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Congenital heart defects: A familial, genetic and environmental perspective

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Abstract

Congenital heart disease (CHD) is the most common birth defect, affecting approximately 1% of all live births. The population of adults with CHD has surpassed the prevalence of pediatric CHD due to remarkable achievements in medical care in the last few years, not only diagnostic but therapeutic as well. The etiology of CHD follows a multifactorial model, in which a complexity of genes and non-genetic factors interact. However, the precise genetic, epigenetic and environmental mechanisms for these perturbations remains poorly understood, despite the recent advances in genetic knowledge associated to CHD.

This review has the aim to explore the etiology of CHD, namely genetics, including syndromic and nonsyndromic cardiac defects, familiar risk and environmental factors discovered to date. This information is vital to offer a better health management and family planning to the patients and their families. Therefore, parental counselling, echocardiographic and genetic testing, as well as, prenatal screening will also be addressed. A brief review of the CHDs most reported in etiological studies, including epidemiology, pathology and phenotype, will be approached as well.

Key words: Congenital heart disease; syndromic; nonsyndromic; etiology; genetics; familiar risk; environmental factors; genetic counselling; prenatal screening; genetic testing

Resumo

As cardiopatias congénitas (CC) são o defeito mais comum à nascença, afetando aproximadamente 1% de todos os nados-vivos. A população de adultos com CC ultrapassou a prevalência da população pediátrica, devido aos avanços notáveis dos cuidados médicos nos últimos anos, não só ao nível diagnóstico como também terapêutico. A etiologia das CC's segue um modelo multifactorial, no qual uma complexidade de genes e fatores não genéticos interagem entre si. Contudo, ainda pouco é conhecido sobre os mecanismos genéticos, epigenéticos e ambientais precisos para estas doenças, apesar dos avanços recentes no conhecimento genético associado às CC's.

Esta revisão tem como objectivo explorar a etiologia das CC's, nomeadamente genética, incluindo defeitos cardíacos sindrómicos e não-sindrómicos, risco familiar e factores ambientais descobertos até à data actual. Esta informação é vital para oferecer melhores cuidados de saúde e planeamento familiar aos pacientes e suas famílias. Por esse motivo, temas como o aconselhamento parental, testes de rastreio genético e ecocardiográfico, bem como pré-natal, vão ser abordados. Será também apresentado um breve resumo das CC's mais referidas nos estudos etiológicos, incluindo epidemiologia, patologia e fenótipo.

Palavras-chave: Cardiopatias congénitas; sindrómico; não-sindrómico; etiologia; genética; risco familiar; factores ambientais; aconselhamento genético; testes de rastreio pré-natal; testes genéticos.

Abbreviations

AS Aortic Stenosis
ASD Atrial Septal Defect
AVSD Atrioventricular Septal Defect
BAV Bicuspid Aortic Valve
CoA Coarctation of the aorta
CHD Congenital Heart Disease
CHF Congestive Heart Failure
CNV Copy Number Variation
DORV Double-outlet right ventricle
EA Ebstein´s anomaly
HLHS Hypoplastic Left Heart Syndrome
IAA Interruption of the aortic arch
PDA Patent Ductus Arteriosus
PPS Peripheral Pulmonary Stenosis
PVS Pulmonary Valve Stenosis
SNP Single Nucleotide Polymorphism
SVAS Supravalvular Aortic Stenosis
TA Tricuspid Atresia
TGA Transposition of the great arteries
TAPVR Total anomalous pulmonary venous return
PAPVR Partial anomalous pulmonary venous return
TOF Tetralogy of Fallot
VSD Ventricular Septal Defect

Introduction

Congenital heart disease (CHD) is the most common birth defect, accounting for 28% of all congenital malformations.[1] There is a variety of genes, inheritance patterns and other factors, which may have a role in the cause of CHD. Therefore, it is considered a multifactorial disease, in which the interaction between genes and the environment is still poorly understood. The phenotypes of cardiac disease can range as well from the simplest defect, like a small ASD without other comorbidities, to the most complex ones, which may lead to cyanosis and consequently to poor survival after birth.

The interest in exploring the etiology factors of CHD, including inheritance patterns, familiar recurrence risks, environmental factors and available screening and diagnostic tools, is of paramount importance. Taking this knowledge into consideration, professionals can provide the best counselling to patients and their families regarding prevention, diagnosis and treatment of CHD. As such, discovery of the causes for CHD is vital not only for research but also to the healthcare of these patients.

This review has the purpose to reunite the discoveries to date regarding all the etiology factors of CHD mentioned above, and approach parental counselling, genetic testing and prenatal screening of CHD.

Epidemiology

In CHD, the incidence and prevalence quantifications are difficult to measure accurately due to nonstandardized definitions of qualifying pathologies, geographic variability and nonuniform screening methods. The average incidence of CHD is calculated in 8 per 1,000 live births, varying between 1.2 per 1.000 (Columbia) and 17 per 1,000 (Iceland), according to a large systematic review from studies worldwide.[2] Another study reports an incidence between 7 to 10 per 1,000 live births, excluding patent *foramen ovale* and bicuspid aortic valve (BAV), and 30 per 1000, including BAV.[3] The largest population study is from EUROCAT, the European Registry of Congenital Anomalies, which collects data from 20 countries and a population of over 18 million births. The average incidence of CHD found in live infants one week after birth was 6.4 per 1,000. [4]

Reported total CHD birth prevalence increased substantially over time, from 0.6 per 1,000 live births in 1930 to 1934 to 9.1 per 1,000 live births after 1995. It is estimated that 1.35 million newborns are born with CHD every year and 21 million

adults are living with CHD.[5] Considering that the worldwide annual birth rate is around 150 million births, it represents a major public health issue. Asia reported the highest CHD birth prevalence, with 9.3 per 1,000 live births, with relatively more pulmonary outflow obstructions and fewer left ventricular outflow tract obstructions. In Europe, the total CHD birth prevalence was significantly higher than in North America (8.2 vs. 6.9 per 1,000 live births).[6] As a result, CHD complicates around 1% of all live births in the general population, but occurs more frequently in the offspring (about 4-10%, depending on maternal CHD type) of women with CHD.[7]

The population of adults with CHD has been growing at a rampant rate, surpassing the static prevalence of pediatric CHD with a ratio of 2:1. It is estimated that more than 90% of afflicted neonates and children now reach adulthood due to the remarkable advances over the last 60 years in diagnostic methods, medical management, interventional techniques, contemporary surgical procedures and perioperative care.[7,8] It has been reported as well an increased incidence of simpler defects in developed countries, which might reflect an enhanced sensitivity in detecting milder forms of CHD combined with a reduction in the incidence of severe defects, due to pregnancy termination after prenatal diagnosis.[8] From 1987 to 2005 was reported more than 30% reduction in mortality among these patients and a 67% reduction among children with complex disease.[9] In the same period, the median age of death increased from 60 to 75 years and from 2 to 23 years in those with severe defects.[9]

About a quarter of those requiring treatment will need surgery in the first year of life.[10] Most infants and children requiring single interventions can expect to lead a near normal life. A small group of infants with complex lesions require multiple surgical procedures, intensive support and close monitoring during the first few years of life, although their quality of life may still be good. Because true surgical cures are rare, and all repairs, be they palliative or corrective, may leave residua, sequelae, or complications, most require some degree of lifetime expert surveillance.

Women with CHD may now frequently successfully bear children after competent repairs. Although they are at increased risk for peri- and postpartum complications, maternal CHD is generally not considered an absolute contraindication to pregnancy unless the mother has high-risk features, namely cyanosis, pulmonary hypertension, decompensated heart failure, arrhythmias, aortic aneurysm, among others. Consultation with an adult CHD expert is warranted for all females with CHD who desire to become pregnant.[8]

Ongoing efforts to increase awareness, resources, the number of specialty centers and specialists are needed in order to keep up with the growing number of adults with CHD.[7]

Pathology

The anatomic and physiologic changes in the heart and circulation due to any specific CHD lesion are not static but rather progress from prenatal life to adulthood. Malformations that are benign or escape detection in childhood may become clinically significant in the adult.[7]

Congenital heart diseases can be divided into: (1) left-to-right shunt lesions, such as atrial septal defect (ASD), ventricular septal defect (VSD), atrioventricular septal defect (AVSD), patent ductus arteriosus (PDA) and partial anomalous pulmonary venous return (PAPVR); (2) Obstructive lesions, such as pulmonary valve stenosis (PVS), aortic stenosis (AS), coarctation of the aorta (CoA) and interruption of the aortic arch (IAA); (3) and cyanotic defects, such as transposition of the great arteries (TGA), tetralogy of Fallot (TOF), total anomalous pulmonary venous return (TAPVR), tricuspid atresia (TA), hypoplastic left heart syndrome (HLHS), Ebstein´s anomaly (EA), truncus arteriosus, single ventricle, double-outlet right ventricle (DORV) and heterotaxy. Conotruncal defects include TGA, TOF, truncus arteriosus, DORV and IAA.

According to their prognosis, CHD malformations can be divided into simple (Table 1), intermediate (Table 2) or complex (Table 3).[7] This classification was created in order to stratify the CHD according to their severity and consequently suggest when cardiology consultation or advanced CHD specialty care is needed. Simple defects generally are single lesions with a shunt or a valvular malformation. These patients often may be managed by a well-informed internist or general cardiologist, although consultation with a specifically trained adult congenital cardiologist is occasionally advisable. Intermediate defects may have two or more simple defects and should have an initial consultation and subsequent occasional intermittent follow-up with an adult CHD specialist. Complex defects generally have components of an intermediate defect plus more complex cardiac and vascular anatomy, often with cyanosis, and frequently with transposition complexes. These patients should virtually always be managed in conjunction with an experienced specialty adult CHD center.[7] Specific heart defects will be approached later in this review.

Simple adult congenital heart disease
Native disease
Uncomplicated congenital aortic valve disease
Mild congenital mitral valve disease
Uncomplicated small atrial septal defect
Uncomplicated small ventricular septal defect
Mild pulmonic stenosis
Repaired conditions
Previously ligated or occluded ductus arteriosus
Repaired secundum or sinous venosus atrial septal defect without residua
Repaired ventricular septal defect without residua

Table 1. Simple adult CHD. Source: adapted from Kasper et al. (2015)[7]

Intermediate complexity congenital heart disease
Ostium primum or sinous venosus atrial septal defect
Total or partial anomalous pulmonary venous drainage
Atrioventricular canal defects (partial or complete)
Complicated ventricular septal defect (e.g., absent or abnormal valves or with associated obstructive lesions, aortic regurgitation)
Coarctation of the aorta
Moderate to severe pulmonic valve stenosis
Infundibular right ventricular outflow obstruction of significance
Moderate to severe pulmonary valve regurgitation
Moderate to large patent ductus arteriosus (nonclosed)
Sinus of Valsalva fistula/aneurysm
Subvalvular or supravalvular aortic stenosis

Table 2. Intermediate Complexity CHD. Source: adapted from Kasper et al. (2015)[7]

Complex adult CHD
Cyanotic congenital heart diseases (all forms)
Eisenmenger's syndrome
Ebstein's anomaly
Tetralogy of Fallot or pulmonary atresia (all forms)
Transposition of the great arteries
Single ventricle; tricuspid or mitral atresia
Double-outlet ventricle
Truncus arteriosus
Fontan or Rastelli procedures

Table 3. Complex adult CHD. Source: adapted from Kasper et al. (2015)[7]

Etiology

The etiology of CHD remains to be elucidated. In 1968, a hypothesis of multifactorial etiology was proposed, in which the environment and genetic factors interact with each other to cause CHD.[11] A few years later, several separate environmental and genetic causes have been identified.[12,13] The prevailing model involves variations in many different genes, each of which contributes only a small amount to the individual's susceptibility to a particular condition. These interact with each other and with environmental factors to raise the likelihood that an individual will have CHD. Most sporadic cases of CHD (isolated cases of CHD without a family history of the condition) would fall into this category.[10]

Efforts have been made to explore the genetic and environmental factors that may lead to an increased risk of CHD in order to offer a better health management and family planning to the patients and their families, so that the risk of recurrence in the children of patients with CHD can be assessed. It may help as well identify possible complications and risk factors for surgery or treatment, as patients with genetic syndromes or extracardiac anomalies are generally at higher risk of operative mortality and morbidity.[14]

Although advances in understanding genetic risk factors have been made, little is known about nongenetic risk factors for the development of CHDs. Notwithstanding these facts, preventive strategies have been designed and instituted based on the information available, and parents are counseled regarding their risk of having an affected child.[12] Approximately 20% of the cases result from known causes, such as chromosomal anomalies, Mendelian syndromes, nonsyndromic single gene disorders and teratogens. The proportion of syndromic phenotypes with extracardiac manifestations can rise up to 30% of patients diagnosed with CHD, according to some authors.[15]Recognized chromosomal aberrations and mutations of single genes account for less than 10% of all cardiac malformations.[7] Down syndrome and velocardiofacial syndrome are the most commonly seen syndromes in patients with CHD. The other 80% is multifactorial between genetic and environmental contributors.[10] Currently, more than 40 genes have been linked to nonsyndromic forms of CHD.[16] Their contribution to CHD remains unknown but is presumed to be relatively small. Although cardiac defects can occur in the setting of multiple birth defects as part of a syndrome, most are found as isolated defects with no syndromic association.[13]

Genetics of congenital heart disease

Genetic abnormalities appear to be the primary cause of CHD but identifying precise defects has proven challenging, principally because CHD is a complex genetic trait. In the last few years, the novel genetic testing technologies, such as next-generation DNA sequencing, allowed the discovery of new candidate genes that may be involved in cases of CHD with unknown etiology.[13] The human cardiovascular genetics is in the early phase of gene discovery. Molecular genetic studies, in the past two decades, have observed families with multiple affected individuals and have provided insights into the genetic causes of several forms of CHD. Thanks to these discoveries, it was demonstrated that the genetic contributions to CHD are more important than it was believed in the past. Embryos, fetuses, children and adults are being genetically tested in both research and clinical setting and it is expanding swifter than are the surveillance programs, for example.

For the clinician caring for a child with CHD, it is very important to determine whether there is an underlying genetic pattern, because there may be extra-cardiac involvement, as well as prognostic information for clinical outcomes, important genetic reproductive risks and other family members may benefit from genetic testing.[17]

Insights in developmental biology

CHD is a disorder resulting from abnormal heart development, so it is likely that defects in the genetic control of cardiac development underlie a majority of CHD.[18] Molecular pathways have been identified that orchestrate formation of primordial cardiogenic fields, that shape the cardiac crescent and linear heart tube, and that drive atrial, ventricular, inflow and outflow tract morphogenesis.[19] It is difficult to estimate the number of genes required for cardiac development, especially since some essential cardiac genes, such as those involved in Hedgehog signaling, are also essential in very early, essential developmental processes that can obscure the cardiac phenotype. Defects in these general developmental genes may result in very early lethality, or instead, manifest as complex syndromes that have CHD as an important component.[18]

Cells destined to become the heart originate from the mesoderm and to a smaller extent the neural crest. The genetic network regulating cardiac and regional cardiac identity is complex, and any of the genes involved are candidates for CHD-causing genes.[18]

The first major morphogenetic event in heart development is heart looping. The left-right (LR) asymmetry of the heart depends on global LR positional information generated by cilia during gastrulation. Defects in genes encoding ciliary function underlie many mouse models of heterotaxy [20] and ciliary defects have been identified in some humans with heterotaxy. The LR axis is initiated at a transient, evolutionary conserved ciliated structure called the Left-Right Organizer (LRO). LRO function depends on the extensive set of genes required for ciliogenesis, motility and signaling. Subsequent to heart looping, the T-Box transcription factors *TBX 2,3,18,* and *20* play a prominent role in specifying ventricular identity. Recent studies have shown that *Tbx2* plays a key role in the formation of the AV canal, the left ventricular base, and the cardiac conduction system.[21] *Tbx3* and *Tbx18* play key roles in sinoatrial (SA) node development.[22]

The formation of the atrioventricular valves depends on the domains of endocardium that are fated to undergo epithelial-mesenchymal transformation (EMT). Genes involved in valve development include ones that are required for EMT, such as the TGFp family, and others not required in EMT such as *NFATC1* and others that lie within the "Down Syndrome critical region". It is interesting to note that patients with Down syndrome have a high frequency of atrioventricular septal defects (AVSDs) that are phenotypically more uniform than nonsyndromic AVSDs, and that the Down Syndrome-associated AVSDs usually respond remarkably well to surgical repair.[18]

As we can see, the integration of insights from developmental biology has informed why some human CHD mutations produce specific clinical phenotypes. For example, mutations in *ZIC3* cause cardiac laterality defects that are often accompanied by visceral heterotaxy, an association that is explained by evidence that *ZIC3* transcriptionally activates *NODAL,* a critical morphogen that is required for left-right patterning throughout the embryo.[23] Mutations in *GATA4* typically cause ASDs, but because a subset of these mutations disrupts GATA4-SMAD4 interactions that are critical for valve development, some patients have AVSDs.[24] Mutations in *NKX2-5* and *TBX5* cause cardiac malformations that are associated with electrophysiologic deficits, presumably because these transcription factors have been linked to the formation of the conduction system.[25]

There is great genotype-phenotype variability, that is, one single mutation can lead to multiple cardiac phenotypes. This was observed in studies including humans and model organisms, such as mice. One example is the following: All mice with

heterozygous deletion of Tbx1 (model of DiGeorge syndrome) have a specific defect in the development of the 4th aortic arch early in the development. Remarkably, as development proceeds, a number of pups remodel their aorta to approximate normal, while others progress to severe aortic abnormalities.[26]

Familial CHD mutations occur as autosomal dominant, autosomal recessive, or X-linked traits that are expressed with high penetrance and with variable clinical manifestations.[16] An evolutionary perspective of CHD mutations predicts that reduced reproductive fitness and early mortality would cause substantial negative selection that eliminates CHD mutations from human populations. If autosomal dominant or X-linked mutations make a significant contribution to the population prevalence of CHD, many must be *de novo* mutations, resulting in sporadic CHD.[16] Far less is known about recessive models in CHD.[16] Parental consanguinity (especially first-cousin marriages) significantly increases CHD risk,[27] which supports that recessive mutations also cause CHD. The same mutant genes can be associated with different cardiac defects, and different cardiac defects can be associated to the same gene. All this support a polygenic and multifactorial model of CHD.[16]

Genetic findings associated with CHD will be presented, dividing them into chromosomal anomalies, Mendelian syndromes and non-syndromal single gene disorders.

Chromossomal anomalies

The trisomies (trisomy 21, 18, and 13) are the most common chromosomal aberrations associated with heart defects in live births. These trisomies arise de novo in almost all cases.[28]

Down syndrome, as well known as trisomy 21, is associated with AVSD, ASD, VSD and TOF (less common) in 40 to 50% of these patients.[3,10] Extra-cardiac manifestations include characteristic facial dysmorphism, epicanthal fold, simian crease, conductive hearing loss, gastrointestinal defects, variable degrees of intellectual disability, hypotonia and joint laxity. The overall prevalence of Down syndrome has been estimated to be 1/700 live births, although it is highly dependent on maternal age and has decreased significantly as a result of routine prenatal testing.[29]

Pulmonary hypertension develops more often and earlier in patients with CHD. Down syndrome does not increase surgical mortality and morbidity during the repair of the atrioventricular canal defect. The five-year survival rate is almost 70% for

surgically-corrected AVSDs in Down syndrome, being no different from that of nonsyndromic patients.[29] Down syndrome is a significant risk factor for the development of a post-operative atrioventricular block. Echocardiograms should be performed in all patients at diagnosis, regardless of whether a fetal echocardiogram was performed [30] and it is recommended regular monitoring for signs and symptoms of congestive heart failure (CHF) at every visit if the patient has CHD.[30] Conventional karyotyping is indicated in all patients to confirm the diagnosis.

A candidate gene resequencing approach of 26 genes in individuals with Down syndrome with either AVSDs or without CHD identified potentially damaging variants in almost 20% of individuals with Down syndrome with AVSDs but in only 3% of those with Down syndrome without CHD.[31] Six genes were specifically implicated: *COL6A1*, *COL6A2*, *CRELD1*, *FBLN2*, *FRZB*, and *GATA5*. Pathway analysis with these six genes implicated VEGF-A signaling, which was known to have a role in atrioventricular valvuloseptal morphogenesis.

Patients with Edwards or Patau syndrome have poor survival: 80% and 90% die in first year, respectively.[17]Almost all patients with Edwards syndrome (Trisomy 18) have cardiac defects (90-100%), including VSD, ASD, PDA, DORV, TOF, CoA, HLHS, BAV, d-TGA and polyvalvular nodular dysplasia.[10,17] Other clinical features include prenatal growth deficiency, distinctive facial features and overlapping fingers and toes. Patau syndrome (trisomy 13) is associated with CHDs in 80-100% of cases, namely ASD, VSD, PDA, polyvalvular disease, HLHS and laterality defects.[16,17] Orofacial clefts, postaxial polydactyly, microphthalmia are some of the other features.[16]

Turner syndrome is found in girls caused by the absence of, or structural anomalies in, one of the two X chromosomes (Monosomy X). The incidence of Turner syndrome is estimated to be approximately 1:2000 live female births.[29] It includes diverse clinical features, including a short stature, webbed neck, bowed arms, primary amenorrhea and thus infertility, as well as cardiac and renal anomalies, although virtually any organ system can be affected. [29]Conventional karyotyping is indicated in all Turner patients to confirm the diagnosis. This syndrome is associated in 25-45% of cases to CHD, mainly to BAV (16%) and CoA (11-14%)[32] but, AS, HLHS[17], partial anomalous pulmonary venous return (PAPVR) and VSD can also be found.[29] Moreover, aortic dissection (1-2%), systemic hypertension (30-50%), and ischemic heart disease are of concern in adult Turner patients, and are often fatal and the leading causes of increased mortality.[33] Blood pressure measurements are necessary at every visit, and the

aggressive management of hypertension is recommended if hypertension is detected. Therefore, all Turner patients should have a baseline cardiologic evaluation with an echocardiogram or cardiac magnetic resonance imaging (MRI), and be monitored by longitudinal imaging every 5-10 years even when an adult in order to assess their aortic diameters. Echocardiography is generally adequate to visualize the heart valves and aortic arch in children, however MRI is the recommended imaging modality in adolescents and adults.[32]

Klinefelter syndrome (47,XXY) is associated with CHD in 50% of cases, namely ASD, PDA and mitral valve prolapse (MVP). Other features include tall stature, small testes, delayed puberty, emotional and behavioral problems and variable mental retardation.[17]

Cat eye syndrome is a rare condition caused by the short arm (p) and a small section of the long arm (q) of chromosome 22 (22pter-22q11) being present three (trisomic) or four times (tetrasomic) instead of the usual two times.[34] Normally the inheritance is de novo, but it can be autosomal dominant. It is associated with TAPVR, PAPVR and TOF in more than 50% of cases.[16] There is high variability in the phenotype; some clinical features are the coloboma of the iris, anal atresia, renal malformations and periauricular tags or pits.[16]

Cri-Du-Chat syndrome is caused by a deletion in 5p15.2. The gene associated is *CTNND2* and has only *de novo* inheritance. 10-55% of cases are associated with VSD, PDA, ASD and TOF. Other clinical features include microcephaly, prenatal and postnatal growth retardation, severe psychomotor and mental retardation, distinctive facial features (round face, widely spaced eyes, epicanthal fold) and high-pitched cry.[16,17]

Pallister-Killian syndrome is an extremely rare genetic disorder and is due to the presence of the anomalous extra short arm of the chromosome 12. This leads to the development of tetrasomy 12p. Because not all cells have this defect, Pallister-Killian is a mosaic condition. It is associated in 25% of cases with VSD, CoA, PDA, ASD and AS.[10]

Microdeletion syndromes with cardiac phenotypes show autosomal dominant inheritance, although the large proportion of the microdeletions arise de novo.[28]

The most common segmental aneuploidy is the 22q11 microdeletion, usually arising *de novo*, which accounts for substantial 34% of truncus arteriosus and 16% of TOF.[3] DiGeorge/velocardiofacial syndrome (VCFS) is caused by loss of part of

chromosome 22 (del22q11.2), which includes the gene *TBX1*. The incidence is estimated to be 1/4000 live births. Fluorescence *in situ* hybridization (FISH) has been widely used to confirm the diagnosis.[29] Haploinsufficiency (loss of one of the two copies of the gene) of *TBX1* is responsible for many of the clinical features of VCFS, including the cardiac phenotype.[35] 75-85% of patients with VCFS have CHD, which usually includes conotruncal defects, TOF (20%), interrupted aortic arch type B (13%), truncus arteriosus (6%), aortic arch anomalies and VSD.[10] CHD is required for the diagnosis of the syndrome.[16] The common 22q11.2 deletion and the associated TBX1 mutations represent the most frequent genetic diagnosis in non-syndromic TOF as well. It was also found that AVSD associated with TOF is highly suggestive of trisomy 21 and excludes 22q11.2 deletion.[36]

In addition to cardiac defects, palatal defects including cleft palate and bifid uvula, and immunodeficiency are most frequently associated with significant clinical features. Each is identified in approximately 70-75% of cases.[29] Other clinical findings include hypocalcemia (50%), renal anomalies (30%), thymus and parathyroid aplasia or hypoplasia, hypertelorism, micrognathia, low-set posteriorly rotated ears, “fish mouth”, feeding and swallowing problems, hearing loss, seizures and skeletal anomalies. Learning disabilities and borderline mental retardation are common, and the mean IQ score in those affected is reported to be approximately 70-80.[37] In addition, the incidence of psychiatric disorders, including schizophrenia, bipolar disorder, anxiety, and depression, is known to be increased.[37]

All patients with 22q11.2 deletion syndrome should be evaluated with an echocardiogram and electrocardiogram at diagnosis.[29] Moreover, diagnostic tests are recommended for all patients with an interrupted aortic arch and truncus arteriosus, even if they do not show the other physical phenotypes commonly seen in this syndrome. This is because the proportion of 22q11.2 deletion is particularly high in patients with these two anomalies, 50-89% of an interrupted aortic arch and 34-41% of truncus arteriosus.[17] Various medical problems can occur in multiple organ systems throughout the lifetime, so a multidisciplinary evaluation and follow-up is necessary for these patients.[38]

Considerable attention is now devoted not only to deletion but also to microduplication of 22q11.2. It may occur with a frequency approximately half of that of deletions.[39] The phenotype of patients with microduplication 22q11.2 is extremely variable, but significantly overlaps with the features of DiGeorge/VCFS.[39] The

spectrum of CHD associated with microduplication 22q11.2 is vast, including conotruncal defects,VSD, HLHS, valvular anomalies and TAPVR, among others. Interestingly, there is highly variable inter- and intrafamilial expression even among family members with the same size duplication.[39]Consistent with the effects of these chromosomal duplications, Zweier et al. reported that *TBX1* gain of function mutations produce human conotruncal malformations.[40]

Williams-Beuren syndrome consists in a microdeletion (del 7q11.23), in which the elastin gene (*ELN*) resides. This deletion can be detected by FISH.[29] Haploinsufficiency for elastin in this syndrome is responsible for hernias, the husky voice and the cardiac defects (75-80%), mainly supravalvular aortic stenosis (SVAS). *ELN* can also be associated with non-syndromic familial SVAS, pulmonary valve stenosis (PVS), pulmonary artery stenosis (PAS) or AS.[10] CHD is required for the diagnosis of the syndrome.[16] Other characteristics of this syndrome are elfin facies (100%), psychomotor retardation (75%), characteristic cognitive profile with loquacious personality (90%), skeletal and renal anomalies and idiopathic hypercalcemia (15%). Its incidence is estimated to be 1/10000-20000 live births, however partial or mild forms also can exist.[29]

Supravalvular aortic stenosis has been thought to be progressive, but recent reviews report that the lesion remains stable in the majority of patients, and the progression of stenosis is particularly associated with moderate to severe stenosis at diagnosis.[41] Approximately 20% of patients require surgical or transcatheter interventions for cardiovascular anomalies during the follow-up period.[42] Hypertension is also frequently developed in approximately 50% of Williams patients, and the risk for its development increases with age.[29] Thus, all patients with Williams syndrome should be evaluated with an echocardiogram with a Doppler flow study. Moreover, the evaluations for the development of cardiovascular complications by regular imaging studies, electrocardiograms, and blood pressure measurements on an annual basis during childhood, and every 3-5 years in adulthood are recommended.[43]

Jacobsen syndrome results from the deletion of the terminal region of the chromosome 11 (11q-). 43-70% are associated with CHD, such as HLHS, DORV, d-TGA, AS and VSD. Growth and psychomotor retardation, thrombocytopenia, platelet dysfunction, widely spaced eyes, strabismus, broad nasal bridge, thin upper lip, prominent forehead are some of the features. A cardiac “critical region” has been identified in distal 11q that is thought to contain putative genes for CHD.[44] It was

demonstrated that loss of the murine analogue of the *ETS-1* gene, located in the cardiac critical region of 11q, causes VSD and abnormal ventricular morphology in mice.[45] The role of *ETS-1* in CHD will likely be the subject of future investigation in patients with 11q-as well as non-syndromic patients.

Copy number variations (CNV)

Increasing attention is now being given to copy number variants (CNV) in CHD. They are variations in the number of copies of a specific section of DNA present in an individual. These variations consist in gains or losses of contiguous DNA ranging in size from 1 kb to several Mbs and they are considered as another type of chromosomal anomaly.[3] These represent a significant source of inter-individual genetic variation that may explain the variable expression of inherited disorders, variable phenotypical presentation of disorders, and potentially non-syndromic CHD.[46] These CNVs can either be inherited or de novo.[18] Specific CNVs have in the past been associated with diseases such as autism and schizophrenia and, more recently, with CHD. Significant challenges remain in differentiating pathologic CNVs from benign polymorphic ones.[3] Certain CNVs are recurrent and at higher frequency in individuals with CHD, often ones that are also associated with extracardiac abnormalities. Exploration of genes disrupted in large CNVs may reveal further insights in the role of specific CNVs in the pathogenesis of CHD.[18] The most relevant non-syndromic CNVs are represented in Table 4 (see below)[16].

A genome-wide CNV study of various types of CHD compared more than 2,000 CHD cases including around 800 with TOF to nearly 900 controls.[47] They found a significant burden of rare loss CNVs >100 kb containing genes (OR 1.8; population attributable risk of nearly 3.5%). There was a trend towards greater ORs with increasing CNV deletion size and gene content. Significant association with WNT signaling was revealed, which was altered in a broad range of CHD. Examining CNVs that were recurrent, the authors confirmed prior observations of gain and loss CNVs, including *GATA4* at 8p23.1 and deletions at 15q11.2 in 0.5% of cases, an OR of 8.2 compared to controls. The majority of rare de novo CNVs identified in CHD have paternal origin.[47]

10% of sporadic nonsyndromic TOF were found to harbor rare *de novo* CNVs, including recurrent CNVs at 1q21.1, 3p25.1, 7p21.3 and 22q11.2 regions.[48] A recent study found that microduplications of the 1q21.1 region accounted for about 1% of the population attributable risk of TOF and that duplication of the *GJA5* gene, also present

in that region, was associated with a 10-fold increase in risk of TOF.[49] Deletion of 1q21.1 was more common in cases of non-TOF CHD than in controls. 1q21.1 CNVs have previously been associated with other phenotypes such as autism, schizophrenia, and intellectual disabilities.[3] In another study regarding TOF, more than 400 adults with TOF with or without pulmonary atresia (PA) but without known genetic abnormalities like the 22q11 deletion were studied.[50] Large, rare CNVs (>500 kb) were more prevalent in cases compared to controls (8.8% vs. 4.3%) with mostly genomic gains. Especially when the CNVs contained genes, the subjects were more likely to have extracardiac abnormalities.

CNV's were also associated with left ventricular outflow tract defects, including HLHS.[51,52] Of interest, these rare CNVs were found to harbor genes relevant for angiogenesis. After filtering, the authors identified 25 putative candidate genes for left-sided heart defects.

The role of CNVs in atrioventricular septal defects (AVSDs), both in patients with and without Down syndrome, was investigated.[53] The authors concluded that larger rare CNVs altering chromosome 21 do not contribute significantly to the pathogenesis of AVSDs neither in patients without Down syndrome nor in patients with AVSDs and

Locus	Size Range (Kbp)	No of cases	Inheritance	CNV	No of genes	Genes*	Phenotype
1q21.1	418 - 3,981	21	de novo, inherited, n/a	gain, loss	3–45	*PRKAB2, FM05, CHD1L, BCL9, ACP6, GJA5, CD160, PDZK1, NBPF11, FMO5, GJA8*	TOF, AS,CoA, PA, VSD
3p25.1	175 - 12,380	3	de novo, inherited	gain	2	*RAF J, TMEM40*	TOF
3q22.1–3q26.1	680–32134	3	inherited, n/a	gain, loss	0–300	*FOXL2, NPHP3,FAM62C, CEP70, FAIM, PIK3CB, FOXL2, BPESC1*	DORV, TAPVR, AVSD
4q22.1	45	2	de novo inherited,	gain	1	*PPM1K*	TOF
5q14.1-q14.3	4,937 - 5454	2	de novo	gain	41103	*EDIL3, VCAN, SSBP2, TMEM167A*	TOF
5q35.3	264 - 1777	4	de novo, n/a	gain	19–38	*CNOT6, GFPT2, FLT4, ZNF879, ZNF345C, ADAMTS2, NSD1*	TOF
7q11.23	330–348	2	n/a	gain	5–8	*FKBP6*	HLHS, Ebstein's
8p23.1	67–12,000	10	n/a	gain, loss	4	*GATA4, NEIL2, FDFT1, CSTB, SOX7*	AVSD, VSD, TOF, ASD, BAV
9q34.3	190–263	3	de novo	loss	2–9	*NOTCH1, EHMT1*	TOF, CoA, HLHS
11p15.5	256–271	2	n/a	gain	13	*HRAS*	DILV, AS
13q14.11	555–1430	3	n/a, de novo	gain	7	*TNFSF11*	TOF, TAPVR, VSD, BAV
15q11.2	238 - 2,285	12	n/a	loss	4	*TUBGCP5, CYFIP1, NIPA2, NIPA1*	Coa, ASD, VSD, TAPVD, Complex left-sided
16p13.11	1414–2903	3	n/a	gain	11–14	*MYH11*	malformations HLHS
18q11.1 – 18q11.2	308–6118	2	n/a	gain	1–28	*GATA6*	VSD
19p13.3	52–805	3	n/a, de novo	gain, loss	1–34	*MIER2, CNN2, FSTL3, PTBP1, WDR18, GNA11, S1PR4*	TOF
Xp22.2	509–615	2	n/a	gain	2–4	*MID1*	TOF, AVSD

Table 4. CNVs associated with recurrent cases of nonsyndromic CHD.[16]

Down syndrome. However, isolated sporadic AVSDs may be occasionally associated with large de novo CNVs outside of chromosome 21.[53]

Heterotaxy was also found associated with CNV's. It consists in an asymmetric development along the left-right body axis, leading to thoracic and abdominal organ defects. More than 250 patients with heterotaxy and nearly 1000 controls were genotyped with SNP (Single Nucleotide Polymorphism) arrays.[54] There were nearly two-fold more rare CNVs among the heterotaxy cohort compared to the controls (14.5% vs. 7.4%, respectively). In the 38 smaller CNVs identified, 14 of 61 genes altered were relevant to biological processes of relevance for left-right axis development: ciliary function, zinc finger transcriptions factors and TGF-β signaling. Five genes contained within CNV areas were shown to be significant. *NEK2, ROCK2, TCGBR2, GALNT11*, and *NUP188* were verified by knockdown in Xenopus to produce laterality defects.[54]

With new chromosome microarray technology (SNP microarrays and array comparative genomic hybridization)[55] , further "cryptic" rearrangements, such as CNVs, will be unmasked to reveal loci critical for embryonic heart development.

Mendelian syndromes

About 3%–5% of CHD can be attributed to Mendelian syndromes where a single mutation in the DNA results in pathological consequences, following a Mendelian inheritance pattern.[10] Large multigenerational pedigrees demonstrating Mendelian inheritance of CHD are rare.[18] These single gene syndromes show predominantly autosomal dominant inheritance with variable expressivity and pleiomorphic extracardiac abnormalities. However, the majority of single-gene syndromes arise *de novo*.[28]

Noonan syndrome is the second most common genetic syndrome of CHD, followed by Down syndrome.[29] The estimated prevalence of this syndrome is 1/1000-2500.[56] It is an autosomal dominant disorder that can be caused by mutations in various genes, which are members of the RAS-MAPK pathway: *PTPN11, SOS1, KRAS, RAF1, NRAS, BRAF, SHOC2, CBL and RIT1*. Mutations in these genes can be identified in more than 70% of clinically diagnosed Noonan cases.[56] Gain-of-function mutations in the *PTPN11* account for 40–60% of all Noonan cases. Prenatal testing can be done when the fetus is at risk for inheriting a defined *PTPN11* mutation from an affected parent.[17] This gene is more prevalent among familial cases and among patients with PVS. *PTPN11* encodes the Ras signaling cofactor *Shp2*, causing defective endocardial-

mesenchymal transformation in the endocardial cushions, and thus leading to PVS.[57] Penetrance is nearly complete among those with *PTPN11* mutations, although phenotypic variability within families can be substantial.[17] *RAF1* mutations show a higher prevalence of hypertrophic cardiomyopathy (HCM).[58] Apart from PVS (50-65%) and HCM (20%), secundum ASD (6-10%), and more rarely,VSD, PDA and CoA are also asociated.[10] CHD is present in 70–80% of cases. This syndrome encompasses variable extra-cardicac phenotypes including short stature and typical facial features. The main facial findings are hypertelorism with downslanting palpebral fissures, ptosis, and low-set posteriorly rotated ears. Other manifestations are a webbed neck, thorax deformity, cubitus valgus, mild developmental delay, cryptorchidism in males, feeding difficulties in infancy, bleeding tendency and lymphatic dysplasia.[56]

Pulmonary stenosis shown in Noonan syndrome is usually accompanied by dysplastic leaflets, thus interventional balloon dilation is difficult to perform and surgical correction is often required.[14] The severity of the HCM can vary from mild to severe and can progress with age. Approximately 25% of patients with HCM die from heart failure in the first year of life.[29] Accordingly, a clinical assessment, electrocardiogram, and echocardiogram should be performed at the initial evaluation in all suspected patients. There are no consensus guidelines for Noonan syndrome management and follow-up, however several review articles recommend repeated cardiologic evaluations every 2-5 years, even if the initial examination is normal.[56]

Alagille syndrome is an autosomal dominant disorder with an estimated prevalence of 1 in 70,000 newborns. It is caused by mutations in the *JAG1* gene in more than 90% of cases. Another 7% have deletions on 20p12 that include the *JAG1* and few people have mutations in *NOTCH2*.The *JAG1* and *NOTCH2* genes trigger Notch signaling between neighboring cells during embryonic development. The disruption of this signaling results in this syndrome.[59] Patients suspected of having Alagille syndrome should undergo karyotype and FISH analysis and the finding of a deletion or chromosomal rearrangement can be diagnostic.[17] *JAG1* mutation analysis is clinically available for those patients whose karyotype and FISH analyses are normal. The cardiac defects associated are peripheral pulmonary stenosis (PPS), PVS, TOF and, more rarely, ASD.[10,16,17] The cardiac phenotype shows in 85-95% of the cases.[16,17] The finding of PPS or hypoplasia of the branch pulmonary arteries in a child, alone or in combination with TOF, should prompt consideration of testing for Alagille syndrome.[17] The phenotype includes biliary atresia, cholestasis, skeletal abnormalities, ocular disease,

distinctive facial features (broad forehand, hypertelorism and underdeveloped mandible). Alagille syndrome should have cardiac, hepatic, ophthalmologic, orthopedic (butterfly vertebrae), hematologic (bleeding tendency), and renal (structural, cysts, tubular acidosis) evaluations.[17]

Holt-Oram syndrome occurs in approximately 1 per 100 000 individuals and is caused by mutations in *TBX5* on chromosome 12q24. 85% of the individuals have mutation de novo, although it is inherited in an autosomal dominant manner.[60] It is always associated with upper limb deformity, namely preaxial radial ray malformation (eg, triphalangeal, hypoplastic, or absent thumb and/or radial dysplasia)[17] and, in 80% of cases, with CHDs (ASD, VSD, AVSD and/or conduction defects).[16]

Mutational analyses of the *TBX5* gene-coding regions will detect mutations in approximately three fourths of such patients.[17] Management involves a multidisciplinary team of specialists in medical genetics, cardiology, orthopedics, and hand surgery. Surveillance is done by annual ECG for all individuals, annual Holter monitor for individuals with known conduction disease, and echocardiogram every one to five years for those with septal defects.[60] In pregnancies at 50% risk (one parent affected), detailed high-resolution prenatal ultrasound examination may detect upper-limb malformations and/or congenital heart malformations.[60] Prenatal molecular genetic testing may be used to confirm a diagnosis if the*TBX5* pathogenic variant has been identified in an affected relative.[60] Blastocysts can be subjected to preimplantation genetic testing in vitro for TBX5, so that embryos without TBX5 mutation can achieve offspring.[61]

Char syndrome is caused by mutations in *TFAP2B* on chromosome 6p12. Phenotypically includes distinctive facial features (flat nasal bridge and nose tip, ptosis), hand deformities and PDA in term infants in 60-100% of these patients.[10,16]

Smith–Lemli–Opitz syndrome is an autosomal recessive disease caused by mutations in *DHCR7*.[62] It causes a defect in the conversion of 7-dehydrocholesterol to cholesterol, which leads to a deficiency of cholesterol. This syndrome affects an estimated 1 in 20,000 to 60,000 newborns. This condition is most common in whites of European ancestry, particularly people from Central European countries such as Slovakia and the Czech Republic. It is associated witch CHDs in 45% of cases, namely AVSD, primum ASD, VSD and PAPVR.[10] This condition is characterized by distinctive facial features, microcephaly, intellectual disability and behavioral problems, with some children having characteristic features of autism. Lungs, kidneys,

gastrointestinal tract and genitalia are also commonly affected. Infants with Smith-Lemli-Opitz syndrome have hypotonia, experience feeding difficulties, and have growth retardation. Most have syndactyly and some have polydactyly.[62]

Costello syndrome is very rare (affects probably 200 to 300 people worldwide) and is caused by *HRAS* mutations on chromosome 11p15.5.[63] Some people with signs and symptoms of Costello syndrome do not have an identified mutation in the *HRAS* gene. These individuals may actually have cardiofaciocutaneous syndrome or Noonan syndrome, which are caused by mutations in related genes. This condition is characterized by short stature, intellectual disability, loose folds of skin, unusually flexible joints, distinctive facial features including a large mouth, tight Achilles tendons, hypotonia, brain malformation, skeletal abnormalities, dental and vision problems. Heart defects occur in 63% of cases, typically PVS, HCM, and/or atrial tachycardia. Beginning in early childhood, these individuals have an increased risk of developing certain cancerous (rhabdomyosarcoma, neuroblastoma, bladder cancer) and noncancerous tumors (papillomas).[63]

Cardio-facio-cutaneous syndrome (CFCS) is also very rare (affects probably 200 to 300 people worldwide). This syndrome is characterized by mental retardation, facial dysmorphisms (high forehead, short nose, hypertelorism, down-slanting palpebral fissures, eyelid ptosis, small chin and low-set ears), ectodermal abnormalities (dry skin, nevi, wrinkled palms and soles, sparse or absent eyelashes and eyebrows), failure to thrive and heart defects (71% of cases), namely PVS, ASD and HCM.[16] Mutations in *BRAF*, *MEK1/MEK2* and *KRAS* have been implicated in CFCS.[16] CHD incidence, namely mitral valve defects and septal defects, was found to be lower in patients with *MEK1/MEK2* mutations.[64]

The following syndromes are caused by mutations in chromatin remodeling genes. Genes involved in this function were found to be associated with CHD development, such as non-syndromic *de novo* mutations (see below).

CHARGE syndrome is caused by mutations in *CHD7* in 2/3 of cases, and *SEMA3E*. It has an estimated prevalence of 1:10.000.[65] CHARGE stands for coloboma of the eye, heart defect, atresia choanae, retarded growth and development, genital abnormality (micropenis and cryptorchidism), and ear abnormality (hypoplastic semicircular canals, mild to profound hear loss and abnormal middle and external ear). 25% of these patients have no cognitive impairment.[18] Cardiac defects are associated in 50-85% of these patients and include ASD, VSD and valve defects.[10,16]

Kabuki syndrome is caused by mutations in *MLL2* in 55-80% of the cases (autosomal dominant pattern)[66] and *KDM6A* (X-linked dominant pattern)[67]. It is associated with CHD in 31-55% of cases, namely CoA, ASD, VSD, TOF, PDA, HLHS and mitral stenosis (MS)[16]. The phenotype is characterized by a unique facial appearance (arched eyebrows, long eyelashes, long palpebral fissures, eversion of the lower lip at the outside edges, a flat, broadened tip of the nose and large protruding earlobes), growth retardation (postnatal dwarfism), intellectual disability, skeletal abnormalities, spinal deformities (scoliosis), cleft palate and recurrent otitis media.

Schinzel-Giedon syndrome is a very rare and severe condition caused by heterozygous mutation *de novo* of *SETBP1* (chromosome 18q21.1). [68] Most affected individuals die before the age of ten and present severe mental retardation, neurological problems, such as seizures, feeding or hearing impairment, distinctive facial features (midface retraction, high protruding forehead, larger than normal fontanelles, hypertelorism and prominent ear lobes), skeletal abnormalities, genitourinary and renal malformations, and cardiac defects in 43% of cases. It is reported an increased prevalence of tumors, notably neuroepithelial neoplasia.[68]

Kleefstra syndrome is caused by the loss of *EHMT1* on the chromosome 9q34.3. 25% of individuals have mutations in the EHMT1 gene instead of a deletion[69]. This results in a lack of functional euchromatic histone methyltransferase 1 enzyme (histone modifier), and consequently impairs the control of activity of certain genes in many of body's organs and tissues. Clinical features include conotruncal heart defects (50% of cases), developmental delay, intelectual disability, severely limited or absent speech, hypotonia, microcephaly and brachycephaly. Distinctive facial features include eyebrows that grow together in the middle (synophrys), hypertelorism, midface hypoplasia, anteverted nares, prognathism, everted lips and macroglossia. Affected individuals may have a high birth weight and childhood obesity, structural brain abnormalities, genitourinary abnormalities, seizures, and a tendency to develop severe respiratory infections. During childhood they may exhibit features of autism.[70]

Recent studies have implicated cilia in the etiology of CHD and suggest that some CHDs are part of the ciliopathy spectrum of disease (Figure 1).

Nephronophthisis (NPHP) and several associated ciliopathies, Meckel-Gruber syndrome (MGS), Joubert syndrome (JS), and Senior-Loken syndrome (SLS), are characterized by a spectrum of phenotype, including renal cyst disease, retinal degeneration, and brain malformations. Mutations in 18 genes encoding proteins that

localize to the cilium or basal body have been linked to these four ciliopathies.[71] Among these genes, *NPHP2/INVS*, *NPHP3*, *NPHP4* and *NPHP9/NEK8* can also lead to heterotaxy, situs inversus and CHDs. A partial deletion of Nphp2/Invs in mouse results in situs inversus while an interstitial deletion in humans has been linked to transposition of the great arteries (TGA).[72] A complete loss of Nphp3 in mouse results in situs inversus and CHD, while *NPHP3* mutations in humans have been linked to situs inversus, structural heart disease and polydactyly.[73] *NPHP4* mutations in humans are associated with heterotaxy.[74]Loss of Nphp9/Nek8 in mouse and zebrafish results in renal cyst formation and cardiac laterality defects[75] while a homozygous nonsense mutation in humans results in cystic kidneys and CHD.[76]

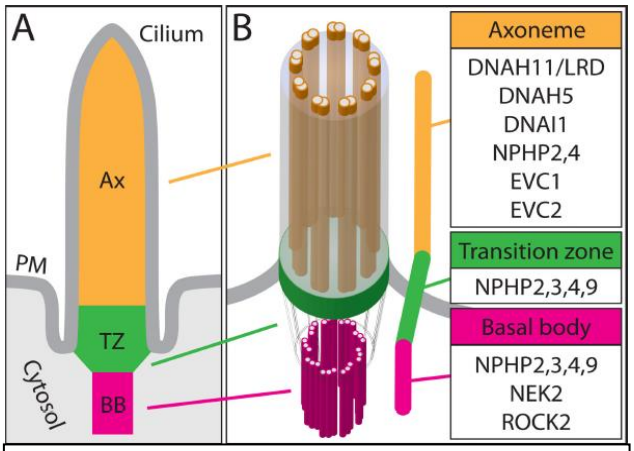

Figure 1. Ciliary genes associated with syndromic CHDs[18]

Ellis–van Creveld syndrome (EVCS) is a recessive skeletal dysplasia disorder that has been classified as a ciliopathy. EVCS is caused by mutations in *EVC1* and *EVC2* on chromosome 4p16. It is characterized by numerous defects, notably dwarfism, polydactyly, dysplastic nails and teeth, and CHD. Among EVCS patients with CHD (60%), 88% of affected individuals display a partial AVSD (primum ASD) and approximately 50% have an ASD producing a common atrium.[77]

Heterotaxy syndrome has X-linked inheritance due to mutations in the ZIC3 transcription factor and is tightly associated with CHD. It includes both cardiac (Dextrocardia, L-TGA, AVSD, TAPVR) and non-cardiac (eg, asplenia, polysplenia) manifestations.[18] Asplenia, also known as right atrial isomerism, is seen predominantly in males and is associated with severe cyanotic CHD (TAPVR, AVSD, TGA, single ventricle) absence of spleen, bilateral trilobed lungs and right atria, symmetrical liver and intestinal malrotation. Most patients die before one year-old. Polysplenia (left atrial isomerism) is seen predominantly in females and can be diagnosed later, as it is less severe than the latter. It is associated with less severe CHD (PAPVR, TAPVR, dextrocardia), multiple spleens, bilateral bilobed lungs and left atria and symmetrical or inverted liver.[78]

Due to the role of motile cilia in determining lateral patterning, defects affecting ciliary motility machinery result in heterotaxy and CHD. Not surprisingly, 6.5% of

patients with Primary Ciliary Dyskinesia (PCD), a disorder defined by abnormal ciliary motility in the airway epithelia, also display heterotaxy, which emphasizes the role of motile cilia in both disorders.[79] Similarly, next-generation sequencing of 13 heterotaxy patients with ciliary dysfunction identified mutations in known dynein components of the ciliary motility machinery: *DNAI1, DNAH5* and *DNAH11*.[80] Novel heterotaxy-causing genes were discovered, some of which have unanticipated roles in cilia structure and function.

Mutations in genes that cause syndromic CHD can occasionally produce isolated heart malformations.[16] Recurrent chromosomal or syndromic heart defects appear to contribute very little to the familiar clustering of same heart defect phenotypes.[28]

Non-syndromal single gene disorders

In the past 20 years, research has primarily focused on gene discovery in non-syndromal, familial forms of CHD. The proportion of cases falling into this group is still unknown, although it is presumed to be relatively small. Many of the genes reported to date encode transcription factors (proteins that regulate gene expression), receptors and ligands, but other types of proteins, particularly structural proteins such as cardiac actins and myosins, have also been implicated.[10] More than 40 genes have since been associated.[16] Many of them are listed in Tables 5[16] and 6[81]. DNA testing for most of the genes for isolated congenital heart defects is unavailable except on a research basis; however, testing of some of these genes is transitioning from the research laboratory to clinical availability.[17]

Autosomal dominant inheritance is most common; however, the pathogenicity of the reported mutations and the role they play in disease phenotype and segregation is, in most cases, not very clear. An example of autosomal inheritance is left-sided obstructive disease ranging from BAV to HLHS due to mutations in *NOTCH1*.[82] Although Mendelian recessive inheritance appears to contribute only a small fraction of the overall population burden of CHD, heterotaxy and AVSD can follow this pattern.[18]It is not the goal of this review to thoroughly address all of the genes discovered. Priority will be given to the latest discoveries in this area.

Gene	Protein	Phenotypes*
Transcription Factors and Co-factors		
ANKRD1	Ankyrin Repeat Domain	TAPVR
CITED2	c-AMP Responsive Element- Binding Protein	ASD; VSD
FOG2/ZFPM2	Friend of GATA	TOF, DORV
GATA4	GATA4 Transcription Factor	ASD, PS, VSD, TOF, AVSD, PAPVR
GATA6	GATA6 Transcription Factor	ASD, TOF, PS, AVSD, PDA, OFT defects, VSD
HAND2	Helix-Loop-Helix Transcription Factor	TOF
IRX4	Iroquois Homeobox 4	VSD
MED13L	Mediator Complex Subunit 13- like	TGA
NKX2-5/NKX2.5	Homeobox Containing Transcription Factor	ASD, VSD, TOF, HLH, CoA, TGA, DORV, IAA, OFT defects
NKX2-6	Homeobox Containing Transcription Factor	PTA
TBX1	T-Box 1 Transcription Factor	TOF, (22q11 deletion syndromes)
TBX5	T-Box 5 Transcription Factor	AVSD, ASD, VSD, (Holt Oram syndrome)
TBX20	T-Box 20 Transcription Factor	ASD, MS, VSD
TFAP2B	Transcription Factor AP-2 Beta	PDA, (Char syndrome)
ZIC3	Zinc Finger Transcription Factor	TGA, PS, DORV, TAPVR, ASD, HLH, VSD, Dextrocardia, L-R axis defects
Receptors, Ligands, and Signaling		
ACVR1/ALK2	BMP Receptor	AVSD
ACVR2B	Activin Receptor	PS, DORV, TGA, Dextrocardia,
ALDH1A2	Retinaldehyde Dehydrogenase	TOF
CFC1/CRYPTIC	Cryptic Protein	TOF; TGA; AVSD; ASD; VSD; IAA; DORV
CRELD1	Epidermal Growth Factor- Related Proteins	ASD; AVSD
FOXH1	Forkhead Activin Signal Transducer	TOF, TGA
GDF1	Growth Differentiation Factor-1	Heterotaxy, TOF, TGA, DORV
GJA1	Connexin 43	ASD, HLH, TAPVR, (Oculodentodigital dysplasia)
JAG1	Jagged-1 Ligand	PAS, TOF, (Alagille syndrome)
LEFTY2	Left-Right Determination Factor	TGA, AVSD, IAA, CoA, L-R axis defects, IVC defects
NODAL	Nodal homolog (TGF-beta superfamily)	TGA, PA, TOF, DORV, Dextrocardia, IVC defect, TAPVR, AVSD
NOTCH1	NOTCH1 (Ligand of JAG1)	BAV, AS, CoA, HLH
PDGFRA	Platelet-Derived Growth Factor Receptor Alpha	TAPVR
SMAD6	MAD-related protein	BAV, CoA, AS
TAB2	TGF-beta Activated Kinase	OFT defects
TDGF1	Teratocarcinoma-Derived Growth Factor 1	TOF, VSD
VEGF	Vascular Endothelial Growth Factor	CoA, OFT defects
Structural Proteins		
ACTC	Alpha Cardiac Actin	ASD
ELN	Elastin	SVAS, PAS, PS, AS, (Williams-Beuren syndrome)
MYH11	Myosin Heavy Chain 11	PDA, Aortic Aneurysm
MYH6	Alpha Myosin Heavy Chain	ASD, TA, AS, PFO, TGA
MYH7	Beta Myosin Heavy Chain	Ebstein Anomaly, ASD, NVM

Table 5. Genes that cause isolated CHD; Phenotypes in parentheses denote syndromes and/or extracardiac manifestations associated with gene mutations.[16]

CHM	BAV/AS	VSD	ASD	PS	TOF	AVSD	TGA	HLHS	DORV	Heterotaxy
Incidence[a]	14 / 0.8	4	1.0	0.7	0.4	0.3	0.2	0.2	0.16	0.1
Genes	*NOTCH1*	*NKX.2.5*[b] *GATA4* *TBX20* *TBX1* *ACTC1* *MYBPC3* *TBX5* *CITED2*	*NKX2.5*[b] *GATA4* *TBX20* *MYH6* *ACTC1* *MYH7* *MYBPC3* *TBX5*[b] *CITED2* *TLL1*	*GATA4* *MYOCD* *JAG1* *GATA6*	*NKX2.5* *GATA4* *TBX1* *FOG2* *CFC1* *NOTCH1* *GDF1* *TDGF1* *JAG1* *FOXH1*	*GATA4* *CRELD1* *CFC1* *GDF1* *NKX2.5*[c] *TBX5*[c] *HEY2*[c] *PTPN11* *ALK2*	*NKX2.5* *THRAP2* *ZIC3* *CFC1* *GDF1* *THRAP2* *FOXH1*	*NKX2.5* *NOTCH1* *HAND1*[c] *GJA1*[c]	*NKX2.5* *GDF1* *CFC1*	*ZIC3* *GDF1* *CFC1* *LEFTY2* *ACVR2B* *NODAL* *NKX2.5* *CRELD1* *FOXH1*

Table 6. Some genes involved in non-syndromic CHD clustered by cardiac defect.[81]

The first two genes to be linked to non-syndromal CHD were *NKX2-5* and *GATA4.*[10] *NKX2.5* mutations are associated with a range of lesions including ASDs associated with later-onset atrioventricular block, conotruncal defects (TOF, TGA, DORV), HLHS, Ebstein´s anomaly and VSD.[3,10] Mutations in *GATA4* have also been identified in patients with ASD and VSD.[13] Both gain-of function and loss-of-function mutations in *TBX20* result in familial ostium secundum ASDs and valve defects.[83] A gain-of-function mutation was found which significantly enhanced transcriptional activity of two target genes, *NKX2.5* and *GATA4/*5. These findings support the idea that the development of the atrial septum is a result of the complex interaction of multiple transcription factors.[83] It is also interesting to mention the great similarity between genes associated to ASD and to VSD.[81]

Non-syndromal single gene disorders often arise *de novo* such that the parents do not harbor the mutation and family history is negative for CHD. For highly penetrant mutations underlying severe forms of CHD, this mechanism is more likely since low reproductive fitness prior to the modern era provides strong negative selection against the accumulation of these mutations in the population.[3]

Exome sequencing was performed for 362 parent-offspring trios in which the offspring had a sporadic conotruncal defect, left ventricular outflow track obstructive lesion, or heterotaxy, and was compared to comparable data from 264 control trios.[84] It was found an excess burden of protein-altering mutations in genes highly expressed in the heart during heart development (OR of 2.53). These excess mutations accounted for 10% of severe CHD cases and led to the estimate that approximately 400 genes underlie these cardiac lesions. After filtering to retain only variants most likely to be deleterious (nonsense, splice site and frameshift defects), the burden among CHD cases increased, attaining an OR of 7.50.[84]

A highly significant enrichment of mutations among genes encoding proteins relevant for chromatin biology was observed, specifically the production, removal or reading of methylation of Lys4 of histone 3 (H3K4me).[84] The phenotypes of the eight subjects harboring H3K4me *de novo* mutations was diverse, both with respect to the form of CHD and extracardiac manifestations. In addition, two independent *de novo* mutations were identified in *SMAD2,* which encodes a protein with relevance for demethylation of H3K27me. SMAD2 contributes to the development of the left-right body axis; both subjects harboring *SMAD2* mutations had dextrocardia with unbalanced complete AVSD with PS. Mutations in genes involved in H3K4 methylation have been

implicated in human neurodevelopmental disorders including autism and Rett syndrome, and cognitive impairment is a prominent feature of the majority of human syndromes associated with mutations in histone modification genes.[18]

Mutations in non-coding regions, such as gene promoters, enhancers, locus control regions, microRNAs, and even intergenic regions are likely to be relevant for CHD causality, although not much has been discovered to date.[3] A recent study focused on the identification of cis-regulatory elements for *TBX5*, the gene mutated in Holt-Oram syndrome.[85] A 700-kb region around *TBX5* was scanned and three enhancer elements were sequenced in 260 unrelated individuals with ASDs, VSDs, or AVSDs, the predominant cardiac defects of Holt-Oram syndrome. It was identified a homozygous mutation in an enhancer of TBX5 in a patient with VSD. The authors provided strong evidence that this single nucleotide variant in a *TBX5* enhancer alters expression of the gene during heart development. Given the population-wide frequency of this variant, it is estimated that 1/100 000 individuals would be homozygous for this variant, highlighting that a significant number of CHD associated with TBX5 dysfunction might arise from non-coding mutations in TBX5 heart enhancers.[85] The challenges, well illuminated through this study, are how to prove causality definitively and how to scale such work as large numbers of candidate variants are identified through whole genome sequencing.

Familiar risk

In a minority of cases, it is possible to provide a precise recurrence risk for CHD, based on known Mendelian inheritance in a family or on risk figures related to a chromosomal anomaly. The transmission risk for autosomal-dominant conditions such as Holt-Oram, Noonan, Alagille, and CHARGE syndromes is 50%. Chromosomal microdeletion is also associated with a 50% risk of transmission, as reported for both 22q11.2 microdeletion and Williams syndrome. For recessive conditions, there is a 25% risk of both parents transmitting the disease-causing mutation to the offspring. However, Mendelian inheritance is observed in a relatively small proportion of families, so recurrence risk is usually based upon individual pedigree.[86]

In the absence of such information, empirical risk estimates must be used. The contribution of CHD family history to the overall prevalence of CHD in the populations was reported in 2.2%, and after the exclusion of chromosomal and extracardiac defects, the attributable risks were 4.2% and 3.6%, respectively.[28] The low population-

attributable risk from a positive family history can be explained by a variety of factors: environmental risk factors for CHD, low-penetrance genes or gene combinations in susceptible individuals; susceptible embryos may develop a heart defect if other important genes along the same pathway in embryonic heart development are disturbed due to additional inherited gene mutations, de novo mutations, copy-number variations, or unfavorable maternal and intrauterine factors. [28]

For the majority of patients with CHD and no family history or chromosomal abnormality, the risk of recurrence of CHD in offspring is around 3%.[86]The risk of recurrence for all types of CHD is threefold higher when a first-degree relative has CHD.[28] Parental consanguinity is associated with a 2-3 fold increased offspring risk of CHD, likely due to the shared genetic variants among parents.[87]

Recurrence risks vary between different types of nonsyndromal CHD with multifactorial inheritance (Table 7).[10] For most lesions, the reported recurrence risk in siblings of an affected individual, when neither parent is affected, is in the range of 1%-6%.[10] If more than one sibling is affected, the recurrence risk can increase to 10%.[10] The recurrence risk in offspring of affected parents is generally significantly higher than that in siblings of affected individuals with unaffected parents. Further, if the mother is the affected parent, the risk of disease transmission is higher (2-20% vs. 1-5% if the father is the only affected).[10] The reason for these differences is unknown, and it is difficult to reconcile them with known genetic mechanisms. However, maternal mitochondrial and cytoplasmatic inheritance has been considered among etiological mechanisms.[88,89] Generally, the recurrence risk increases if a parent rather than a sibling is affected, particularly when the affected parent is the mother.

Cardiac lesion	RR in siblings with unaffected parents		RR in children of affected parents	
	1 child affected	⩾2 children affected	Mother affected	Father affected
VSD	3%	10%	9%–10%	2%–3%
ASD	2%–3%	8%	6%	1%–2%
TOF	2%–3%	8%	2%–5%	1%–2%
CoA	2%	6%	4%	2%–3%
AS	2%	6%	12%–20%	5%
PS	2%	6%	6%–7%	2%
HLHS	3%	10%	nr	nr
AVSD	3%–4%	nr	10%–14%	1%
PA	1%	3%	nr	nr
TA	1%	3%	nr	nr
TGA	1%–2%	5%	nr	nr
L-TGA	5%–6%	nr	nr	nr
Ebstein anomaly	1%	3%	6%	nr
Heterotaxy	5%–6%	nr	nr	nr
Overall	**1%–6%**	**3%–10%**	**2%–20%**	**1%–5%**

ASD = atrial septal defect. AS = aortic stenosis. AVSD = atrioventricular septal defect. CoA = coarctation of the aorta. HLHS = hypoplastic left heart syndrome. L-TGA = congenitally corrected transposition of the great arteries. nr = not reported. PA = pulmonary atresia. PS = pulmonary stenosis. TA = truncus arteriosus. TGA = transposition of the great arteries. TOF = tetralogy of Fallot. VSD = ventricular septal defect.

Table 7. Recurrence risk (RR) of different types of congenital heart disease (CHD)[10]

Wang et al. (2014) analyzed a total of 61 familial pedigrees with CHD, and 134 patients out of 761 family members had a diagnosis of CHD confirmed.[90] The present study revealed that the

prevalence of CHD in first-degree relatives (55/249, 22.0%) was significantly higher than that in second-degree relatives (18/526, 3.4%). Additionally, the recurrence rate of CHD in families in which the patient's mother or sister had CHD was significantly higher than in cases with the father or brother having CHD. This study confirmed the previous findings and was the first study to show that the recurrence risk was higher in patients whose sister was affected than in those whose brother was affected.[90]

Interestingly, the approximated relative risk for CHD in monochorionic/diamniotic (MC/DA) twins was much stronger than in the general population (OR: 9.18, 5.51–15.29 ;$P < .001$).[91] In addition, MC/DA twin gestations affected by twin-twin transfusion syndrome (TTTS) were more likely to be complicated by CHDs than those that did not have TTTS (OR: 2.78, 1.03–7.52).[91] VSDs were the most frequent heart defects. PVS and ASD were significantly more prevalent in pregnancies complicated with TTTS.[91] Therefore, fetal echocardiography may be considered for all MC/DA twin gestations.[91] The relative risks in dizygotic twins and in singletons with a family history of any CHD in first-degree relatives were similar. The excess relative risk of CHD in monozygous twins and not in dizygotic twins indicates that twinning per se predisposes to CHDs, whereas the shared in utero conditions do not appear to play a big role.[91]

The first study of the recurrence of CHD in the population of mothers referred for fetal echocardiography for a family history reported a total recurrence rate of 1.9% after one previously affected child and 10% after two previously affected children.[92] More recently, Fesslova et al. (2011) studied 1634 pregnancies screened prenatally by fetal echocardiography because of a family history of CHD in either first degree or more distant relatives.[89] They pursued a final cardiologic examination of the child at least at 6 months of age or later, in order to detect even those smaller anomalies that are difficult to visualize in the fetus.[93] A higher recurrence rate of CHD than in previously published data was demonstrated (3.98%), mainly thanks to an improved detection of even milder forms of CHD both in utero and after birth.[89] The recurrence rate would have been lower if it was considered only cases diagnosed during fetal life (3.2%), more similar to the results of Gill et al.[92] Fesslova et al. (2011) considered as recurrent even the milder congenital heart lesions, such as small VSDs, discovered after birth that are often not detectable by fetal echocardiography as well as ASD that cannot usually be seen in the fetus, unless they are truly large.[89] The occurrence of these defects is important for the

family that is usually very anxious after a previous experience with a cardiac problem and also for the own knowledge of the recurrence.

Concordance of recurrent CHD (the same subtype of CHD) within members of the same family can vary substantially between different types of CHD. Being exactly concordant means that the cardiac defect was identical between two family members and concordant for the group if the defect belonged to the same spectrum of CHD, that is, group of shunts, conotruncal lesions, left and right heart obstructive lesions, and laterality defects/dextrocardia. The overall exact concordance of recurrent CHD is reportedly 37%, with a group concordance (within the same spectrum of CHD) of 44%, according to an analysis of 6,640 consecutive pregnancies evaluated by fetal echocardiography.[92] This study found that in families where there were two or more recurrences, the exact concordance rate was 55%, and these were particularly high for isolated AVSDs (80%) and laterality defects (64%).[92] According to Fesslova et al., complete concordance of recurrent CHD was found in 21.2% and a partial concordance in additional 20% of cases.[89]Among discordant pairs of lesions, some occur more frequently and mainly within the group of septal defects (ASD, VSD, AVSD) or between TOF, TGA, and VSD.[89] Equally, the variability between different types of the left ventricular obstructive lesions has been reported in several studies.[94]

The single-gene causes of familial clustering of nonsyndromic CHD are examples that could underlie the strong recurrence risks of the same heart defect phenotype (concordant defects).[28] They may also give rise to different phenotypic effects (pleiotropy) in the same family[17], which could explain the several case studies on families with clustering of different heart defects (discordant lesions).[28] Among other hypothesis to explain this situation, such as epigenetic and environmental interactions, one should bear in mind of the possibility of maternal and paternal imprinting, in which genes behave differently depending upon paternal versus maternal inheritance.[86]

Chromosomal and syndromic heart defects play only a small role in recurrent heart defects.[28] Although recurrence risk ratios are strong, very few families experience a second heart defect of any type, which is important to recognize for clinical counseling purposes.[28]

Familiar risk by specific CHD

Familial occurrence of AVSD is reported both in the presence of euploidy and aneuploidy (trisomy 21), with a rate in siblings about 3-4%,[10] the recurrent lesions being

often concordant. In another study, the rate in siblings was 6.5%, all affected cases being nonsyndromic. In some families there were reported discordant lesions such as VSD, ASD, patent ductus arteriosus, and hypoplastic right ventricle with PS.[95] Vertical transmission found in selected pedigrees suggests an autosomal-dominant mechanism, with monogenic or oligogenic inheritance.[89] Recurrence risk is about 10% for an affected parent, higher for mothers (14%) and lower for fathers (1%).[10]

Familial recurrence of CHD among patients with nonsyndromic TOF was reported of 2–5.5%[10,89], 2-5% in affected mothers and 1-2% in affected facthers.[10] An etiologic relationship was reported for the conotruncal lesions, PS, and VSD.[94]

TGA is generally considered to have a sporadic occurrence in families, with a total risk of around 3%[89], and 1-2% in siblings.[10,89] However, a study showed concordant cardiac defects within affected family members with TGA, suggesting that this disease is not always sporadic in families and supports monogenic or oligogenic inheritance of TGA in certain kindreds.[96] Another study found recurrent discordant lesions, namely VSD and PS.[89]

Corrected transposition of great arteries (l-TGA) has a total risk of recurrence of 6.7%[89] and a recurrence of 5-10% in siblings.[10,89]

Aortic stenosis (AS) has a total recurrence of 4.3%[89], a recurrence in siblings of 2%[10] and 7.4-20% in offspring when the mother is affected[10,89] and 5-5.9% when the father is affected.[10,89] CoA has a documented total recurrence of 2.2%[89] and a recurrence among affected mothers of 4-5.9%[10,89] and among affected fathers of 2-14.3%.[10,89] The highest recurrence rate observed in the offspring among affected fathers, may be due to the low number of affected parents observed in that study.[89] HLHS has a 5% risk of association with BAV in a parent and a sibling recurrence risk between 2 and 9%.[17] Obstructive lesions of the left outflow tract have a high degree of heritability and are presumed to be due to an altered embryonic blood flow. They may occur in families with variable degrees of severity starting with bicuspid aortic valve (BAV), AS, CoA, and HLHS suggesting a genetic predisposition to the flow alterations.[95,97] An autosomal recessive transmission has been suggested.[98] It is reported that up to 20% of the asymptomatic first-degree relatives of patients with obstructive left heart lesions may have undiagnosed CHD, in particular bicuspid aortic valve (BAV).[99] These data suggest that cardiac screening of relatives of patients with obstructive left heart lesions without noncardiac anomalies is justified. This may help prevent sudden,

unexpected, cardiac death or life-threatening complications in relatives with undetected BAV.[99]

PA and TA have each a recurrence in siblings reported as 1-3%.[10] Pulmonary stenosis is known to be frequently associated with Noonan syndrome and has a total recurrence rate documented between 2%[89] and 9.1%.[100] The recurrence in siblings is 2-5%.[10,89]

VSD has a total recurrence rate between 1.7 and 4.2%[89], 1.59-3% in siblings, 7-10% in affected mothers and 2-7% in affected fathers.[10,89]

Familial ASD is usually due to an autosomal dominant transmission. However, the genetics of ASD is considered to be heterogeneous.[100] The recurrence of ASD is reported between 2.5 and 4.1%[10,89], lower in fetuses from affected siblings (2-3%)[10,89] and higher in fetuses from affected mothers and fathers (6% and 1-5%).[10,89]

Recurrence for the laterality defects has been reported to be between 2 and 4.75%[89] and 5-6% in siblings. It was found a concordant recurrence in siblings of 5.3% with left isomerism and AVSD, but with different associated intracardiac lesions.[89] High relative risk ratio for heterotaxy recurrence among first-degree relatives was reported (79.1, 32.9-190).[28] Out of 22 cases with dextrocardia, either with situs solitus and or inversus, recurrent lesions occurred in 2 cases (total recurrence 8.7% and rate in siblings 2/14–14.3%).[89]

Environmental factors

Several environmental factors have been found that might cause CHD. However, less information is available regarding the potential adverse effect of modifiable risk factors on the fetal heart, which has made it difficult to develop population-based strategies targeting CHD development and to assist couples in making lifestyle choices to reduce the likelihood of having a child with a CHD. The proportion of CHDs potentially preventable through changes in the fetal environment is unknown, but it is suggested that the fraction of cases attributable to identifiable and potentially modifiable factors may be as high as 30 % for some defects.[12]

The modifiable risk factors are limited to the periconceptional period, which is defined as the 3 months before pregnancy through the third month of pregnancy, because the critical period for cardiac development is between 2 and 7 weeks of gestational age.[101]

Two population-based studies merit further mention because they are the source for the majority of information regarding risk factors for the development of CHD. The Baltimore-Washington Infant Study (BWIS), conducted between 1981 and 1989, was a case–control study designed to further characterize the epidemiology of CHD (59, 60). The case infants were live-born infants with a CHD from the Baltimore-Washington area. During the study period, 4,390 cases and 3,572 control patients were enrolled in the study. The National Birth Defects Prevention Study (NBDPS), which began in 1997, is designed to identify infants with and without major birth defects and to evaluate genetic and environmental factors associated with the occurrence of birth defects (35). This ongoing case-control study includes case and control infants from birth defect surveillance registries in 10 states (Arkansas, California, Georgia, Iowa, Massachusetts, New Jersey, New York, North Carolina, Texas, and Utah). As of December 2009, 27,812 cases and 10,200 control subjects are included in the database.

Parental age

Findings have shown that maternal age, both advanced and younger, is associated with CHD. Advanced maternal age was found to be a risk factor with an OR (95% CI) varying from 1.2 (1.1-1.3) for women with 35 years or older[102] to 4.0 (1.7-9.2) for women with 40 years or older compared with women aged 20-24 years.[103] According to Hollier et al. (2000), the additional age-related risk of nonchromosomal malformations was approximately 1% in women 35 years of age or older.[103] Advanced maternal age was found to be associated with EA, TGA, ASD, VSD, CoA and TOF.[12] Maternal age <20 years old was associated with lateraliy defects[102], TA[104] and total anomalous pulmonary venous return (TAPVR).[105]

A clear steady pattern of increasing relative risk with advancing paternal age from 35 years of age onwards was found for heart malformation (OR: 1.2, 1.1-1.4).[106] A matched case-control study performed in Egypt identified an association between advanced paternal age and VSDs.[107] However, given the weak association, paternal age appears to play a small role in the etiology of CHD.

Pre-gestational diabetes

Pre-gestational diabetes is highly associated with multiple congenital cardiac defects (OR varying between 2.2, 1.9-2.6,[108] and 4.6, 2.9-7.5[109]), including ASD, VSD, AVSD, CoA, double-outlet right ventricle (DORV), pulmonary atresia (PA), TAPVR,

TGA, TOF and truncus arteriosus.[12] It is hypothesized that pre-gestational diabetes inhibits expression of Pax3 in neuroepithelium through hyperglycemia-induced oxidative stress. This gene is required for cardiac neural crest (CNC) migration to the heart and for outflow tract septation. Therefore, its inhibition can lead to outflow tract defects.[110] It appears to induce malformation before the seventh weak of gestation, during the critical period of organogenesis, and is considered as an independent risk factor for mortality among infants with CHD.[12] The prevalence of birth defects among diabetic women with good glycemic control is similar to that in general population, so it appears that euglycemia reduces the risk of birth defects.[111] However, achieving and maintaining euglycemia in the early pregnancy remains a challenge because many women with diabetes do not plan their pregnancies and do not achieve adequate glycemic control before conception. Therefore, glycemic control has been proposed as a prevention strategy for birth defects, but as the prevalence of diabetes and risk factors for diabetes continues to rise, it appears that this may not be effective only by itself.[12] The associations between CHD with Gestational Diabetes Mellitus (GDM) were weaker and generally limited to offspring of women with a prepregnancy BMI $\geq$25.0 kg/m^2, which is consistent with the hypothesis that associations of birth defects with GDM may reflect associations with undiagnosed type 2 diabetes mellitus.[109]

Febrile illness

Maternal febrile illness during the first trimester of pregnancy has been investigated for its role in the development of CHDs. Nevertheless, it is difficult to evaluate febrile illness as a risk factor, due to its complex definition. It can be caused by respiratory infections, influenza, urinary infections and so on. Even limiting the definition of fever to the presence of these etiologies has not identified consistent associations, suggesting the need for further investigations. It was found in the Hungarian population an association between acute inflammatory pelvic disease (APID) in the second or third month of pregnancy and CHD (OR: 2.6, 1.2-5.4), particularly secundum ASD, with a 4.2 fold increase, but more studies are needed to support this finding.[112] Maternal fever or influenza were found to be associated with a significantly increased risk of having an offspring with a right-sided obstructive heart defect, especially TA and PA with intact ventricular septum, and AVSD in patients with Down Syndrome.[113] Antipyretic use might offer a protective effect and suggests that

symptomatic treatment of febrile illness and influenza in pregnant women might have beneficial effects on the developing fetus.[113]

The possible mechanisms underlying the associations observed between maternal fever or influenza and specific types of CHD are unclear. Associations between maternal urinary tract infections and cardiac defects were found, namely left ventricular outflow tract obstructive defects and AVSDs, which remained among women who did not have fever or used sulfonamides. However, the findings were not conclusive yet.[114]

Maternal rubella infection during the first trimester of pregnancy commonly results in multiple anomalies, including PDA, VSD, ASD, PS and TOF. Infections by cytomegalovirus, herpesvirus, and coxsackievirus B are suspected to be teratogenic if they occur in early pregnancy, but if they occur later in pregnancy they may cause myocarditis. Human immunodeficiency virus infection (in illicit drug users) has been associated with infantile cardiomyopathy.[78]

Phenylketonuria

Untreated maternal phenylketonuria is associated with a 6-fold-increased risk of heart defects.[115,116] The most frequent defects are TOF, VSDs, PDA and single ventricle. Fortunately, with strict diet control before conception and during pregnancy, this excess risk can be reduced.[116]

Hyperhomocysteinemia

Folate is essential to the cardiovascular development. Reduced levels of folate or vitamin B12 results in hyperhomocysteinemia. Homocysteine causes cellular oxidative stress through the production of reactive oxygen species and binds to nitric oxide or leads to the accumulation of its precursor, a potent inhibitor of biologic transmethylations, Three studies demonstrated an increased risk of CHD in infants born to mothers with high homocysteine levels (OR varying between 1.5 and 3.5), but their sample sizes were not big enough to reach any definite conclusion.[12]

Hypertension

It was identified an approximate twofold increased risk of CHDs among infants born to mothers with hypertension, but no association by defect was found, and it is still being investigated whether the underlying hypertension or the antihypertensive drugs is

the basis for the increased risk. Nonetheless, it is suggested that the apparent increased risk of cardiac malformations is more likely to be caused by the underlying hypertension (OR 1.4, 1.3-1.5) rather than the antihypertensives used during the first trimester of pregnancy (OR: 1.12, 0.76 - 1.64).[117]

Prepregnancy weight

The prevalence of overweight and obesity is increasing at an alarming rate. Increased prepregnancy weight is associated with a slightly increased risk of CHDs. There was a highly significant trend of increasing risk for CHDs with increasing maternal obesity: Body Mass Index (BMI) 30-34.9 (OR 1.16, 1.07-1.25); BMI 35-39.9 (OR 1.25, 1.13-1.39); BMI ≥ 40 (OR 1.49, 1.32-1.69). Overweight (BMI between 25 and 30 kg/m^2) was not associated as a risk factor (OR: 1.00, 0.94-1.06), but it is not conclusive, so further studies are needed to confirm this conclusion.[118] The association was greatest for left and right ventricular outflow tract defects and no association was found for conotruncal defects (OR 1.04, 0.82-1.33)[119]. Each category of BMI was associated with different CHDs. Overweight mothers were more likely to have an infant with CoA[120], although the sample size of the study was very limited. Obese mothers were more likely to have an infant with ASD, AS, HLHS, PVS or truncus arteriosus.[118] A BMI greater than 40 kg/m^2 was more associated with DORV.[118]

Reproductive history

History of infertility and use of assisted reproductive techniques (ART), which include inductors of ovulation (IO), in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), have been identified as having a 1.3 fold increased risk of CHDs of any type and 1.4 fold increased risk of CHD without chromosomal abnormalities.[121]

In contrast, it was not found any statistically significant association between IO and CHD. The cardiac defects most reported are ASD, AS, CoA, TOF and VSD.[121] It was found an association between clomiphene citrate use in CoA, AS and VSD as a subfertility treatment in the period from 2 months before conception through the first month of pregnancy.[122] Although the mechanism is not clear, couples considering ART should be informed of all potential risks and benefits.

Multiparous women have a greater risk than mothers of infants without structural heart defects.[12] Nulliparous mothers in general do not have an elevated risk of

having an infant with CHD, however when stratified by defect, significant associations were found with ASD, VSD and TOF.[123]

Socioeconomic Status

Low maternal and paternal socioocupational status was associated with infant CHDs in an investigation of more than 81,000 births in Denmark (OR: 1.4-1.6).[124] A 3.4 fold risk was shown as well in mothers with low socioeconomical status (SES) in a small case-control study in Lithuania.[125] A large multicentered case-control study was conducted in 1,841 cases and 2,551 nonmalformed liverborn controls delivered in 1997-2000. Particular characteristics, such as unemployed mother or father, low education of either parent, low household income and maternal occupation were associated with increased risks of TGA and TOF. However, no marker of socioeconomic status was statistically significant.[126] This study revealed consistently increased risks of selected birth defects in association with household SES index but not individual SES measures.

Stress

One potential mechanism whereby stressful life events may lead to birth defects is through increased production of maternal corticotrophin-releasing hormone and corticosteroids during pregnancy. High levels of these hormones are associated with hyerinsulinemia, insulin resistance and teratogenicity. Stress can result as well in increased catecholamine production, which leads to decreased uterine blood flow and increased fetal hypoxia.[12] A case-control study in China identified a threefold greater risk of CHDs[127] and an association with TGA was identified.[128] The maternal periconceptional stressful life events, social support, and the two factors in combination were at most modestly, if at all, associated with risks of conotruncal heart defects. However, social support was associated with reduced risks, most notably for TGA.[129]

Maternal medications

Thalidomide is known to be a cardiac teratogen and therefore contraindicated during pregnancy and among women planning a pregnancy. Thalidomide embryopathy includes CHD from VSDs and ASDs to complex conotruncal defects.[130] No safe dose of thalidomide treatment during the critical period of gestation has been established, and

cases of thalidomide embryopathy have been described after maternal ingestion of as little as one 50-mg capsule during this time.

Maternal intake of isotretinoin has been shown to cause congenital cardiac defects in addition to other malformations. Characteristic features of isotretinoin embryopathy include central nervous system malformations, micrognathia, cleft palate, thymic and eye anomalies, and cardiac and great vessel defects. The frequency of congenital anomalies does not appear to be increased among children of women who discontinue therapy before conception. These medications are contraindicated during pregnancy and among women planning a pregnancy.[115]

The use of antidepressants during the periconceptional period , such as selective serotonin-reuptake inhibitors (SSRI's), serotonin-epinephrine reuptake inhibitors (SNRI's) and tryciclic antidepressants (TCAs), is associated with CHD development.[131,132,133] When evaluated by defect, ASD (paroxetine[134] and venlafaxine use [132]), VSD (fluoxetine use)[131] and CoA (venlafaxine use)[132] remained significantly associated. However, given the weak associations noted, additional investigations are needed.

Antihypertensives were associated with a two- to threefold increase in the risk of CHD.[135,136] However, more recent studies have not reported significant associations[117] and conflicting results have been reported when examining specific types of antihypertensive medications, such as beta-blockers and ACE inhibitors.[117,135,136] According to the NBDPS database, secundum ASD, CoA, Ebstein´s anomaly and PVS were found associated with first trimester use; secundum ASD, perimembranous VSD and PVS were associated to antihypertensive treatment after the first trimester.[135] A modest nonsignificant elevation in the risk for all CHDs was observed not only to first-trimester ACE inhibitor use, but for antiadrenergic, beta-blockers and, in a lesser extent, to diuretics as well.[135] Calcium-channel blockers were not associated with increased risk.[135] These findings contrast with those reported by Cooper et al.[137] In that study, first-trimester ACE inhibitor use was associated with risk of CHDs (OR: 3.7, 1.9-7.3), whereas use of other medication classes was not, leading these authors to attribute their findings to ACE inhibitor exposure and not to the underlying hypertension.

Anti-infection medications, including antibiotics and antifungals, are commonly prescribed during the periconceptional period of pregnancy. Two large studies using the NBDPS and the Swedish Medical Birth Register were unable to identify a significant association between general maternal use of antibiotics and the development of

CHDs.[138] However, increased risk of CHD[139], CoA and HLHS[138] have been reported for mothers using sulfonamide medications (folic acid antagonists). These increased risks also were reduced if the mother concomitantly took folic acid supplementation.[140] The BWIS identified a significant association between metronidazole use and the development of CHDs (OR: 2.5, 1.1-5.8).[141] TOF[142] and VSD[143] were found associated with metronidazole use. Examination of the NBDPS data did not identify an increased risk among mothers who reported using metronidazole.[144]

No consistent associations between maternal use of any type of NSAID and the development of CHDs have been reported.[145,146] Various types of NSAIDs also have been evaluated for their role in CHD formation. Although aspirin is not significantly associated with CHDs as a group, maternal use of aspirin is reported to be associated with interrupted aortic arch (IAA).[147] The BWIS data showed an association between ibuprofen use and an increased risk of CHDs.[141] Stratification by defect identified a significant association between ibuprofen and Down syndrome associated with AVSD, DORV, and TGA.[142] An association between maternal use of naproxen and CHDs overall has been demonstrated, namely PVS.[145,148]

Amphetamines have been associated with VSD, PDA, ASD and TGA.[78] Anticonvulsivants are suspecting of causing CHD. Specifically, phenytoin has been associated with PVS, AS, CoA and PDA.[78] Valproic acid may be associated with various heart defects such as ASD, VSD, AS, PA with intact ventricular septum, and CoA.[154] Trimethadione has been associated with TGA, TOF and HLHS[78] and lithium has been associated with Ebstein's anomaly.[152] Retinoic acid may cause conotruncal anomalies, namelyTGA, as observed in murine and chick embryo hearts.[153] Other medications suspected of causing CHD (VSD, TOF, TGA) include progesterone and estrogen.[78]

Multivitamin supplements containing folic acid may reduce the risk for some types of CHD, similar to the known risk reduction of neural tube defects seen with folic acid. The use of periconceptional folic acid supplements was related to approximately 20% reduction in the prevalence of any CHD.[149]TGA[150] and VSD[143] exhibited significant risk reduction. Reduction in risk was present when the multivitamin supplementation was used at about the time of conception or early in the first month of pregnancy but not when it was started during the second or third months of pregnancy, suggesting that the underlying mechanism of folate is most effective during the critical period of cardiac development.[12] The exact mechanism of folic acid's protective effect

has yet to be elucidated. One hypothesis is that folic acid prevents congenital defects by stimulating cellular methylation reactions. Folic acid-deficient rats have been reported to produce offspring with VSDs and defects of the outflow tract and great vessels, which corroborates the risk reduction of TGA and VSD.[151]

Parental nontherapeutic drug exposures

Alcohol may have an impact on heart development through its contribution to impaired conversion of retinol to retinoic acid, antagonism of the N-methyl-D-aspartate (NMDA) receptor, compromised nutritional status, and vascular disruptive events.[12] Conflicting results have been reported for maternal alcohol consumption during the periconceptional period. A majority of studies have not reported a significant association between maternal alcohol use and CHDs. However, a case-control study reported a threefold risk for CHDs among mothers who reported periconceptional alcohol use.[155] Increased risks were observed for ASD[156], TGA[157], and VSD.[158] The majority of studies evaluating the adverse outcomes related to alcohol have focused on maternal consumption, but paternal consumption also has been investigated for its role in CHDs. One study identified a fourfold increase in risk for VSDs among fathers who reported alcohol use.[158]

Caffeine is known to cross the placenta, so concerns have led to inform pregnant women to limit their caffeine intake.[115] Using data from the NBDPS, the consumption of coffee, tea, soda, and chocolate was examined for an association with select CHDs, and no evidence for a teratogenic effect of caffeine was identified.[159]

Various studies, including BWIS database, did not identify a significant association between maternal periconceptional cigarette smoking and the development of CHDs as a group.[160] However, a study using the NBDPS database did identify an increased risk for the development of CHDs among mothers who reported use of cigarettes (OR: 1.2, 1.1-1.4).[161] A population-based study showed as well that offspring of mothers reporting cigarette use in the first trimester of pregnancy were more likely to be born with a CHD (OR 1.16, 1.08-1.24).[163] Subtypes at increased risk are: AVSD (strongest association in mothers who smoked more than 25 cigarettes/day)[162], ASD, PVS, VSD[161], truncus arteriosus (OR: 1.90, 1.04-3.45), and levo-transposition of the great arteries (l-TGA) (OR: 1.79, 1.04-3.10).[160] The association was stronger with increasing number of daily cigarettes, namely more than ten cigarettes daily, and among older (35+ years) mothers compared with younger mothers.[163] It was also demonstrated

an association between maternal passive smoke exposure and AVSDs.[162] Few studies have evaluated the effect of paternal smoking, and the results have been conflicting, similar to the results for maternal smoking. The BWIS did not identify a significant association between paternal cigarette smoking and CHDs as a group or any CHD subtype.[141] A study performed in Italy demonstrated an increased risk of CHDs as a group among fathers who reported smoking (OR 1.7, 1.1-2.6).[164] The mechanisms by which cigarette smoke or the chemical compounds contained within the smoke might result in CHDs remain to be elucidated.

Data from the BWIS showed an association between maternal and paternal cocaine use and CHDs (OR: 1.6, 1.1-2.3; OR: 1.7, 1.3-2.2, respectively).[141] Stratified by defect, VSD, TA, ASD and AVSD were associated with cocaine use, remaining AVSD as the only not associated with paternal cocaine use.[142] Cocaine may exert direct toxic effects on fetal myocytes. Although maternal marijuana use is not reported to be associated with CHDs as a group, findings have demonstrated its association with VSDs[158] and Ebstein's anomaly[142]. However, data from the BWIS have demonstrated that paternal marijuana is associated with an increased risk for the development of any type of CHDs (OR: 1.2, 1.1-1.4)[141], more specifically VSD[165] and TA[142]. Although these studies did demonstrate positive associations, it should be noted that the number of individuals who reported illicit drug use was extremely small, making interpretation of these results limited.

Studies have examined the effects of maternal environmental air pollutant exposure and subsequent CHD development and there was little or no association.[166,167]

Exposure to occupational chemicals, especially during the periconceptional period, influences the reproductive system in both men and women and may lead to adverse health effects in children. Plausible mechanisms include reduced sperm quality, disrupted epigenetic programming during maturation of sperm cells, impaired maturation of oocytes, and impaired embryogenesis.[168] However, the relationship between chemical exposure and CHDs is not clear.[169] Associations between maternal exposure to organic solvents during the periconceptional period were observed for CoA, HLHS, and TGA.[142] Maternal exposure to cyanide or heavy metals is associated with CHDs (OR: 2.2, 1.3-3.9; OR: 1.5, 1.1-2.3, respectively).[170] A study showed an association between paternal exposure to phthalates or alkylphenolic compounds during the periconceptional period and an increased risk of infant CHDs in general (OR: 1.7, 1.1-2.5; OR: 1.8, 1.1-3.0, respectively).[171] In this study, paternal exposure to phthalates

was associated with VSDs, exposure to polychlorinated compounds was associated with AVSDs, and exposure to alkylphenolic compounds was associated with CoA.[171] Chemical exposures were difficult to define in these studies, and the associations identified were weak, suggesting that these results need to be replicated for a definition of the true risk they represent.

Multiple studies have examined the relationship between CHDs and maternal exposure to contaminated water with trichloroethylene and dichloroethylene. Trichloroethylene, a hydrocarbon solvent used as a metal degreasing agent, is an intermediate product in the production of polyvinyl chloride.[172] It also has uses as an anesthetic, antiseptic, solvent for dry cleaning, and coffee decaffeination. Dichloroethylene is a chemical used for synthesis of polyvinyl chloride and plastic packaging. Gestational dichloroethylene exposure has been demonstrated to result in a significant proportion of CHD (OR: 2.8, 1-.3-5.9).[173,174]

A six-fold increased risk of CHD in infants born to mothers older than 38 years exposed to trichloroethylene compared with those born to nonexposed younger mothers was identified (OR: 6.2, 2.6-14.5).[175] However, two large reviews have reported no association between maternal exposure to trichloroethylene contaminated water and CHDs.[172,173]

Significant associations between multiple risk factors and CHDs have been identified. Studies investigating a majority of these risk factors have yielded conflicting results, suggesting that additional investigations need to be performed. Most of the studies have had small samples due to the rarity of specific types of CHDs. It is also difficult to perform precise measurements of some exposures, such as occupational and environmental exposures. It is interesting to note that a large proportion of the risk factors were observed to be associated with a variety of CHDs, suggesting that chance associations may have been observed as opposed to true associations. Mechanisms for these associations are difficult to define because multiple categories of defects were found to be associated with a specific risk factor, further implying that these associations occurred by chance.[12]

Specific congenital heart defects

In the following section, the majority of specific congenital heart defects will be approached, namely regarding general considerations, including epidemiology,

environmental factors associated and phenotypes. Management of CHDs remains beyond the scope of this review.

Left-to-Right Shunt lesions

Atrial septal defect (ASD)

Atrial septal defect (ASD) occurs as an isolated anomaly in 5 % to 10% of all congenital heart defects, may be first encountered in the adult and occurs more frequently in females (male/female ratio of 1:2). About 30% to 50% of children with CHDs have an ASD as part of the cardiac defect.[78] There are four types of ASD: Ostium secundum defect, ostium primum defect, sinus venous defect and coronary sinus ASD, a very rare defect (Figure 2). Patent foramen ovale (PFO) does not ordinarily produce intracardiac shunts; it is considered a common normal variant, which lacks total obliteration of the foramen ovale after birth.

Environmental factors for ASD include advanced maternal age (OR: 2.5, 1.5-4.1)[105], pre-gestational diabetes (OR varying from 2.3, 1.8-2.8 [108], to 8.5, 4.4-16.4 [109]), gestational diabetes (OR: 2.2, 1.5-3.2)[109], prepregnancy obesity (OR: 1.2, 1.1-1.4)[118], infertility/ART (OR: 3.4, 1.6-6.2)[176], nulliparity (OR: 1.3, 1.1-1.5)[123], paroxetine(OR: 5.7, 1.4-23.5)[134] and venlafaxine use (OR: 2.9, 1.2-6.9)[132], antihypertensive use during first and/or other semesters (OR: 2.4, 1.3-4.4)[135], amphetamines[78], valproic acid[154], alcohol (OR: 2.0, 1.1-3.4)[156], cigarette use (OR: 2.0, 1.5-2.6)[161] and paternal cocaine use (OR: 2.3, 1.3-4.2).[142]

Patients with ASD are usually asymptomatic in early life although there may be some physical underdevelopment (relatively slender body build) and an increased tendency for respiratory infections; Cardiorespiratory symptoms occur in many older

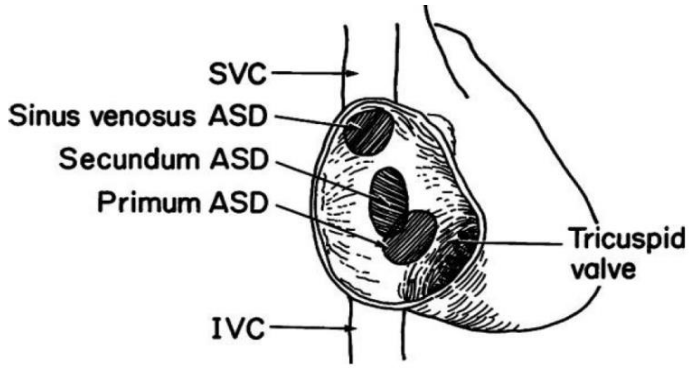

Figure 2. Anatomic types of atrial septal defects (ASDs) viewed with the right atrial wall removed. IVC, inferior vena cava; SVC, superior vena cava.[78]

patients. During the fifth and sixth decades, the incidence of progressive symptoms, often leading to severe disability, increases substantially. If a large defect is left untreated, a significant number of patients beyond the third and fourth decades of life develop atrial arrhythmias, pulmonary arterial hypertension and right heart failure. Patients with sinus venosus or ostium secundum ASDs rarely die before the fifth decade.[7]. The defect may decrease in size or even close spontaneously in some patients.

Ventricular septal defect (VSD)

VSD is the most common of all cardiac birth defects and accounts for 15% to 20% of all such defects, either as an isolated defect or as a component of a combination of anomalies, not including those occurring as part of cyanotic CHDs. The VSD is usually single and situated in the membranous or midmuscular portion of the septum, but it can occur in other regions of the septum. Only small- or moderate-size VSDs are seen initially in adulthood, as most patients with an isolated large VSD come to medical or surgical attention early in life.[7]

Environmental risk factors for VSD include advanced maternal age (OR: 2.5, 1.8-3.5)[105], advanced paternal age (OR: 1.7, 1.3-2.3) based on a study conducted in the children's hospitals of Alexandria, Egypt[107], pre-gestational diabetes (OR varying between 1.5, 1.2-1.8 [108], and 2.9, 1.3-6.6 [109]), infertility and ART (OR: 1.4, 1.1-1.8)[121], clomiphene citrate use as a subfertility treatment (OR: 1.6, 1.1-2.4)[122], nulliparity (OR: 1.4, 1.2-1.6)[123], fluoxetine (OR: 1.7, 1.1-2.4)[131], antihypertensive treatment after the first trimester for perimembranous VSDs (OR: 2.3, 1.2-4.6)[135], metronidazole (OR: 1.6, 1.2-2.3)[143], amphetamine, progesterone and estrogen use[78], valproic acid[154], maternal alcohol use (OR: 3.1, 1.2-8.2)[158], paternal alcohol use (OR: 4.0, 1.6-9.9)[158], cigarette use (OR: 1.3, 1.1-1.7)[161], maternal cocaine use (OR: 2.9, 1.7-4.8)[142], paternal cocaine use (OR: 2.3, 1.6-3.3)[142], maternal marijuana use (OR: 2.4, 1.4-3.9)[158], paternal marijuana use (OR: 2.2, 1.1-4.4)[165] and paternal exposure to phthalates (OR: 2.8, 1.4-5.9).[171] VSDs exhibited significant risk reduction with folic acid use (OR: 0.8, 0.8-0.9).[143]

The defects vary in size, ranging from tiny defects without hemodynamic significance which can close spontaneously to large defects with accompanying CHF, pulmonary hypertension and death in infancy.

Atrioventricular septal defect (AVSD)

There are two forms of AVSD: complete and partial. Complete AVSD (also called complete endocardial cushion defect or AV communis) occurs in 2% of all congenital heart defects. Of patients with complete AVSD, about 70% are children with Down syndrome. Of children with Down syndrome, about 40% have congenital heart defects and 50% of the defects are AVSD. AVSD is also a component of heart defects in asplenia and polysplenia syndromes. It consists in an ostium primum ASD, inlet VSD and clefts in the anterior mitral valve and the septal leaflet of the tricuspid valve (forming the common AV valve). Partial AVSD (also called partial endocardial cushion defect or ostium primum ASD) occurs in 1% to 2% of all congenital heart defects and consists in an ASD with two AV valve orifices present without an interventricular shunt.[78]

Risk factors for AVSD include pre-gestational diabetes (OR varying from 2.2, 1.2-4.0[108], to 12.4, 3.7-41.5[109]), maternal urinary tract infections, although not conclusive yet (OR: 2.3, 1.1-4.7)[114] and maternal fever (OR: 1.9, 1.1-3.4) and influenza (OR: 1.7, 1.04-2.6) in infants with Down Syndrome.[113] It is unclear why AVSD in patients with Down syndrome may be associated with fever and influenza and AVSD in patients without Down syndrome is not. This finding may represent an important gene–environment interaction that warrants further investigation, or the observed association may be only incidental.[113] Other environmental risk factors are: ibuprofen associated with Down Syndrome-AVSD (OR: 2.4, 1.1-4.2)[142], cigarette use (OR: 1.5, 1.1-2.1) with the strongest association in mothers who smoked more than 25 cigarettes/day[162], maternal passive smoke exposure (OR: 1.5, 1.1-2.1)[162], cocaine use (OR: 3.5, 1.1-11.4)[142] and paternal exposure to polychlorinated compounds (OR: 4.2, 1.2-14.4).[171]

Abnormalities seen in complete AVSD may result in interatrial and interventricular shunts, LV-to-RA shunt, and AV valve regurgitation. Clinical manifestations include failure to thrive, repeated respiratory infections, and signs of CHF. For patients with complete AVSD, heart failure occurs 1 to 2 months after birth and recurrent pneumonia is common. Without surgical intervention, most patients die by the age of 2 to 3 years. In the latter half of the first year of life, the survivors begin to develop pulmonary vascular obstructive disease. These survivors usually die in late childhood or as young adults. Infants with Down syndrome are particularly susceptible to the early development of pulmonary vascular obstructive disease during infancy. As a result, surgery should be performed during infancy.[78]

Pulmonary outflow obstruction

Pulmonary valve stenosis (PVS)

Obstruction to RV outflow may be localized to the supravalvular, valvular, or subvalvular levels or occur at a combination of these sites. Multiple sites of narrowing of the peripheral pulmonary arteries are a feature of rubella embryopathy and may occur with both the familial and sporadic forms of supravalvular aortic stenosis. Valvular pulmonic stenosis is the most common form of isolated RV obstruction.[7]

Environmental risk factors include prepregnancy obesity (OR: 1.3, 1.1-1.6)[118], antihypertensive treatment during the first trimester of pregnancy (OR: 2.6, 1.3-5.4)[135] or after first trimester (OR: 2.4, 1.1-5.4) [135], naproxen (OR: 2.4, 1.3-4.5)[148], phenytoin[78] and cigarette use (OR: 2.3, 1.1-4.8).[161]

Patients with mild PVS are generally asymptomatic and demonstrate little or no progression in the severity of obstruction with age. In patients with more significant stenosis, the severity may increase with time. Symptoms vary with the degree of obstruction. Fatigue, dyspnea, RV failure, and syncope may limit the activity of older patients, in whom moderate or severe obstruction may prevent an augmentation of cardiac output with exercise.[7]

Pulmonary atresia (PA)

Pulmonary atresia with intact ventricular septum accounts for fewer than 1% of all congenital heart defects.[78] A history of severe cyanosis since birth is present.

Risk factors for PA include pre-gestational diabetes (OR: 7.2, 1.6-31.4)[12] and maternal influenza (OR: 2.7, 1.2-6.3)[113].

Without appropriate management (which includes PGE1 infusion and surgery), the prognosis is exceedingly poor. About 50% of these patients die by the end of the first month if not managed properly; about 80% die by 6 months of age.[78]

Aortic outflow obstruction

Malformations that cause obstruction to LV outflow include AS, discrete subaortic stenosis, SVAS, BAV, CoA and HLHS. Obstructive left heart lesions generally have noticeably higher recurrence risks in siblings of unaffected parents and/or offspring of affected parents compared with other types of CHD.[177] The phenotypes are often discordant between first-degree relatives.[15]

Aortic valve stenosis (AS)

Aortic stenosis (AS) occurs in about one-fourth of all patients with chronic valvular heart disease; approximately 80% of adult patients with symptomatic, valvular AS are male.[7]

Risk factors for congenital AVS include pre-gestational diabetes (OR: 5.0, 1.1-22.9)[109], prepregnancy obesity (OR: 2.2, 1.2-4.0)[118], infertility and clomiphene citrate use as a subfertility treatment (OR: 2.6, 1.2-5.3)[122].

AS is rarely of clinical importance until the valve orifice has narrowed to approximately 1 cm^2. Even severe AS may exist for many years without producing any symptoms because of the ability of the hypertrophied LV to generate the elevated intraventricular pressures required to maintain a normal stroke volume. Once symptoms occur, valve replacement is indicated. Exertional dyspnea, angina pectoris, and syncope are the three cardinal symptoms. Often, there is a history of insidious progression of fatigue and dyspnea associated with gradual curtailment of activities and reduced effort tolerance.[7] Diagnosis is made by echocardiography, which reveals the morphology of the aortic valve and aortic root and quantitates severity of stenosis or regurgitation.[7]

Supravalvular aortic stenosis

This is a localized or diffuse narrowing of the ascending aorta originating just above the level of the coronary arteries at the superior margin of the sinuses of Valsalva.[7] It is the most commonly associated cardiac defect in WilIiams-Beuren syndrome (mutations of ELN gene on chromosome 7), but it can also occur as non-syndromic CHD.[10]

Bicuspid aortic valve (BAV)

Bicuspid aortic valve is the most common congenital heart valve defect, occurring in 0.5–1.4% of the population, and is more common in males than in females (2-4:1). The congenital bicuspid aortic valve may initially be functionally normal and may go undetected in early life.[7]

The inheritance pattern appears to be autosomal dominant with incomplete penetrance, although some have questioned an X-linked component as suggested by the prevalence of BAV disease among patients with Turner's syndrome.[29] The prevalence of BAV disease among first-degree relatives of an affected individual is approximately 24%.[17] It is a frequent finding in parents of children with other left-sided obstructive

defects and there is a 19.3% prevalence of cardiac anomalies in first-degree relatives with BAV.[17] A single gene defect to explain the majority of cases has not been identified, although a mutation in the *NOTCH1* gene has been described in some families.[7]

Coarctation of the aorta (CoA)

Narrowing or constriction of the lumen of the aorta may occur anywhere along its length but is most common distal to the origin of the left subclavian artery near the insertion of the ligamentum arteriosum. Coarctation occurs in around 7% of patients with CHD, is more common in males than females, and is particularly frequent in patients with Turner syndrome.[7]

Environmental risk factors for CoA include advanced maternal age (OR: 1.5, 1.1-2.2)[102], pre-gestational diabetes (OR: 1.9, 1.2-3.1)[108], prepregnancy overweight (OR: 3.9, 1.1-13.8)[120], infertility, ART and clomiphene citrate use (OR: 2.3, 1.4-3.8)[122], venlafaxine (OR: 4.5, 1.4-12.5)[132], antihypertensive treatment during the first trimester (OR: 3.0, 1.3-6.6)[135], sulfonamide (OR: 2.7, 1.3-5.6)[138], phenytoin[78], valproic acid[154], organic solvents (OR: 3.2, 1.3-7.9)[142] and paternal exposure to alkylphenolic compounds (OR: 3.9, 1.2-12.7).[171]

Most children and young adults with isolated, discrete coarctation are asymptomatic. Headache, epistaxis, chest pressure, and claudication with exercise may occur, and attention is usually directed to the cardiovascular system when a heart murmur or hypertension in the upper extremities and absence, marked diminution, or delayed pulsations in the femoral arteries are detected on physical examination.

The chief hazards of proximal aortic severe hypertension include cerebral aneurysms and hemorrhage, aortic dissection and rupture, premature coronary arteriosclerosis, aortic valve failure, and LV failure; infective endarteritis may occur on the coarctation site or endocarditis may settle on an associated bicuspid aortic valve, which is estimated to be present in 50% of patients.[7]

Hypoplastic Left Heart Syndrome (HLHS)

HLHS occurs in 1% of all congenital heart defects. HLHS includes a group of closely related anomalies characterized by hypoplasia of the LV and encompasses atresia or critical stenosis of the aortic or mitral valves, or both, and hypoplasia of the ascending aorta and aortic arch.[78]

Risk factors include prepregnancy obesity (OR: 1.7, 1.2-2.5)[118], sulfonamides (OR: 3.2, 1.3-7.6)[138], trimethadione[78] and organic solvents (OR: 3.4, 1.6-6.9).[142]

Echocardiography findings are diagnostic and usually obviate the need for cardiac catheterization and angiocardiography. Pulmonary edema and CHF develop in the first week of life. Circulatory shock and progressive hypoxemia and acidosis result in death, usually in the first month of life.[78] However, with the proper medical and surgical care, it is estimated that 70% of HLHS patients will reach adulthood.[178]

Laterality defects

General considerations about heterotaxy syndrome can be seen above. Among genes associated with these defects, the X-linked inheritance through mutations in *ZIC3* (chromosome Xq26.2) is most reported. Maternal age <20 years was found to be a risk factor (OR: 2.06; 1.2-3.5).[102]

Conotruncal defects

Tetralogy of Fallot (TOF)

The four components of the tetralogy of Fallot are malaligned VSD, obstruction to RV outflow, aortic override of the VSD, and RV hypertrophy due to the RV's response to aortic pressure via the large VSD.

Risk factors for TOF include advanced maternal age (OR: 2.2, 1.4-3.3)[105], pre-gestational diabetes (OR: 4.9, 2.2-11.0)[109], gestational diabetes (OR: 1.8, 1.1-2.9)[109], infertility/ART (OR: 3.6, 1.9-6.9)[142], nulliparity (OR: 1.3, 1.1-1.6)[123], metronidazole (OR: 6.0, 1.8-20.7)[142], trimethadione, progesterone and estrogen use[78] and low socioeconomic status.[126]

The severity of RV outflow obstruction determines the clinical presentation. The severity of hypoplasia of the RV outflow tract varies from mild to complete (pulmonary atresia). Pulmonary valve stenosis and supravalvular and peripheral pulmonary arterial obstruction may coexist. When the RV outflow obstruction is severe, pulmonary blood flow is reduced markedly, and a large volume of desaturated systemic venous blood shunts right-to-left across the VSD. Severe cyanosis and erythrocytosis occur, and symptoms of systemic hypoxemia are prominent. In many infants and children, the obstruction is mild but progressive.

For a variety of reasons, only a few adults with tetralogy of Fallot have not had some form of previous surgical intervention. Reoperation in adults is most commonly for severe pulmonary regurgitation or pulmonary stenosis. Long-term concerns about ventricular function persist. Ventricular and atrial arrhythmias occur, respectively, in 15% and 25% of adults and may require medical treatment, electrophysiologic study and ablation, defibrillator placement, or transcatheter or surgical intervention, usually including pulmonary valve replacement.

Truncus arteriosus

Persistent truncus arteriosus occurs in less than 1% of all congenital heart defects. Only a single arterial trunk with a truncal valve leaves the heart and gives rise to the pulmonary, systemic, and coronary circulations. A large perimembranous, infundibular VSD is present directly below the truncus. The truncal valve may be bicuspid, tricuspid, or quadricuspid, and it is often incompetent.[78]

Risk factors for truncus arteriosus include pre-gestational diabetes (OR: 2.8, 1.2-6.9)[108], pre-pregnancy obesity (OR 6.3; 1.6-24.8)[12] and cigarette use (OR: 1.9, 1.04-3.45).[160]

Most infants present with CHF during the first 2 weeks; 85% of untreated children die by 1 year of age. Death occurs around the third decade of life.

Because of the frequent association of DiGeorge syndrome, supplementation of calcium and magnesium may be indicated. Only irradiated blood product should be used for an urgent surgery (because of insufficient time for evaluation of immune status accurately). Because of the thymus-based immune deficiency, treatment and prophylaxis against pneumococcal and streptococcal infections are important. Immunization with live vaccine should be avoided.[78]

Transposition of the great arteries (TGA)

This condition is commonly called dextro-transposition of the great arteries. The aorta arises rightward anteriorly from the RV, and the pulmonary artery emerges leftward and posteriorly from the LV, which results in two separate parallel circulations; some communication between them must exist after birth to sustain life. Most patients have an ASD, two-thirds have a patent ductus arteriosus, and about one-third have an associated VSD. TGA is more common in males and accounts for approximately 10% of cyanotic heart disease.[7]

Environmental risk factors for TGA include advanced maternal age (OR: 1.7, 1.1-2.5)[102], pre-gestational diabetes (OR varying from 2.0, 1.2-3.2[108], to 3.3, 1.1-10.1[109]), low socioeconomical status[126], maternal periconceptional stressful life events (most modestly, if at all, associated)[129], ibuprofen (OR: 2.5, 1.2-4.9)[142], amphetamines, trimethadione, progesterone and estrogen use[78], alcohol use (OR: 1.9, 1.1-3.2)[157], cigarette use relatively to levo-transposition of the great arteries (l-TGA) (OR: 1.79, 1.04-3.10)[160] and organic solvents (OR: 3.4, 1.5-7.5).[142] Folic acid (OR: 0.4, 0.2-0.9)[150] and social support[129] were associated with reduced risks .

Patients who do not undergo surgical palliation generally do not survive to reach adulthood.[7]

Double-outlet right ventricle (DORV)

DORV occurs in less than 1% of all congenital heart defects. DORV occurs frequently in patients with heterotaxy in association with other complex cardiac defects. Both the aorta and the PA arise from the RV. The only outlet from the LV is a large VSD. Infants without pulmonary stenosis (PS) can develop CHF and later pulmonary vascular obstructive disease if left untreated. When PS is present, complications common to cyanotic congenital heart defects, like polycythemia and cerebrovascular accident may develop.[78]

Risk factors for DORV include pre-gestational diabetes (OR: 12.3, 2.8-55.2)[12], prepregnancy severe obesity (OR: 2.5, 1.1-5.8)[118] and ibuprofen (OR: 3.6, 1.1-12.2).[142]

Surgical treatment is necessary, followed by a long-term, regular follow-up at 6- to 12-month intervals in order to detect and manage complications of surgery.[78]

Tricuspid atresia (TA)

This malformation is characterized by atresia of the tricuspid valve, an interatrial communication, and frequently, hypoplasia of the RV and pulmonary artery. The clinical picture is usually dominated by severe cyanosis due to obligatory mixture of systemic and pulmonary venous blood in the LV.[7]

Environmental risk factors for TA include maternal age <20 years (OR: 2.6; 1.4-5.1)[104], maternal fever (OR: 7.5, 2.6-21.8) and influenza (OR: 6.0, 2.4-15.4)[113], paternal cocaine use (OR: 4.8, 1.6-14.0)[142] and paternal marijuana use (OR: 2.7, 1.3-5.8).[142]

Atrial septostomy and palliative operations to increase pulmonary blood flow, often by anastomosis of a systemic artery or vein to a pulmonary artery, may allow

survival to the second or third decade. A Fontan atriopulmonary or total cavopulmonary connection may then allow functional correction in patients with normal or low pulmonary arterial resistance pressure and good LV function.[7]

Ebstein's anomaly (EA)

Ebstein's anomaly of the tricuspid valve occurs in less than 1% of all congenital heart defects. This anomaly is characterized by a downward displacement of the tricuspid valve into the RV, due to anomalous attachment of the tricuspid leaflets, resulting in tricuspid regurgitation. The abnormally situated tricuspid orifice produces an "atrialized" portion of the RV lying between the atrioventricular ring and the origin of the valve, which is continuous with the RA chamber. Often, the RV is hypoplastic.[7]

Risk factors for Ebstein's anomaly include advanced maternal age (OR: 2.6, 1.4-4.8)[142], antihypertensive treatment during the first trimester of pregnancy (OR: 11.4, 2.8-34.1)[135], benzodiazepines (OR: 5.3, 1.5-18.5)[142], lithium[152] and marijuana use (OR: 3.6, 1.6-8.5).[142]

Although the clinical manifestations are variable, some patients come to initial attention because of progressive cyanosis from right-to-left atrial shunting, symptoms due to tricuspid regurgitation and RV dysfunction or paroxysmal atrial tachyarrhythmias with or without Wolff-Parkinson-White syndrome. The median age at death is about 20 years. Surgical approaches include prosthetic replacement of the tricuspid valve when the leaflets are tethered or repair of the native valve.[7]

Total anomalous pulmonary venous return (TAPVR)

TAPVR accounts for 1% of all congenital heart defects. No direct communication exists between the pulmonary veins and the LA. Instead, they drain anomalously into the systemic venous tributaries or into the RA.[78] Clinical manifestations differ, depending on whether there is obstruction to the pulmonary venous return. In patients without obstruction, CHF with growth retardation and frequent pulmonary infection are common in infancy and a history of mild cyanosis from birth is present. In patients with pulmonary venous obstruction, pulmonary arterial hypertension, progressive pulmonary venous congestion, hypoxemia, and systemic hypoperfusion occur. An interatrial communication, either an ASD or PFO, is necessary for survival. Most patients do not have restricted flow across the atrial septum. The left

side of the heart is relatively small. Without surgical repair, two thirds of the infants without obstruction die before reaching 1 year of age. They usually die from superimposed pneumonia.[78]

Risk factors for TAPVR include maternal age <20 years (OR: 2.3; 1.3-4.0)[105] and pre-gestational diabetes (OR: 7.1, 2.0-25.4).[109]

Corrective surgery is necessary for all patients with this condition. An office evaluation every 6 to 12 months is recommended for such late complications as pulmonary vein obstruction and atrial arrhythmias.[78]

Prenatal and family screening

Fetal echocardiographic screening is indicated if either parent is afflicted with any form of CHD. It should be performed in a specialized center at 18-22 weeks of gestation.[179]Earlier fetal echocardiography may be recommended for families with high rates of CHD recurrence and more severe cardiac defects.[86] 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, reducing mortality and morbidity.[179] Termination of a pregnancy can be an option if the fetus is affected.[28] Reported pregnancy termination rates for severe CHD identified by prenatal screening were 45% in the Netherlands[179] and 86% in Switzerland.[180] Factors associated with pregnancy termination included severity of CHD, gestational age at diagnosis, presence of chromosomal abnormalities, and parental ethnicity.[181]

Although prenatal detection of neural tube defects is high, the sensitivity for detecting CHDs is still low[182], which means that a large proportion of CHDs cannot be avoided by detection and termination of affected pregnancies. The couple receiving prenatal counseling and surveillance should be informed of the impossibility to exclude smaller or evolutive lesions as coarctation in examined fetuses and the final prognosis of the affected cases may be sometimes completed only after birth.[89] Fetal echocardiography may be considered for all MC/DA twin gestations, as discussed before.[91] The associations of birth defects with gestational diabetes mellitus (GDM) previously noticed highlight the importance of follow-up evaluation and counseling of women who are diagnosed with GDM during and subsequent to the index pregnancy. Pregnancies that are complicated by GDM among women with a history of above average weight before pregnancy might warrant consideration for prenatal screening for

malformations with ultrasound scans and fetal echocardiogram, even though the quality of such examinations might be rendered less than optimal by the presence of abdominal adiposity.[109]

In some circumstances, prenatal or preimplantation genetic screening could identify fetuses or embryos at high risk for CHD.

Fetal genetic screening for CHD is possible, including genome-wide high-resolution SNP arrays to identify CNVs[183] and competitive genomic hybridization to detect submicroscopic chromosomal aberrations.[184] A prenatal diagnostic test can be performed after chorionic villus sampling before 14 weeks of gestation or through amniocentesis between 15 and 18 weeks of gestation. However, these procedures carry a fetal loss rate of 0.5-1%.[86] 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). Cytogenetic testing has been recommended as well for establishing a prenatal diagnosis when CHD is identified by fetal echocardiography.[17] It has been recommended to screen all children with SVAS or PVS for Williams-Beuren syndrome (prenatal testing for 7q11.23 microdeletion) 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 (prenatal testing for 22q11 deletion).[17] It could also be performed for any severe monogenic disease if the result could influence the decision to terminate pregnancy.[15]

Preimplantation diagnostic testing could be proposed in selected cases, particularly for women with a history of multiple therapeutic abortions. Thus, it could be used to identify embryos free of specific genetic abnormalities prior to embryo transfer for severe inherited disorders.[86] It has already been used for Holt Oram (as it was referred before) and Marfan syndromes.[61] Beyond syndromes such as trisomy 21, 18 or 13, prenatal or preimplantation genetic screening remains controversial. Ethical issues 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.[15]

Echocardiographic screening of relatives of patients with obstructive left heart lesion, including BAV, CoA and/or aortic dilation without extracardiac anomalies is justified owing to the possibility of presence of an asymptomatic and undetetected BAV in the family.[99] The rationale for family screening is that early detection may help avert complications related to aortic dilatation (6-fold higher risk of aortic dissection), aortic

insufficiency, endocarditis, AS and CoA, such as arterial hypertension. Early detection may lead to lifestyle recommendations, for example to limit isometric exercises, enhanced monitoring, namely for progressive aortic dilatation, or preventive surgery, prior to aortic dissection for example. Age at screening remains controversial. It should generally be proposed to adults if not previously performed during childhood.[15] At present, systematic screening of first-degree relatives is not recommended for other forms of nonsyndromic CHD.[15]

Genetic counselling

Cardiovascular genetic counseling was first recognized by the National Society of Genetic Counselors as a specialty in 2006. Consequently, most CHD cardiologists will be required to participate in the genetic counseling of their patients at some stage.[86] More than simply estimating the risk of recurrence in families with CHD, genetic counseling is a process that aims to help people understand and adapt to the medical, psychological and familial implications of genetic contributions to disease.[86]

Genetic counseling is important before and after genetic testing.[15] Prior to testing, interpretation of family and medical histories should be addressed to assess the chance of disease occurrence or recurrence. The family should be educated about inheritance, testing, management, prevention, resources, and research, so that informed choices and adaptation to the risk or condition can be promoted.[86] First, the patient 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. The potential impact that test results may have on family dynamics should be discussed before testing is undertaken. Informed consent is obtained after each of these issues is addressed.[86]

After genetic testing, counseling is important to review the results, explain the genetic variant, and discuss implications with the patient and family.[185] Given the complexity of the medical and ethical issues surrounding a genetic diagnosis in CHD, close collaboration between cardiologists, genetic counselors, and geneticists is recommended.

Genetic testing and indications

Monogenic and chromosomal abnormality models account for a substantial proportion of CHD, which enhances the potential value of genetic investigation and testing.[15] Positive consequences of genetic testing include relief of uncertainty surrounding a diagnosis, more accurate estimation of transmission risk, and identification of asymptomatic at-risk family members. Genetic confirmation may also enable preemptive screening and treatment of complications such as aortic dilation in Marfan syndrome[186], HCM in Noonan syndrome[187] and neuropsychiatric disease in 22q11.2 microdeletion syndrome.[188]Potential harms of genetic testing include difficulty in acquiring insurance, potential job discrimination, and negative self-image.[189]

The selection of a genetic test is contingent upon the particular diagnostic hypothesis generated by clinical examination.[15] Genetic investigation in CHD may carry the potential to improve prognosis by yielding valuable information regarding personalized medical care, confidence in the clinical diagnosis, and/or targeted patient followup. 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.

The first clinical situation to consider genetic testing in CHD is the presence of a syndromic phenotype. As it was shown above, many syndromic conditions associated with CHD, such as Down, Patau, Edward, Noonan, Alagille, Williams-Beuren, Holt-Oram and DiGeorge/VCFS syndromes, have genetic testing available in the clinical setting, which can confirm the diagnosis. In general, genetic consultation is recommended when a probable syndromic phenotype is identified.[15] It is important to identify defects which arise suspicion of a syndrome and those that may benefit from early surgical intervention.[15]

The second clinical situation to consider genetic testing is in the context of a family in which a person diagnosed with CHD has an afflicted first- or second-degree relative.

The clinical investigation includes a detailed assessment of past medical, surgical and family histories (at least three generations to differentiate *de novo* from inherited disease).[86] Themes that should be questioned to the families include familiar screening for cardiac diseases, consanguinity, particular phenotypes such as dysmorphias, aborted pregnancies, infertility and early deaths.[15]

For non-syndromic CHD, the family tree may orient the clinician towards a genetic etiology and a specific pattern of inheritance. 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. Identification of a specific mutation in a multiplex family with CHD may allow for targeted screening of additional family members.[15]

Specific genetic tests include search for chromosomal anomalies, such as trisomy 21, 18, 13 or monosomy X (Turner syndrome) through karyotype analysis. Fluorescence in situ hybridization (FISH) is the predominant technique used to identify Williams-Beuren, DiGeorge, and Alagille syndromes. Subtelomere FISH analyses are less commonly used today and detect abnormalities in subtelomere and telomere DNA regions.[17] These studies should be ordered if the karyotype is normal in a patient with dysmorphic facial features, congenital anomalies, developmental delay, and mental retardation. 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.[190] As many as 50% of families can have other individual members with subtelomeric abnormalities.[191]

Array-based comparative genomic hybridization (aCGH) is used to detect structural and numerical chromosomal abnormalities. 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.[15] 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.[15] Gene sequencing through Next-generation sequencing technologies target coding and non-coding parts of the genome and can be helpful in conditions such as Noonan syndrome, Alagille syndrome with a normal FISH analysis, Holt-Oram syndrome, and several other diseases.[15]

Genes and prognosis

The genetic environment could modulate the prognosis of various forms of CHD, helping to elucidate risks of developing some defects, such as conduction defects and systolic disfunction (*NKX2.5* mutations associated with ASD, atrioventricular block and dilated cardiomyopathy)[192] and diastolic dysfunction (*TBX5* gene, implicated in Holt-Oram syndrome). Genes implicated in RASopathy syndromes (members of the

RAS-MAPK pathway), responsible for Noonan, cardiofaciocutaneous, Costello and Leopard syndromes, are also responsible for cardiac hypertrophy in later development.[187]

TBX20 mutation may have an underlying ASD, VSD or mitral valve disease or may present exclusively with pulmonary hypertension or cardiomyopathy.[193]Mutations in *MYH6* (alpha-cardiac myosin heavy chain) are associated with various forms of CHD but also dilated and hypertrophic cardiomyopathy.[194] Moreover, mutations in MYH7 have been reported in patients with autosomal dominant inheritance Ebstein's anomaly and left ventricular noncompaction.[195] Some family members may have CHD whereas others could develop progressive cardiomyopathy or electrophysiologic disorders.

22q11.2 deletion (DiGeorge syndrome) in patients with TOF predicts a longer cardiopulmonary bypass time and a greater length of stay in intensive care.[196] While several explanations have been proposed, potential factors include a higher prevalence of aortopulmonary shunts and respiratory problems prior to surgical repair in these patients, resulting in longer mechanical ventilatory support. Therefore, one can predict that genetic profile may be used in the future for pre-operative risk assessment.[15]

The interpretation of genetic tests must be carefully assessed and placed in context of the clinical and family evaluation, because genetic variants may be identified in unaffected family members due to variable penetrance and expressivity.[15]

Limitations of genetics in CHD

Despite the fact that CHD is the most common birth defect, 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. Families with recurrent CHD and strong phenotypic penetrance were the most used cases in genetic studies, which represent the minority of cases of CHD.[197] The relatively low familial recurrence risk may be due, in part, to *de novo* mutations, incomplete penetrance, and other etiological factors such as environmental influences.

In the presence of environmental interactions, incomplete penetrance and variable expressivity, patterns of inheritance may be difficult to find. Moreover, a large number of members with consanguinity is required for genetic analysis based on individual families.[198] The functional validation of mutations that may involve non-coding regions of the DNA, which was reported as having a role in CHD, is more difficult and resource consuming, which can limit as well the current knowledge of

genetics. Rare and unique mutations to individual families can complicate as well the establishment of genotype-phenotype correlations. In fact, most CHD mutations identified to date appear to be private or do not recur. Notwithstanding these 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.[15]

Prevention of CHD

According to the present information regarding environmental factors associated with CHD, some principles for prevention could be useful. In fact, parents should be advised for the following measures. The use of a multivitamin containing folate daily in the pre- and peri-conception period is highly recommended, as well as ensuring that rubella vaccination has been completed before pregnancy. Avoidance of individuals with flu or febrile illness to minimize the chance of infection, avoidance of organic solvents, and avoidance of alcohol, tobacco, and illicit drugs are also important things to have in mind. Health care providers should provide close monitoring of women receiving regular medications, such as thalidomide, antihypertensives, anticonvulsivants and anti-inflammatory drugs, among others, and even over-the-counter medications.

Careful management of women with phenylketonuria and diabetes should be pursued, through maintenance of good glycaemic control in mothers with diabetes.[10] A diagnosis of gestational diabetes mellitus (GDM) actually may represent undiagnosed type 2 DM and GDM is associated with an increased risk of GDM in subsequent pregnancies and with type 2 DM later in life. Therefore, women who are diagnosed with GDM might benefit from follow-up evaluations, family planning, and counseling regarding diabetes mellitus management if they are found to have type 2 DM and of diabetes mellitus prevention education otherwise.[109]

In addition, screening for CHDs should be performed when these situations or exposures are present.

Conclusion

Although much remains to be discovered in the world of CHD genetics, recent advances in technology provide us with the potential to better understand the etiology of CHD and the various phenotypes. Revolutionary genetic techniques still harbor many

challenges, including the difficulty in distinguishing benign variants from disease causing mutations, but the possible identification of disease etiology greatly outweighs these challenges. Novel genetic tests, including prenatal ones, are becoming gradually available, allowing families to plan and prepare themselves in advance, as well as to reduce anxiety from the possibility of having offspring with CHD. Genetic diagnosis and the assessment of risk for syndromic patients are important in order to effectively plan the surgical and medical management and multidisciplinary follow-up, helping in the reduction of mortality and morbidity.

An accurate family history can provide valuable clues about possible causation and inheritance, which is particularly relevant to families with multiple affected individuals, and a referral to a genetics service should be considered.[10] Working alone or in collaboration with clinical geneticists and genetic counselors, adult CHD specialists will continue to play an important role in identifying patients who may benefit from genetic testing, and in caring for those patients.

Although significant associations between multiple non-genetic risk factors and CHDs have been identified, many of these risk factors have yielded conflicting results. Gene/environment interactions are still poorly understood as well, suggesting that additional investigations need to be performed.

Finally, researchers and physicians will need to translate genetic findings and the new pathophysiological insights into meaningful medical advances, such as the development of new potential therapeutic targets in long-term to prevent or repair heart malformations, and thus leading to a better quality of life for the patients and families worldwide.

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Bibliography

1. Dolk H, Loane M, Garne E. Congenital heart defects in Europe: prevalence and perinatal mortality, 2000 to 2005. *Circulation*. 2011;123(8):841-849.
2. Bernier P-L, Stefanescu A, Samoukovic G, Tchervenkov CI. The challenge of congenital heart disease worldwide: epidemiologic and demographic facts. *Semin Thorac Cardiovasc Surg Pediatr Card Surg Annu*. 2010;13(1):26-34.
3. Gelb BD, Chung WK. Complex genetics and the etiology of human congenital heart disease. *Cold Spring Harb Perspect Med*. 2014;4(7):a013953.
4. Dolk H; Loane M. EUROCAT Steering Committee: Congenital heart defects in Europe: 2000-2005. Newtownabbey, Northern Ireland: University of Ulster; March 2009.
5. van der Bom T, Zomer AC, Zwinderman AH, Meijboom FJ, Bouma BJ, Mulder BJM. The changing epidemiology of congenital heart disease. *Nat Rev Cardiol*. 2011;8(1):50-60.
6. van der Linde D, Konings EEM, Slager MA, et al. Birth prevalence of congenital heart disease worldwide: a systematic review and meta-analysis. *J Am Coll Cardiol*. 2011;58(21):2241-2247.
7. Kasper D, Fauci A, Hauser S, Longo D, Jameson J LJ. *Harrison's Principles of Internal Medicine*. 19th ed. McGraw Hill Education; 2015.
8. Ávila P, Mercier L-A, Dore A, et al. Adult congenital heart disease: a growing epidemic. *Can J Cardiol*. 2014;30(12 Suppl):S410-S419.
9. Khairy P, Ionescu-Ittu R, Mackie AS, Abrahamowicz M, Pilote L, Marelli AJ. Changing mortality in congenital heart disease. *J Am Coll Cardiol*. 2010;56(14):1149-1157.
10. Blue GM, Kirk EP, Sholler GF, Harvey RP WD. Congenital heart disease: current knowledge about causes and inheritance. *Med J Aust*. 2012;197(3):155-159.
11. Nora JJ. Multifactorial inheritance hypothesis for the etiology of congenital heart diseases. The genetic-environmental interaction. *Circulation*. 1968;38(3):604-617.
12. Patel SS, Burns TL. Nongenetic risk factors and congenital heart defects. *Pediatr Cardiol*. 2013;34(7):1535-1555.
13. Wolf M, Basson CT. The molecular genetics of congenital heart disease: a review of recent developments. *Curr Opin Cardiol*. 2010;25(3):192-197.
14. Formigari R, Michielon G, Digilio MC, et al. Genetic syndromes and congenital heart defects: how is surgical management affected? *Eur J Cardiothorac Surg*. 2009;35(4):606-614.
15. Chaix MA, Andelfinger G, Khairy P. Genetic testing in congenital heart disease: A clinical approach. *World J Cardiol*. 2016;8(2):180-191.
16. Fahed AC, Gelb BD, Seidman JG, Seidman CE. Genetics of congenital heart disease: The glass half empty. *Circ Res*. 2013;112(4):707-720.
17. Pierpont ME, Basson CT, Benson DW, et al. Genetic Basis for Congenital Heart Defects: Current Knowledge A Scientific Statement From the American Heart Association Congenital Cardiac Defects Committee, Council on Cardiovascular Disease in the Young: Endorsed by the American Academy of Pediatrics. *Circulation*. 2007;115(23):3015-3038.
18. Shiaulou Yuan, Samir Zaidi and MB. Congenital Heart Disease: emerging themes linking genetics and development. 2014;23(3):352-359.
19. Vincent SD, Buckingham ME. How to make a heart: the origin and regulation of cardiac progenitor cells. *Curr Top Dev Biol*. 2010;90:1-41.

20. Nakamura T, Hamada H. Left-right patterning: conserved and divergent mechanisms. *Development*. 2012;139(18):3257-3262.
21. Aanhaanen WTJ, Brons JF, Domínguez JN, et al. The Tbx2+ primary myocardium of the atrioventricular canal forms the atrioventricular node and the base of the left ventricle. *Circ Res*. 2009;104(11):1267-1274.
22. Wiese C, Grieskamp T, Airik R, et al. Formation of the sinus node head and differentiation of sinus node myocardium are independently regulated by Tbx18 and Tbx3. *Circ Res*. 2009;104(3):388-397.
23. Ware SM, Harutyunyan KG, Belmont JW. Heart defects in X-linked heterotaxy: evidence for a genetic interaction of Zic3 with the nodal signaling pathway. *Dev Dyn*. 2006;235(6):1631-1637.
24. Moskowitz IP, Wang J, Peterson MA, et al. Transcription factor genes Smad4 and Gata4 cooperatively regulate cardiac valve development. [corrected]. *Proc Natl Acad Sci U S A*. 2011;108(10):4006-4011.
25. Moskowitz IPG, Kim JB, Moore ML, et al. A molecular pathway including Id2, Tbx5, and Nkx2-5 required for cardiac conduction system development. *Cell*. 2007;129(7):1365-1376.
26. Lindsay EA, Baldini A. Recovery from arterial growth delay reduces penetrance of cardiovascular defects in mice deleted for the DiGeorge syndrome region. *Hum Mol Genet*. 2001;10(9):997-1002.
27. Shieh JTC, Bittles AH, Hudgins L. Consanguinity and the risk of congenital heart disease. *Am J Med Genet A*. 2012;158A(5):1236-1241.
28. Øyen N, Poulsen G, Boyd HA, Wohlfahrt J, Jensen PKA, Melbye M. Recurrence of congenital heart defects in families. *Circulation*. 2009;120(4):295-301.
29. Ko JM. Genetic Syndromes associated with Congenital Heart Disease. *Korean Circ J*. 2015;45(5):357-361.
30. Bull MJ. Health supervision for children with Down syndrome. *Pediatrics*. 2011;128(2):393-406.
31. Ackerman C, Locke AE, Feingold E, et al. An excess of deleterious variants in VEGF-A pathway genes in Down-syndrome-associated atrioventricular septal defects. *Am J Hum Genet*. 2012;91(4):646-659.
32. Bondy CA. Care of girls and women with Turner syndrome: a guideline of the Turner Syndrome Study Group. *J Clin Endocrinol Metab*. 2007;92(1):10-25.
33. Davenport ML. Approach to the patient with Turner syndrome. *J Clin Endocrinol Metab*. 2010;95(4):1487-1495.
34. Rosias PR, Sijstermans JM, Theunissen PM, et al. Phenotypic variability of the cat eye syndrome. Case report and review of the literature. *Genet Couns*. 2001;12(3):273-282.
35. Merscher S, Funke B, Epstein JA, et al. TBX1 is responsible for cardiovascular defects in velo-cardio-facial/DiGeorge syndrome. *Cell*. 2001;104(4):619-629.
36. Rauch R, Hofbeck M, Zweier C, et al. Comprehensive genotype-phenotype analysis in 230 patients with tetralogy of Fallot. *J Med Genet*. 2010;47(5):321-331.
37. Oskarsdóttir S, Belfrage M, Sandstedt E, Viggedal G, Uvebrant P. Disabilities and cognition in children and adolescents with 22q11 deletion syndrome. *Dev Med Child Neurol*. 2005;47(3):177-184.
38. Bassett AS, McDonald-McGinn DM, Devriendt K, et al. Practical guidelines for managing patients with 22q11.2 deletion syndrome. *J Pediatr*. 2011;159(2):332-339.e1.
39. Ou Z, Berg JS, Yonath H, et al. Microduplications of 22q11.2 are frequently inherited and are associated with variable phenotypes. *Genet Med*. 2008;10(4):267-277.

40. Zweier C, Sticht H, Aydin-Yaylagül I, Campbell CE, Rauch A. Human TBX1 missense mutations cause gain of function resulting in the same phenotype as 22q11.2 deletions. *Am J Hum Genet*. 2007;80(3):510-517.
41. Collins RT. Cardiovascular disease in Williams syndrome. *Circulation*. 2013;127(21):2125-2134.
42. Collins RT, Kaplan P, Somes GW, Rome JJ. Long-term outcomes of patients with cardiovascular abnormalities and williams syndrome. *Am J Cardiol*. 2010;105(6):874-878.
43. American Academy of Pediatrics: Health care supervision for children with Williams syndrome. *Pediatrics*. 2001;107(5):1192-1204.
44. Grossfeld PD, Mattina T, Lai Z, et al. The 11q terminal deletion disorder: a prospective study of 110 cases. *Am J Med Genet A*. 2004;129A(1):51-61.
45. Ye M, Coldren C, Liang X, et al. Deletion of ETS-1, a gene in the Jacobsen syndrome critical region, causes ventricular septal defects and abnormal ventricular morphology in mice. *Hum Mol Genet*. 2010;19(4):648-656.
46. Beckmann JS, Estivill X, Antonarakis SE. Copy number variants and genetic traits: closer to the resolution of phenotypic to genotypic variability. *Nat Rev Genet*. 2007;8(8):639-646.
47. Soemedi R, Wilson IJ, Bentham J, et al. Contribution of global rare copy-number variants to the risk of sporadic congenital heart disease. *Am J Hum Genet*. 2012;91(3):489-501.
48. Greenway SC, Pereira AC, Lin JC, et al. De novo copy number variants identify new genes and loci in isolated sporadic tetralogy of Fallot. *Nat Genet*. 2009;41(8):931-935.
49. Soemedi R, Topf A, Wilson IJ, et al. Phenotype-specific effect of chromosome 1q21.1 rearrangements and GJA5 duplications in 2436 congenital heart disease patients and 6760 controls. *Hum Mol Genet*. 2012;21(7):1513-1520.
50. Silversides CK, Lionel AC, Costain G, et al. Rare copy number variations in adults with tetralogy of Fallot implicate novel risk gene pathways. *PLoS Genet*. 2012;8(8):e1002843.
51. Hitz M-P, Lemieux-Perreault L-P, Marshall C, et al. Rare copy number variants contribute to congenital left-sided heart disease. *PLoS Genet*. 2012;8(9):e1002903.
52. Payne AR, Chang S-W, Koenig SN, Zinn AR, Garg V. Submicroscopic chromosomal copy number variations identified in children with hypoplastic left heart syndrome. *Pediatr Cardiol*. 2012;33(5):757-763.
53. Priest JR, Girirajan S, Vu TH, Olson A, Eichler EE, Portman MA. Rare copy number variants in isolated sporadic and syndromic atrioventricular septal defects. *Am J Med Genet A*. 2012;158A(6):1279-1284.
54. Fakhro KA, Choi M, Ware SM, et al. Rare copy number variations in congenital heart disease patients identify unique genes in left-right patterning. *Proc Natl Acad Sci U S A*. 2011;108(7):2915-2920.
55. Slavotinek AM. Novel microdeletion syndromes detected by chromosome microarrays. *Hum Genet*. 2008;124(1):1-17.
56. Roberts AE, Allanson JE, Tartaglia M, Gelb BD. Noonan syndrome. *Lancet (London, England)*. 2013;381(9863):333-342.
57. Tartaglia M, Kalidas K, Shaw A, et al. PTPN11 mutations in Noonan syndrome: molecular spectrum, genotype-phenotype correlation, and phenotypic heterogeneity. *Am J Hum Genet*. 2002;70(6):1555-1563.
58. Lee BH, Kim J-M, Jin HY, Kim G-H, Choi J-H, Yoo H-W. Spectrum of mutations in

Noonan syndrome and their correlation with phenotypes. *J Pediatr*. 2011;159(6):1029-1035.
59. Turnpenny PD, Ellard S. Alagille syndrome: pathogenesis, diagnosis and management. *Eur J Hum Genet*. 2012;20(3):251-257.
60. McDermott DA, Fong JC, Basson CT. Holt-Oram Syndrome. Available in: http://www.ncbi.nlm.nih.gov/books/NBK1111/. GeneReviews® [Internet]: University of Washington, Seattle; 1993-2016. Updated: October 8 2015.
61. He J, McDermott DA, Song Y, Gilbert F, Kligman I, Basson CT. Preimplantation genetic diagnosis of human congenital heart malformation and Holt-Oram syndrome. *Am J Med Genet A*. 2004;126A(1):93-98.
62. Bianconi SE, Cross JL, Wassif CA, Porter FD. Pathogenesis, Epidemiology, Diagnosis and Clinical Aspects of Smith-Lemli-Opitz Syndrome. *Expert Opin orphan drugs*. 2015;3(3):267-280.
63. Gripp KW, Lin AE, Stabley DL, et al. HRAS mutation analysis in Costello syndrome: genotype and phenotype correlation. *Am J Med Genet A*. 2006;140(1):1-7.
64. Dentici ML, Sarkozy A, Pantaleoni F, et al. Spectrum of MEK1 and MEK2 gene mutations in cardio-facio-cutaneous syndrome and genotype-phenotype correlations. *Eur J Hum Genet*. 2009;17(6):733-740.
65. Sanlaville D, Verloes A. CHARGE syndrome: an update. *Eur J Hum Genet*. 2007;15(4):389-399.
66. Ng SB, Bigham AW, Buckingham KJ, et al. Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. *Nat Genet*. 2010;42(9):790-793.
67. Miyake N, Mizuno S, Okamoto N, et al. KDM6A point mutations cause Kabuki syndrome. *Hum Mutat*. 2013;34(1):108-110.
68. Hoischen A, van Bon BWM, Gilissen C, et al. De novo mutations of SETBP1 cause Schinzel-Giedion syndrome. *Nat Genet*. 2010;42(6):483-485.
69. Kleefstra T, Smidt M, Banning MJG, et al. Disruption of the gene Euchromatin Histone Methyl Transferase1 (Eu-HMTase1) is associated with the 9q34 subtelomeric deletion syndrome. *J Med Genet*. 2005;42(4):299-306.
70. Stewart DR, Kleefstra T. The chromosome 9q subtelomere deletion syndrome. *Am J Med Genet C Semin Med Genet*. 2007;145C(4):383-392.
71. Sang L, Miller JJ, Corbit KC, et al. Mapping the NPHP-JBTS-MKS protein network reveals ciliopathy disease genes and pathways. *Cell*. 2011;145(4):513-528.
72. van Bon BWM, Koolen DA, Pfundt R, van der Burgt I, de Leeuw N, de Vries BBA. Transposition of the great vessels in a patient with a 2.9 Mb interstitial deletion of 9q31.1 encompassing the inversin gene: clinical report and review. *Am J Med Genet A*. 2008;146A(9):1225-1229.
73. Bergmann C, Fliegauf M, Brüchle NO, et al. Loss of nephrocystin-3 function can cause embryonic lethality, Meckel-Gruber-like syndrome, situs inversus, and renal-hepatic-pancreatic dysplasia. *Am J Hum Genet*. 2008;82(4):959-970.
74. French VM, van de Laar IMBH, Wessels MW, et al. NPHP4 variants are associated with pleiotropic heart malformations. *Circ Res*. 2012;110(12):1564-1574.
75. Manning DK, Sergeev M, van Heesbeen RG, et al. Loss of the ciliary kinase Nek8 causes left-right asymmetry defects. *J Am Soc Nephrol*. 2013;24(1):100-112.
76. Frank V, Habbig S, Bartram MP, et al. Mutations in NEK8 link multiple organ dysplasia with altered Hippo signalling and increased c-MYC expression. *Hum Mol Genet*. 2013;22(11):2177-2185.
77. Hills CB, Kochilas L, Schimmenti LA, Moller JH. Ellis-van Creveld syndrome and

congenital heart defects: presentation of an additional 32 cases. *Pediatr Cardiol*. 2011;32(7):977-982.
78. Myung P. *Pediatric Cardiology for Practitioners*. 6th ed. Mosby Elsevier; 2015.
79. Kennedy MP, Omran H, Leigh MW, et al. Congenital heart disease and other heterotaxic defects in a large cohort of patients with primary ciliary dyskinesia. *Circulation*. 2007;115(22):2814-2821.
80. Nakhleh N, Francis R, Giese RA, et al. High prevalence of respiratory ciliary dysfunction in congenital heart disease patients with heterotaxy. *Circulation*. 2012;125(18):2232-2242.
81. Wessels MW, Willems PJ. Genetic factors in non-syndromic congenital heart malformations. *Clin Genet*. 2010;78(2):103-123. doi:10.1111/j.1399-0004.2010.01435.x.
82. Garg V, Muth AN, Ransom JF, et al. Mutations in NOTCH1 cause aortic valve disease. *Nature*. 2005;437(7056):270-274.
83. Posch MG, Gramlich M, Sunde M, et al. A gain-of-function TBX20 mutation causes congenital atrial septal defects, patent foramen ovale and cardiac valve defects. *J Med Genet*. 2010;47(4):230-235.
84. Zaidi S, Choi M, Wakimoto H, et al. De novo mutations in histone-modifying genes in congenital heart disease. *Nature*. 2013;498(7453):220-223.
85. Smemo S, Campos LC, Moskowitz IP, Krieger JE, Pereira AC, Nobrega MA. Regulatory variation in a TBX5 enhancer leads to isolated congenital heart disease. *Hum Mol Genet*. 2012;21(14):3255-3263.
86. Burchill L, Greenway S, Silversides CK, Mital S. Genetic counseling in the adult with congenital heart disease: What is the role? *Curr Cardiol Rep*. 2011;13(4):347-355.
87. Yunis K, Khalid Y, Mumtaz G, et al. Consanguineous marriage and congenital heart defects: a case-control study in the neonatal period. *Am J Med Genet A*. 2006;140(14):1524-1530.
88. Calcagni G, Digilio MC, Sarkozy A, Dallapiccola B, Marino B. Familial recurrence of congenital heart disease: An overview and review of the literature. *Eur J Pediatr*. 2007;166(2):111-116.
89. Fesslova V, Brankovic J, Lalatta F, et al. Recurrence of Congenital Heart Disease in Cases with Familial Risk Screened Prenatally by Echocardiography. *J Pregnancy*. 2011;2011(January 1995):1-9.
90. Wang X, Li P, Chen S, et al. Influence of genes and the environment in familial congenital heart defects. *Mol Med Rep*. 2014;9(2):695-700.
91. Bahtiyar MO, Dulay AT, Weeks BP, Friedman AH, Copel JA. Prevalence of congenital heart defects in monochorionic/diamniotic twin gestations: a systematic literature review. *J Ultrasound Med*. 2007;26(11):1491-1498.
92. Gill HK, Splitt M, Sharland GK, Simpson JM. Patterns of recurrence of congenital heart disease: an analysis of 6,640 consecutive pregnancies evaluated by detailed fetal echocardiography. *J Am Coll Cardiol*. 2003;42(5):923-929.
93. Allan LD. Diagnosis of cardiac anomalies in offspring of women with congenital heart disease: is fetal echocardiography enough? *Nat Clin Pract Cardiovasc Med*. 2008;5(9):522-523.
94. Corone P, Bonaiti C, Feingold J, Fromont S, Berthet-Bondet D. Familial congenital heart disease: How are the various types related? *Am J Cardiol*. 1983;51(6):942-945.
95. Hinton RB, Martin LJ, Tabangin ME, Mazwi ML, Cripe LH, Benson DW. Hypoplastic Left Heart Syndrome Is Heritable. *J Am Coll Cardiol*. 2007;50(16):1590-1595.
96. Digilio MC, Casey B, Toscano A, et al. Complete transposition of the great arteries:

patterns of congenital heart disease in familial precurrence. *Circulation*. 2001;104(23):2809-2814.
97. Cripe L, Andelfinger G, Martin LJ, Shooner K, Benson DW. Bicuspid aortic valve is heritable. *J Am Coll Cardiol*. 2004;44(1):138-143.
98. Loffredo CA, Chokkalingam A, Sill AM, et al. Prevalence of congenital cardiovascular malformations among relatives of infants with hypoplastic left heart, coarctation of the aorta, and d-transposition of the great arteries. *Am J Med Genet A*. 2004;124A(3):225-230.
99. Kerstjens-Frederikse WS, Du Marchie Sarvaas GJ, Ruiter JS, et al. Left ventricular outflow tract obstruction: should cardiac screening be offered to first-degree relatives? *Heart*. 2011;97(15):1228-1232.
100. Ferencz C, Boughman JA, Neill CA, Brenner J, Perry LW and TB-WISG. Congenital cardiovascular malformations: questions on inheritance. *J Am Coll Cardiol*. 1989;14:756-763.
101. Srivastava D. Genetic assembly of the heart: implications for congenital heart disease. *Annu Rev Physiol*. 2001;63:451-469.
102. Miller A, Riehle-Colarusso T, Siffel C, Frías JL, Correa A. Maternal age and prevalence of isolated congenital heart defects in an urban area of the United States. *Am J Med Genet A*. 2011;155A(9):2137-2145.
103. Hollier LM, Leveno KJ, Kelly MA, MCIntire DD, Cunningham FG. Maternal age and malformations in singleton births. *Obstet Gynecol*. 2000;96(5 Pt 1):701-706.
104. Loane M, Dolk H, Morris JK. Maternal age-specific risk of non-chromosomal anomalies. *BJOG*. 2009;116(8):1111-1119.
105. Gill SK, Broussard C, Devine O, Green RF, Rasmussen SA, Reefhuis J. Association between maternal age and birth defects of unknown etiology: United States, 1997-2007. *Birth Defects Res A Clin Mol Teratol*. 2012;94(12):1010-1018.
106. Yang Q, Wen SW, Leader A, Chen XK, Lipson J, Walker M. Paternal age and birth defects: how strong is the association? *Hum Reprod*. 2007;22(3):696-701.
107. Bassili A, Mokhtar SA, Dabous NI, Zaher SR, Mokhtar MM, Zaki A. Risk factors for congenital heart diseases in Alexandria, Egypt. *Eur J Epidemiol*. 2000;16(9):805-814.
108. Garne E, Loane M, Dolk H, et al. Spectrum of congenital anomalies in pregnancies with pregestational diabetes. *Birth Defects Res A Clin Mol Teratol*. 2012;94(3):134-140.
109. Correa A, Gilboa SM, Besser LM, et al. Diabetes mellitus and birth defects. *Am J Obstet Gynecol*. 2008;199(3):237.e1-e9.
110. Morgan SC, Relaix F, Sandell LL, Loeken MR. Oxidative stress during diabetic pregnancy disrupts cardiac neural crest migration and causes outflow tract defects. *Birth Defects Res A Clin Mol Teratol*. 2008;82(6):453-463.
111. Dunne F, Brydon P, Smith K, Gee H. Pregnancy in women with Type 2 diabetes: 12 years outcome data 1990-2002. *Diabet Med*. 2003;20(9):734-738.
112. Ács N, Bánhidy F, Puhó EH, Czeizel AE. Possible association between acute pelvic inflammatory disease in pregnant women and congenital abnormalities in their offspring: a population-based case-control study. *Birth Defects Res A Clin Mol Teratol*. 2008;82(8):563-570.
113. Oster ME, Riehle-Colarusso T, Alverson CJ, Correa A. Associations between maternal fever and influenza and congenital heart defects. *J Pediatr*. 2011;158(6):990-995.
114. Cleves MA, Malik S, Yang S, Carter TC, Hobbs CA. Maternal urinary tract infections and selected cardiovascular malformations. *Birth Defects Res A Clin Mol Teratol*. 2008;82(6):464-473.

115. Jenkins KJ, Correa A, Feinstein JA, Botto L, Britt AE, Daniels SR, Elixson M, Warnes CA WC. Noninherited risk factors and congenital cardiovascular defects: current knowledge: a scientific statement from the American Heart Association Council on Cardiovascular Disease in the Young: endorsed by the American Academy of Pediatrics. *Circulation*. 2007;115(23):2995-3014.
116. Levy HL, Guldberg P, Güttler F, et al. Congenital heart disease in maternal phenylketonuria: report from the Maternal PKU Collaborative Study. *Pediatr Res*. 2001;49(5):636-642.
117. Li D-K, Yang C, Andrade S, Tavares V, Ferber JR. Maternal exposure to angiotensin converting enzyme inhibitors in the first trimester and risk of malformations in offspring: a retrospective cohort study. *BMJ*. 2011;343:d5931.
118. Mills JL, Troendle J, Conley MR, Carter T, Druschel CM. Maternal obesity and congenital heart defects: a population-based study. *Am J Clin Nutr*. 2010;91(6):1543-1549.
119. Madsen NL, Schwartz SM, Lewin MB, Mueller BA. Prepregnancy body mass index and congenital heart defects among offspring: a population-based study. *Congenit Heart Dis*. 8(2):131-141.
120. Watkins ML, Rasmussen SA, Honein MA, Botto LD, Moore CA. Maternal obesity and risk for birth defects. *Pediatrics*. 2003;111(5 Pt 2):1152-1158.
121. Tararbit K, Houyel L, Bonnet D, et al. Risk of congenital heart defects associated with assisted reproductive technologies: a population-based evaluation. *Eur Heart J*. 2011;32(4):500-508.
122. Reefhuis J, Honein MA, Schieve LA, Rasmussen SA. Use of clomiphene citrate and birth defects, National Birth Defects Prevention Study, 1997-2005. *Hum Reprod*. 2011;26(2):451-457.
123. Duong HT, Hoyt AT, Carmichael SL, et al. Is maternal parity an independent risk factor for birth defects? *Birth Defects Res A Clin Mol Teratol*. 2012;94(4):230-236.
124. Varela MMM-S, Nohr EA, Llopis-González A, Andersen A-MN, Olsen J. Socio-occupational status and congenital anomalies. *Eur J Public Health*. 2009;19(2):161-167.
125. Kuciene R, Dulskiene V. Maternal socioeconomic and lifestyle factors during pregnancy and the risk of congenital heart defects. *Medicina (Kaunas)*. 2009;45(11):904-909.
126. Yang J, Carmichael SL, Canfield M, Song J, Shaw GM. Socioeconomic status in relation to selected birth defects in a large multicentered US case-control study. *Am J Epidemiol*. 2008;167(2):145-154.
127. Liu S, Liu J, Tang J, Ji J, Chen J, Liu C. Environmental risk factors for congenital heart disease in the Shandong Peninsula, China: a hospital-based case-control study. *J Epidemiol*. 2009;19(3):122-130.
128. Carmichael SL, Shaw GM. Maternal life event stress and congenital anomalies. *Epidemiology*. 2000;11(1):30-35.
129. Carmichael SL, Ma C, Tinker S, Rasmussen SA, Shaw GM. Maternal stressors and social support as risks for delivering babies with structural birth defects. *Paediatr Perinat Epidemiol*. 2014;28(4):338-344.
130. Smithells RW, Newman CG. Recognition of thalidomide defects. *J Med Genet*. 1992;29(10):716-723.
131. Malm H, Artama M, Gissler M, Ritvanen A. Selective serotonin reuptake inhibitors and risk for major congenital anomalies. *Obstet Gynecol*. 2011;118(1):111-120.
132. Polen KND, Rasmussen SA, Riehle-Colarusso T, Reefhuis J. Association between reported venlafaxine use in early pregnancy and birth defects, national birth defects

prevention study, 1997-2007. *Birth Defects Res A Clin Mol Teratol*. 2013;97(1):28-35.
133. Reis M, Källén B. Delivery outcome after maternal use of antidepressant drugs in pregnancy: an update using Swedish data. *Psychol Med*. 2010;40(10):1723-1733.
134. Bakker MK, Kerstjens-Frederikse WS, Buys CHCM, de Walle HEK, de Jong-van den Berg LTW. First-trimester use of paroxetine and congenital heart defects: a population-based case-control study. *Birth Defects Res A Clin Mol Teratol*. 2010;88(2):94-100.
135. Caton AR, Bell EM, Druschel CM, et al. Antihypertensive medication use during pregnancy and the risk of cardiovascular malformations. *Hypertension*. 2009;54(1):63-70.
136. Lennestål R, Otterblad Olausson P, Källén B. Maternal use of antihypertensive drugs in early pregnancy and delivery outcome, notably the presence of congenital heart defects in the infants. *Eur J Clin Pharmacol*. 2009;65(6):615-625.
137. Cooper WO, Hernandez-Diaz S, Arbogast PG, et al. Major congenital malformations after first-trimester exposure to ACE inhibitors. *N Engl J Med*. 2006;354(23):2443-2451.
138. Crider KS, Cleves MA, Reefhuis J, Berry RJ, Hobbs CA, Hu DJ. Antibacterial medication use during pregnancy and risk of birth defects: National Birth Defects Prevention Study. *Arch Pediatr Adolesc Med*. 2009;163(11):978-985.
139. Matok I, Gorodischer R, Koren G, Landau D, Wiznitzer A, Levy A. Exposure to folic acid antagonists during the first trimester of pregnancy and the risk of major malformations. *Br J Clin Pharmacol*. 2009;68(6):956-962.
140. Czeizel AE, Rockenbauer M, Sørensen HT, Olsen J. The teratogenic risk of trimethoprim-sulfonamides: a population based case-control study. *Reprod Toxicol*. 15(6):637-646.
141. Ferencz C, Rubin JD, Loffredo C MC. Epidemiology of congenital heart disease: the Baltimore Washington Infant Study: 1981-1989. *Futur Publ Co, Mt Kisco, NY*. 1993.
142. Ferencz C, Correa-Villasenor A LC. Genetic and environmental risk factors of major cardiovascular malformations: the Baltimore Washington Study: 1981-1989. *Futur Publ Co, Armonk, NY*. 1997:p. 463.
143. Csáky-Szunyogh M, Vereczkey A, Kósa Z, Urbán R, Czeizel AE. Association of maternal diseases during pregnancy with the risk of single ventricular septal defects in the offspring--a population-based case-control study. *J Matern Fetal Neonatal Med*. 2013;26(8):738-747.
144. Carter TC, Druschel CM, Romitti PA, Bell EM, Werler MM, Mitchell AA. Antifungal drugs and the risk of selected birth defects. *Am J Obstet Gynecol*. 2008;198(2):191.e1-e7.
145. Källén BAJ, Otterblad Olausson P. Maternal drug use in early pregnancy and infant cardiovascular defect. *Reprod Toxicol*. 17(3):255-261. http://www.ncbi.nlm.nih.gov/pubmed/12759093.
146. van Gelder MMHJ, Roeleveld N, Nordeng H. Exposure to non-steroidal anti-inflammatory drugs during pregnancy and the risk of selected birth defects: a prospective cohort study. *PLoS One*. 2011;6(7):e22174.
147. Loffredo CA, Ferencz C, Wilson PD, Lurie IW. Interrupted aortic arch: an epidemiologic study. *Teratology*. 2000;61(5):368-375.
148. Hernandez RK, Werler MM, Romitti P, Sun L, Anderka M. Nonsteroidal antiinflammatory drug use among women and the risk of birth defects. *Am J Obstet Gynecol*. 2012;206(3):228.e1-e8.
149. van Beynum IM, Kapusta L, Bakker MK, den Heijer M, Blom HJ, de Walle HEK. Protective effect of periconceptional folic acid supplements on the risk of congenital

heart defects: a registry-based case-control study in the northern Netherlands. *Eur Heart J*. 2010;31(4):464-471.
150. Botto LD, Khoury MJ, Mulinare J, Erickson JD. Periconceptional multivitamin use and the occurrence of conotruncal heart defects: results from a population-based, case-control study. *Pediatrics*. 1996;98(5):911-917.
151. Botto LD, Mulinare J, Erickson JD. Occurrence of congenital heart defects in relation to maternal mulitivitamin use. *Am J Epidemiol*. 2000;151(9):878-884.
152. Ortigado Matamala A, García García A, Jiménez Bustos JM. [Ebstein's anomaly and lithium exposure during pregnancy]. *An Pediatr (Barcelona, Spain 2003)*. 2006;65(6):626-627.
153. Unolt M, Putotto C, Silvestri LM, et al. Transposition of great arteries: new insights into the pathogenesis. *Front Pediatr*. 2013;1:11.
154. Tanoshima M, Kobayashi T, Tanoshima R, Beyene J, Koren G, Ito S. Risks of congenital malformations in offspring exposed to valproic acid in utero: A systematic review and cumulative meta-analysis. *Clin Pharmacol Ther*. 2015;98(4):417-441.
155. Mateja WA, Nelson DB, Kroelinger CD, Ruzek S, Segal J. The association between maternal alcohol use and smoking in early pregnancy and congenital cardiac defects. *J Womens Health (Larchmt)*. 2012;21(1):26-34.
156. Tikkanen J, Heinonen OP. Risk factors for atrial septal defect. *Eur J Epidemiol*. 1992;8(4):509-515.
157. Grewal J, Carmichael SL, Ma C, Lammer EJ, Shaw GM. Maternal periconceptional smoking and alcohol consumption and risk for select congenital anomalies. *Birth Defects Res A Clin Mol Teratol*. 2008;82(7):519-526.
158. Williams LJ, Correa A, Rasmussen S. Maternal lifestyle factors and risk for ventricular septal defects. *Birth Defects Res A Clin Mol Teratol*. 2004;70(2):59-64.
159. Browne ML, Bell EM, Druschel CM, et al. Maternal caffeine consumption and risk of cardiovascular malformations. *Birth Defects Res A Clin Mol Teratol*. 2007;79(7):533-543.
160. Alverson CJ, Strickland MJ, Gilboa SM, Correa A. Maternal smoking and congenital heart defects in the Baltimore-Washington Infant Study. *Pediatrics*. 2011;127(3):e647-e653.
161. Malik S, Cleves MA, Honein MA, et al. Maternal smoking and congenital heart defects. *Pediatrics*. 2008;121(4):e810-e816.
162. Patel SS, Burns TL, Botto LD, et al. Analysis of selected maternal exposures and non-syndromic atrioventricular septal defects in the National Birth Defects Prevention Study, 1997-2005. *Am J Med Genet A*. 2012;158A(10):2447-2455.
163. Sullivan PM, Dervan LA, Reiger S, Buddhe S, Schwartz SM. Risk of congenital heart defects in the offspring of smoking mothers: a population-based study. *J Pediatr*. 2015;166(4):978-984.e2.
164. Cresci M, Foffa I, Ait-Ali L, et al. Maternal and paternal environmental risk factors, metabolizing GSTM1 and GSTT1 polymorphisms, and congenital heart disease. *Am J Cardiol*. 2011;108(11):1625-1631.
165. Ewing CK, Loffredo CA, Beaty TH. Paternal risk factors for isolated membranous ventricular septal defects. *Am J Med Genet*. 1997;71(1):42-46.
166. Dadvand P, Rankin J, Rushton S, Pless-Mulloli T. Ambient air pollution and congenital heart disease: a register-based study. *Environ Res*. 2011;111(3):435-441.
167. Dolk H, Armstrong B, Lachowycz K, et al. Ambient air pollution and risk of congenital anomalies in England, 1991-1999. *Occup Environ Med*. 2010;67(4):223-227.

168. Anway MD, Skinner MK. Epigenetic programming of the germ line: effects of endocrine disruptors on the development of transgenerational disease. *Reprod Biomed Online*. 2008;16(1):23-25.
169. Desrosiers TA, Herring AH, Shapira SK, et al. Paternal occupation and birth defects: findings from the National Birth Defects Prevention Study. *Occup Environ Med*. 2012;69(8):534-542.
170. Shaw GM, Schulman J, Frisch JD, Cummins SK, Harris JA. Congenital malformations and birthweight in areas with potential environmental contamination. *Arch Environ Health*. 47(2):147-154.
171. Snijder CA, Vlot IJ, Burdorf A, et al. Congenital heart defects and parental occupational exposure to chemicals. *Hum Reprod*. 2012;27(5):1510-1517.
172. Watson RE, Jacobson CF, Williams AL, Howard WB, DeSesso JM. Trichloroethylene-contaminated drinking water and congenital heart defects: a critical analysis of the literature. *Reprod Toxicol*. 2006;21(2):117-147.
173. Hardin BD, Kelman BJ, Brent RL. Trichloroethylene and dichloroethylene: a critical review of teratogenicity. *Birth Defects Res A Clin Mol Teratol*. 2005;73(12):931-955.
174. Bove FJ, Fulcomer MC, Klotz JB, Esmart J, Dufficy EM, Savrin JE. Public drinking water contamination and birth outcomes. *Am J Epidemiol*. 1995;141(9):850-862.
175. Yauck JS, Malloy ME, Blair K, Simpson PM, McCarver DG. Proximity of residence to trichloroethylene-emitting sites and increased risk of offspring congenital heart defects among older women. *Birth Defects Res A Clin Mol Teratol*. 2004;70(10):808-814.
176. Reefhuis J, Honein MA, Schieve LA, Correa A, Hobbs CA, Rasmussen SA. Assisted reproductive technology and major structural birth defects in the United States. *Hum Reprod*. 2009;24(2):360-366.
177. Lewin MB, McBride KL, Pignatelli R, et al. Echocardiographic evaluation of asymptomatic parental and sibling cardiovascular anomalies associated with congenital left ventricular outflow tract lesions. *Pediatrics*. 2004;114(3):691-696.
178. Feinstein JA, Benson DW, Dubin AM, et al. Hypoplastic left heart syndrome: current considerations and expectations. *J Am Coll Cardiol*. 2012;59(1 Suppl):S1-S42.
179. van Velzen CL, Clur SA, Rijlaarsdam M, et al. Prenatal detection of congenital heart disease-results of a national screening programme. *BJOG*. 2016;123(3):400-407.
180. Rossier M-C, Mivelaz Y, Addor M-C, Sekarski N, Meijboom EJ, Vial Y. Evaluation of prenatal diagnosis of congenital heart disease in a regional controlled case study. *Swiss Med Wkly*. 2014;144:w14068.
181. Chenni N, Lacroze V, Pouet C, et al. Fetal heart disease and interruption of pregnancy: factors influencing the parental decision-making process. *Prenat Diagn*. 2012;32(2):168-172.
182. Stoll C, Alembik Y, Dott B, Roth MP. Impact of prenatal diagnosis on livebirth prevalence of children with congenital anomalies. *Ann génétique*. 45(3):115-121.
183. Liao C, Li R, Fu F, et al. Prenatal diagnosis of congenital heart defect by genome-wide high-resolution SNP array. *Prenat Diagn*. 2014;34(9):858-863.
184. Yan Y, Wu Q, Zhang L, et al. Detection of submicroscopic chromosomal aberrations by array-based comparative genomic hybridization in fetuses with congenital heart disease. *Ultrasound Obstet Gynecol*. 2014;43(4):404-412.
185. Resta R, Biesecker BB, Bennett RL, et al. A new definition of Genetic Counseling: National Society of Genetic Counselors' Task Force report. *J Genet Couns*. 2006;15(2):77-83.
186. Cañadas V, Vilacosta I, Bruna I, Fuster V. Marfan syndrome. Part 1: pathophysiology

and diagnosis. *Nat Rev Cardiol*. 2010;7(5):256-265.
187. Hickey EJ, Mehta R, Elmi M, et al. Survival implications: hypertrophic cardiomyopathy in Noonan syndrome. *Congenit Heart Dis*. 6(1):41-47.
188. Huertas-Rodríguez CK, Payán-Gómez C, Forero-Castro RM. [22q11.2DS Syndrome as a Genetic Subtype of Schizophrenia]. *Rev Colomb Psiquiatr*. 44(1):50-60.
189. Javitt GH. Policy implications of genetic testing: not just for geneticists anymore. *Adv Chronic Kidney Dis*. 2006;13(2):178-182.
190. Anderlid B-M, Schoumans J, Annerén G, et al. Subtelomeric rearrangements detected in patients with idiopathic mental retardation. *Am J Med Genet*. 2002;107(4):275-284.
191. Adeyinka A, Adams SA, Lorentz CP, Van Dyke DL, Jalal SM. Subtelomere deletions and translocations are frequently familial. *Am J Med Genet A*. 2005;135(1):28-35.
192. Costa MW, Guo G, Wolstein O, et al. Functional characterization of a novel mutation in NKX2-5 associated with congenital heart disease and adult-onset cardiomyopathy. *Circ Cardiovasc Genet*. 2013;6(3):238-247.
193. Kirk EP, Sunde M, Costa MW, et al. Mutations in cardiac T-box factor gene TBX20 are associated with diverse cardiac pathologies, including defects of septation and valvulogenesis and cardiomyopathy. *Am J Hum Genet*. 2007;81(2):280-291.
194. Granados-Riveron JT, Ghosh TK, Pope M, et al. Alpha-cardiac myosin heavy chain (MYH6) mutations affecting myofibril formation are associated with congenital heart defects. *Hum Mol Genet*. 2010;19(20):4007-4016.
195. Vermeer AMC, van Engelen K, Postma A V, et al. Ebstein anomaly associated with left ventricular noncompaction: an autosomal dominant condition that can be caused by mutations in MYH7. *Am J Med Genet C Semin Med Genet*. 2013;163C(3):178-184.
196. Mercer-Rosa L, Pinto N, Yang W, Tanel R, Goldmuntz E. 22q11.2 Deletion syndrome is associated with perioperative outcome in tetralogy of Fallot. *J Thorac Cardiovasc Surg*. 2013;146(4):868-873.
197. Barnett P, Postma A V. Genetics of congenital heart disease: Beyond half-measures. *Trends Cardiovasc Med*. 2015;25(4):302-304.
198. Aburawi EH, Aburawi HE, Bagnall KM, Bhuiyan ZA. Molecular insight into heart development and congenital heart disease: An update review from the Arab countries. *Trends Cardiovasc Med*. 2015;25(4):291-301.