, particularly for women with a history of multiple therapeutic abortions. It has already been used for Holt Oram and Marfan syndromes[67]. Beyond syndromes such as trisomy 21, 18 or 13, prenatal or preimplantation genetic screening remains controversial. Ethical dilemmas may arise as a result of uncertainties in interpreting tests, potential for false positives, and the inability to predict disease severity, penetrance and expressivity of a mutation, and concordant or discordant phenotypes.

Limitation of genetics in CHD

Despite the fact that CHD is the most common birth defect, the genetic etiology remains unknown in the majority of cases, with slower progress than for other forms of heart disease such as inherited arrhythmia syndromes and hypertrophic and dilated cardiomyopathy. Genetic studies in CHD were traditionally restricted to multiplex families with strong phenotypic penetrance, which represent the minority of cases[40]. The relatively low familial recurrence risk is not fully understood but may be due, in part, to *de novo* mutations, incomplete penetrance, and other etiological factors such as environmental influences. Patterns of inheritance may be difficult to sort out in the presence of environmental interactions, age-dependent or incomplete penetrance, and variable expressivity. In addition, genetic analysis based on individual families requires a large number of members or consanguinity[11]. Moreover, mutations may involve non-exonic DNA, such as regulatory regions, the functional validation of which is more difficult and resource consuming. Establishing genotype-phenotype correlations may be further complicated by mutations that are rare and unique to individual families[2]. In fact, most CHD mutations identified to date appear to be private or do not recur. Despite these numerous limitations, genetics has and will hopefully continue to provide insights into the etiology of CHD, embryonic heart development, potential therapeutic targets, risk assessment, and patterns of inheritance.

Future perspective

Objetives of genetic testing for clinical reasons differ from research goals. From a clinical perspective, a genetic test should be directly relevant to a patient by serving the purpose of establishing or confirming a diagnosis, providing prognostic information, informing therapeutic decisions, and/or assisting with family planning. In contrast, genetic testing for research purposes may provide pathophysiological insights into a disease entity and identify potential therapeutic targets, thereby carrying the potential to impact care at a longer-term horizon. Nevertheless, genetic results derived from research studies are generally communicated to the clinical team and may directly contribute to the care of a given family[68]. It is important, therefore, for the clinical team to be well versed in the domain in order to effectively communicate with the patient, explain results, and establish an appropriate surveillance plan. In parallel, genetic testing within clinical laboratories may discover new mutations in known genes and novel implicated genes, particulary when modern technologies that sequence a broad array of genes are applied. In the future, therefore, enhanced partnerships between clinical and research teams could maximize the potential for progress. Resources available for research, including highly qualified personnel, informatics infrastructures, laboratory equipment, novel platforms, and more rapid time to analyses could complement the

clinical laboratory setting in enhancing clinical care. Greater integration between clinical and research teams could also contribute to ensuring that discoveries are progressive and clinically meaningful, with direct applications to patient care. Along these lines, the multicenter prospective "CHD GENES" study was initiated in December 2010 to explore relationships between genetic factors, clinical features, and outcomes in patients with CHD[2].

CONCLUSION

Despite major inroads over the past few decades in genetics related to CHD, the majority of patients with CHD are without a genetic diagnosis such that the etiology of their CHD remains incompletely understood. In this article, we discussed the multifaceted implications of genetics in CHD including the potential for personalized care, confidence in the clinical diagnosis, prognostic implications, early identification of non-cardiac organ involvement and associated complications, and tailoring clinical follow-up. Genetic testing could also provide valuable information in predicting recurrence risk, defining the pattern of inheritance, screening family members, and family planning. Various methodologies are available to diagnose chromosomal abnormalities and gene mutations. The challenge lies in first identifying potential genetic etiologies, selecting the appropriate test, and interpreting the test within the context of available knowledge. Collaboration between clinicians and genetics researchers offers the best opportunity for progress in clinical care and innovative breakthroughs[69,70]. Much remains to be discovered in tapping the potential of genetics in CHD.

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