Gynecology and Obstetrics Clinical Medicine 2 (2022) 109–123

Contents lists available at ScienceDirect

Gynecology and Obstetrics Clinical Medicine

journal homepage: www.keaipublishing.com/en/journals/gynecology-and-obstetrics-clinical-medicine

Review Article

Genetic detection of congenital heart disease

Sumathi I. Rachamadugu [a,b,1], Kristen A. Miller [c,1], Ina H. Lee [d], Ying S. Zou [b,e,f,*]

[a] Genetics DNA Diagnostic Lab, Johns Hopkins Genomics, USA
[b] Department of Genomic Medicine, Johns Hopkins University, Baltimore, MD, USA
[c] Department of Obstetrics-Gynecology, Johns Hopkins University School of Medicine, Baltimore, MD, USA
[d] Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA
[e] Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, USA
[f] Clinical Cytogenomics Laboratory, Johns Hopkins Hospital, Baltimore, MD, USA

ARTICLE INFO

Keywords:
Congenital heart disease
Next generation sequencing
Whole-genome sequencing
Circulating cell-free DNA Screening
Gene variant

ABSTRACT

Congenital heart disease (CHD) is the most common congenital anomaly and is an important cause of infant morbidity and mortality. Besides the epigenetic and environmental basis of CHD, genetics plays a central role in CHD pathogenesis. Traditional genetic testing strategies including conventional chromosome analysis, fluorescence *in situ* hybridization, and Sanger sequencing have largely focused on syndromic CHD or selected CHD phenotypes that are strongly associated with a particular genotype. The landscape of clinical genetic testing in CHD is rapidly evolving due to technical advances in genetic testing, including the identification of copy number variants by chromosomal microarray and nucleotide level alterations/variants by next-generation sequencing (NGS), which are essential to detect genetic causes of CHD and identify associations between genotypes and longitudinal clinical phenotypes. Whole-exome and whole-genome NGS not only reveal pathogenic variants in CHD genes, but also identify non-coding variants that influence the expression of CHD genes. Given the increasing availability and cost-effectiveness of clinical NGS to provide information on the causes of CHD and to detect incidental findings that are clinically actionable, the guidance of genetic counselors or experienced clinicians is essential. The identification of definitive causal CHD variants influences patient care and helps to inform the risk of recurrence, prenatal genetic counseling, and pre-implantation testing for the family of a CHD infant and adults with repaired/palliated CHD. Prenatally, circulating cell-free DNA screening as a non-invasive approach is available as early as 9 weeks of gestation and can screen for the common aneuploidies, which may underlie CHD. In this review, we present past and recent genetic testing in CHD based on our increased understanding of the pathogenesis of CHD along with current challenges with the interpretation of *de novo* genetic variants. Identification of a genetic diagnosis can help to predict and potentially improve clinical outcomes in CHD patients.

1. Introduction

Congenital heart disease (CHD) is the most common (and often severe) congenital anomaly at birth, with an estimated prevalence of 1 (range from 0.2 to 6) in 500 newborns.[1–3] CHD encompasses a broad spectrum of heart malformations and may also be accompanied by extra-cardiac malformations. CHD can be divided into syndromic and non-syndromic CHD depending on the involvement of extra-cardiac congenital abnormalities. Non-syndromic CHD has congenital abnormalities isolated to the heart (along with cardiovascular effects), and syndromic CHD has congenital abnormalities in the heart and other organs (extra-cardiac malformations that are independent from the CHD). Advanced medical interventions/treatments during infancy have significantly reduced CHD mortality and the majority (>90%) of current

* Corresponding author. Johns Hopkins University, USA.
E-mail address: yzou19@jhmi.edu (Y.S. Zou).

Publishing services by Elsevier on behalf of KeAi

[1] Co-first authors who contributed equally to this review.

https://doi.org/10.1016/j.gocm.2022.07.005
Received 30 June 2022; Received in revised form 28 July 2022; Accepted 31 July 2022
Available online 26 August 2022

patients with CHD survive to adulthood.[4] Newborns with CHD who have extra-cardiac structural or functional defects may also develop neurodevelopmental delays in childhood.[5,6] Therefore, understanding the etiologies and mechanisms of CHD are important for prognosis and treatment strategies.

The etiology of CHD is most often multifactorial, and genetics has a central role in CHD pathogenesis (Fig. 1).[7–10] Conventional chromosome analysis revealed that trisomies 13, 18 and 21 (Down syndrome) as well as monosomy X (Turner syndrome), are frequently associated with CHD.[11–13] Fluorescence *in situ* hybridization (FISH) can be used to reveal a recurrent small deletion of chromosome 22, resulting in 22q11.2 deletion syndrome (DiGeorge or Velocardiofacial syndrome), which is the most common deletion syndrome identified in CHD.[14] Chromosomal microarray assay (CMA) extended the molecular insights into sub-chromosomal copy number variants (CNVs), and more chromosome deletion or duplication syndromes are revealed using this analysis.[15–17]

Besides genome-wide linkage analyses of familial CHD to identify inherited CHD genes, advances in sequencing technologies have substantially accelerated the discovery of genes associated with CHD.[18–21] Next generation sequencing (NGS) technologies not only have transformed our understanding of CHD, but also are now widely adopted in clinical genetic laboratories as primary testing.[22] It is now cost-effective to analyze an individual with CHD-targeted gene panels, whole exome sequencing (WES), or whole genome sequencing (WGS) to contribute to the diagnosis of CHD.[23] CHD-targeted NGS gene panels include known CHD genes and CHD predisposition genes, and can identify pathogenic variants such as deleterious missense, loss-of-function variants, small insertions, and small deletions in CHD patients. WES sequences the protein coding regions of all 22,000 human genes (≈1% of the entire genome) and has become a frontline diagnostic tool for CHD. WGS obtains genetic information from the entire human genome. WGS platforms, advanced bioinformatics analytical tools and public, available, population-based sequencing datasets together enable the identification of not only pathogenic single nucleotide variants, but also CNVs and structural variants (SVs) in CHD patients.[22]

In the complex developmental processes of heart formation, CHD involves numerous essential genes and pathways, many of which are expressed during early embryonic development. Prenatally, circulating cell-free DNA screening is available as early as 9 weeks of gestation, and is a more sensitive screening tool for the most common chromosomal trisomies as compared to traditional biochemical-based prenatal aneuploidy screening methods.[24,25] As prenatal screening ultrasonography reveals heart anomalies,[26] advanced NGS technologies and powerful familial trio pipelines assist in genetic diagnosis of CHD.[27] The genetic counseling process helps communicate genetic and other relevant information such as recurrence risk that can influence patient care. In this review, we summarize overall genetic causes of CHD, discuss different genetic testing modalities to identify pathogenic variants in patients with CHD, and highlight advances in the use of CHD-targeted gene panels, WES, and WGS in the current genetic diagnosis of CHD.

2. Etiology of CHD

2.1. Overview

The etiology of CHD is multifactorial, and both genetic and environmental factors are contributors.[9,19,28] Genetic variation contributes to an estimated 40% of CHD cases, environmental factors contribute to an estimated 5%, and the remaining 55% of CHD cases are of unknown etiology (Fig. 1).

2.2. Genetic causes in CHD

2.2.1. Overview

Higher risk of CHD within families compared with the general population,[29] Mendelian inheritance patterns,[8] increased occurrences in consanguinity,[30] and higher monozygotic twin concordance rates[31] have long corroborated the role of genetics in CHD. Genetic causes of CHD are very heterogeneous, from chromosome abnormalities to single gene alterations, including CNVs (estimated 15%: range from 3% to 25% in syndromic CHD and 3%–10% in non-syndromic CHD), chromosomal anomalies or aneuploidies (estimated 13%, range from 9% to 18%), and single gene disorders (estimated 12%).[8,18,32] Most single gene disorders are attributable to *de novo* pathogenic variants (estimated 10.5% in CHD) rather than inherited pathogenic variants (estimated 1.5% in CHD) (Fig. 1).[6]

2.2.2. Chromosomal aneuploidy with CHD

Identified in the 1950's, the earliest genetic defect associated with CHD was chromosomal aneuploidy,[33] which result from a gain (trisomy) or loss (monosomy) of an entire chromosome. Whole chromosome aneuploidy is estimated to account for between 9 and 18% of all cases of

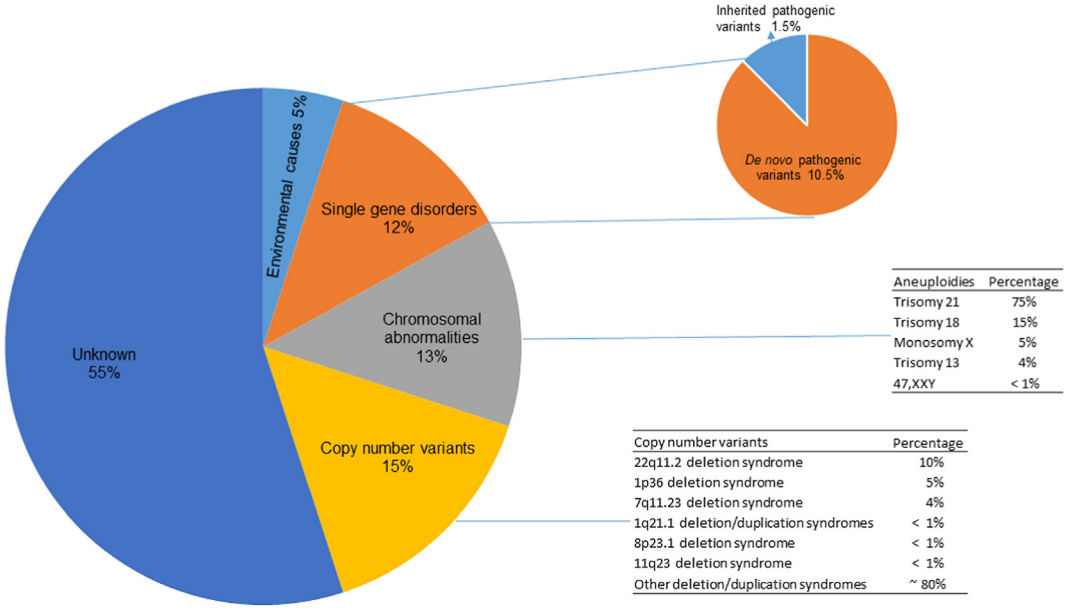

Fig. 1. Etiology of congenital heart disease (CHD).

CHD.[13] Trisomy 21 (Down syndrome) is the most frequent genetic diagnosis in CHD, and together with trisomy 18, trisomy 13, and monosomy X comprise the majority of chromosomal aneuploidies identified in infants with CHD (Fig. 1).

The large number of genes that are dysregulated in the setting of aneuploidy results in phenotypes with a broad range of effects in various body systems; 98% of fetuses with CHD and cytogenetic abnormalities have at least one extra-cardiac abnormality.[34,35] Down syndrome, for example, results from an extra copy of chromosome 21 that encompasses 225 genes,[36] and classically presents with multiple features that include intellectual disability, hypotonia, blood disorders, and hypothyroidism in addition to cardiac anomalies and distinct facies that can at times be detected by ultrasonography.[11]

Hartman et al. assessed the frequency of chromosomal abnormalities among live-born infants and fetal deaths with CHD in the Metropolitan Atlanta Congenital Defects Program.[13] They found that 1 in 8 infants with CHD had a chromosome abnormality. The specific types of CHDs most likely to be associated with a chromosome abnormality were interrupted aortic arch (type B or not specified), atrioventricular septal defect, and double outlet right ventricle. Heterotaxy, Ebstein's anomaly, and pulmonary valve stenosis were least likely to have a chromosome abnormality. This study was conducted at a time when CMA was not routinely available.

Data from Donnelly et al. suggests a 15.58% incremental yield of CMA over karyotype analysis in fetuses with cardiac anomalies.[37] This study also suggests that, depending on the specific cardiac anomaly identified in the fetus, the incremental yield of CMA over karyotype may be as high as 30%. Wang et al. found a diagnostic yield of 20.8% for fetuses with CHD using CMA.[38]

2.2.3. Deletion/duplication syndromes with CHD

CNVs comprise deletions or duplications that involve only a portion of the chromosome that ranges in size from 1 kilobase pairs to several megabase pairs and can lead to altered dosage of genes. CNVs occur in both heritable and *de novo* settings, which can present as non-syndromic CHD or a syndrome affecting multiple tissue systems, and account for 10–15% of CHD.[39] Lander et al. estimate that CNVs contribute to between 3 and 25% of CHD with extra-cardiac abnormalities, as compared to 3–10% of non-syndromic CHD.[40]

CNVs are best detected with CMA, and more recently, whole-genome and whole-exome sequencing. A large cohort-wide analysis of 538 CHD trios by WES and genotype array revealed *de novo* CNVs in 9.8% patients, who previously had no identified pathogenic genetic findings.[32] Continued gains in speed, accuracy, and resolution of sequencing technologies will inevitably identify additional novel alterations that will account for cases that currently have no known genetic cause. Furthermore, the ever-increasing ability to identify CNVs in CHD patients continues to reveal affected genes and the range of cellular functions involved in CHD, which include structural proteins and a host of regulatory and repair elements. For example, chromosome 7q11.23 deletion syndrome results in haploinsufficiency of the Elastin structural protein, which leads to supravalvar aortic and pulmonary stenosis in William Syndrome.[41]

The most common human microdeletion associated with CHD is chromosome 22q11.2 deletion syndrome (Del22q11), also known as DiGeorge Syndrome and Velocardio-facial syndrome, which presents with a variable phenotype encompassing CHD, palate abnormalities, hypocalcemia, immunodeficiency, characteristic facial features, and neurodevelopmental abnormalities such as learning disabilities and psychiatric disorders.[42,43] Del22q11 includes the T-Box transcription factor *TBX1*, in which haploinsufficiency results in the cardio-pharyngeal phenotype.[44–46] Enrichment of H3K4me1 was shown in *Tbx1* haploinsufficient mice, suggesting interplay of *TBX1* and chromatin regulation in Del22q11.[47] Chromosome 8p23 deletion syndrome is another well characterized CNV which results in a range of CHD along with developmental delays, which may stem from loss of the cardiac transcription factor *GATA4*.[48] Other CNVs in syndromic CHD include chromosome 1p36 deletion syndrome (OMIM: 607872), 1q21.1 deletion/duplication syndrome (OMIM: 612474 and 612475), and 11q23 deletion syndrome (Jacobsen syndrome, OMIM: 147791).

Large scale investigations of microdeletions and microduplications have identified recurrently altered genes, implicating them in the CHD pathogenesis. In one study, 240 CNVs were detected in genes with known involvement in cardiac development including *NRP1, NTRK3, MESP1, ADAM19*, and *HAND1*.[49] Another cohort study found that distinct CNVs of particular cardiac-specific genes showed differential enrichment in patients with tetralogy of Fallot, atrial ventricular septal defect, truncus arteriosus, subaortic stenosis, and atrial ventricular canal,[50] providing further insight into specific pathogenic mechanisms for CHD subtypes. Interestingly, additional CNVs and/or environmental factors in patients with Down syndrome could contribute to the CHD risk.[51]

2.2.4. Monogenic causes of CHD

While it is estimated that about 400 human genes are involved in causing or contributing to structural CHD,[6,52,53] < 200 genes are currently recognized as being definitively associated,[54] implying that most CHD-associated genes are yet to be identified. Monogenic contributors to various non-syndromic and syndromic forms of CHD have been recognized, and include genes encoding morphogenetic factors, transcription factors, signal transducers, histone/chromatin modifiers, sarcomeric and other cardiac structural proteins that are important for cardiac development.[55]

While single gene variants contribute least to CHD among the various genetic etiologies, their contribution is still significant, accounting for 8–30% of CHD.[8,54,56] Based on a systematic review of 18 studies which utilized prenatal WES for fetuses with CHD, the incremental yield from monogenic testing was shown to be at the higher end of the aforementioned range, at 21%.[57] Most CHD-causing variants occur *de novo* rather than being transmitted from parent to child.[18,28,57] Overall, it appears that *de novo* variants contribute to 8–10% of all CHD, while inherited or transmitted variants (which can occur in genes such as *GDF1*, *MYH6* and *FLT4,* among others) account for about 1.8% of CHD cases.[8,50] In recent years, it has been recognized that *de novo* variants contributing to CHD may sometimes be identified in the mosaic state, where the variant is present in some but not all somatic cells.[53]

Similar to what is observed in the case of chromosomal aneuploidy and CNVs, the likelihood of identifying a monogenic disorder is higher for syndromic vs. non-syndromic CHD.[18,58,59] Most single gene variants that contribute to syndromic CHD occur *de novo,* while inherited variants seem to play a more significant role in non-syndromic CHD.[28] It has been shown that 10–28% of syndromic CHD cases are attributable to single gene variants that arose *de novo*.[6,8,18] On the other hand, only 2–10% of non-syndromic sporadic CHD is attributable to *de novo* coding variants in known and candidate CHD genes.[6,8,60] The difference in the trend in yield from single gene disorders observed in postnatal cases highlighted above holds true for prenatal testing as well, where yield of 49% for syndromic CHD vs. approximately 11% for non-syndromic CHD has been demonstrated.[57] The yield from monogenic testing in multiplex families with apparently non-syndromic CHD may be as high as 31–46%.[61,62]

Syndromic or non-syndromic CHD attributable to single gene dysfunction may be inherited in an autosomal dominant, autosomal recessive, or X-linked manner, depending on the inheritance pattern associated with the particular gene/disorder. Syndromic CHD tends to have high or complete penetrance, while incomplete penetrance is most notable in non-syndromic familial CHD.[28,63] Variable presentation may be seen in affected family members regardless of whether the gene is associated with syndromic or non-syndromic CHD, and if disease expression is very mild, it may go unrecognized.[55,64] As a result of

reduced penetrance and variable expressivity, what appears as sporadic CHD may at times be familial.

Importantly, both familial as well as sporadic CHD may occur due to pathogenic variants in genes associated with syndromic and/or non-syndromic CHD.[8] While some genes such as *FLT4* are known to only cause non-syndromic CHD,[8,65] other genes can result in either apparently non-syndromic or syndromic CHD. For example, the patient with a *CHD7*-mediated congenital heart defect with no other classic features of CHARGE syndrome,[52] patients with *JAG1*-related CHD that had no other features of Alagille syndrome,[66] and families with *TBX5*-related CHD who did not display other features of Holt-Oram syndrome.[61,62] Further, some genes may contribute to more than one type of cardiac lesion or dissimilar cardiac phenotypes among affected individuals,[52,67,68] and for many genes associated with syndromic CHD, individuals with extra-cardiac features of the syndrome may not have CHD; for example, 15–25% of individuals with CHARGE syndrome do not develop CHD,[67] and some individuals in a family with Holt-Oram syndrome did not have cardiac defects but had other features of the syndrome.[69]

Monogenic CHD can be tested for via Sanger sequencing of a single gene when there is strong suspicion for a particular genetic contributor, or via broader testing such as a targeted CHD panel, WES or WGS when the differential diagnosis list is long or when the constellation of syndromic findings in an individual do not fit a known disorder. The more expansive sequencing methods are described in subsequent sections of this article. Importantly, what appears as non-syndromic CHD may in fact be syndromic, and is especially relevant when apparently non-syndromic CHD is diagnosed in a fetus, as *in utero* imaging techniques may miss certain extra-cardiac fetal anomalies, and features of neuro-developmental disorders cannot be recognized in the prenatal period.[57,70] Therefore, the absence of extra-cardiac features should not preclude testing for syndromic CHD genes. Testing for monogenic causes is indicated when karyotype and CMA are negative in case of fetal anomalies suspected to be due to genetic etiology, including sporadically occurring syndromic or non-syndromic CHD.[71] Testing for single gene dysfunction is also indicated for familial CHD.[61,62]

2.2.5. Non-coding variants in CHD

Ongoing technological improvements will continue to overcome previous limitations and add to the list of detectable genetic alterations resulting in dysregulation of gene expression, however newer research shows that variants in noncoding regulatory elements may also contribute to CHD with unknown etiology. Richter et al. assessed the contribution of noncoding *de novo* variants in 749 CHD probands with no previously detected genetic cause.[72] Using neural network prediction algorithms, the group showed that the CHD probands had significantly more *de novo* variants affecting noncoding transcriptional elements such as enhancers and RNA-binding protein regulatory sites, compared with unaffected individuals. This study further identified recurrently implicated genes with noncoding *de novo* variants, which included *SHOC2, ZNRF3, CPSF3, MAP4K4*, and *COL1A2*.72.

MicroRNAs (miRNAs) (~20–30 nucleotides long) and long non-coding RNAs (LncRNAs) (>200 nucleotides long) are non-coding nucleic acid molecules that influence gene expression and post-transcriptional regulation.[73–75] Both miRNA and LncRNA are essential to tissue and organ differentiation and morphogenesis, including that of the myocardium.[76–78] Dysregulation of miRNAs by alterations in the miRNA binding site of a gene[79] or within the miRNA proper[80] can result in severe CHD.[81] For instance, a single nucleotide variant in miRNA-499, which normally regulates a key enzyme in folate metabolism by expression inhibition, results in abnormal early development of the heart.[82] Consequently, miR-499 has become useful as a diagnostic biomarker for CHD.[82,83] Likewise, aberrant expression of LncRNA has been found in cardiac tissue of patients with VSDs,[84] as well as in the plasma of pregnant mothers whose fetuses had echocardiography confirmed CHD.[85] It is foreseeable that the role of miRNAs and LncRNAs in diagnostics, prognosis, and perhaps even treatment will continue to grow.

2.2.6. Risk factors for CHD

CHD has also been associated with advanced paternal age (>45 years)[86] and genetic risk factors, such as polymorphic alleles in *MTHFR* (g.677C > T and g.1298A > C),[87] *MTRR* (g.66A > G and g.2756A > G),[88] *GATA4* (g.354A > C),[89] *NKX2-5* (g.63A > G),[90] etc.

2.3. Environmental contributors to CHD

Environmental causes are implicated in an estimated 5% (range from 2% to 10%) of CHD cases (Fig. 1).[9] CHD has been associated with environmental risk factors including maternal/paternal environmental exposures to air pollution or toxic chemicals, parental smoking, maternal history of infectious diseases during pregnancy, pre-gestational and gestational diabetes mellitus, maternal obesity, maternal drug use, and pregnancy through artificial reproductive technologies.[9,91–94] The association of maternal alcohol or coffee consumption with CHD was not significant, however, maternal folic acid supplementation seems to have a preventive effect on CHD.[95] Although the underlying mechanisms by which environmental factors disrupt molecular pathways during cardiac development to cause CHD remain unknown, CHD has been shown to be caused by gene-environment interactions resulting in increased incidence of CHD in animal models.[96] Table 1 highlights some of the most common environmental/teratogenic explanations and their associated CHDs. For pregnancies with known risk factors such as those listed in the table, fetal echocardiography may be indicated to screen for CHDs.

Table 1
Common environmental exposures/teratogens and associated CHDs.

Teratogen	Commonly Associated CHDs:

Assisted reproductive technology	• Mild CHD more common than severe:
	  • Ventricular septal defects
	  • Ventricular free wall thickening
	  • Pericardial effusion
	  • Tricuspid regurgitation
	  • Displaced apex

Maternal diabetes	• Ventricular septal defects • Atrioventricular septal defects • Tetralogy of Fallot • Transposition of the great arteries • Truncus arteriosus • Hypoplastic left heart • Patent ductus arteriosus
Maternal lupus	• Fetal heart block
Maternal phenylketonuria (PKU)	• Ventricular septal defects • Tetralogy of Fallot • Patent ductus arteriosus • Hypoplastic left heart syndrome
Rubella	• Patent ductus arteriosus • Ventricular septal defects • Atrial septal defects • Peripheral pulmonary stenosis
Alcohol	• Ventricular septal defects • Atrial septal defects • Patent ductus arteriosus
Lithium	• Ebstein's anomaly (10%) • Tricuspid atresia
Hyperthermia	• Conotruncal and obstructive defects
Antiepileptics	• Ventricular septal defects • Atrial septal defects • tetralogy of Fallot • Pulmonary valve atresia • Hypoplastic right heart
Vitamin A	• Pulmonary stenosis and outflow tract abnormalities
Paroxetine	• Ventricular septal defects • Atrial septal defects • Outflow tract obstructions
NSAIDs, such as Ibuprofen	• Transposition of the great arteries • Ventricular septal defects • Bicuspid aortic valve

Sources for table: Lynch et al. and Patil et al.[152,153]

2.4. Complex inheritance, incomplete penetrance, and genetic modifiers of CHD

Over half of CHD cases have unknown etiology and some known CHD genes display an inheritance pattern characterized by incomplete penetrance,[8] which highlights the genetic complexity of CHD. Incomplete penetrance of CHD genes (e.g., *NOTCH1*, *ROBO4,* and *SMAD6*) happens in some families when certain individuals express CHD phenotype while others do not, even though they carry a pathogenic (disease-causing) variant in a CHD gene. There can also be a sex bias in penetrance.[97–99] Genetic or environmental modifiers could contribute to clinical variabilities (severities) in CHD presentation. Besides classic recessive or dominant transmitted models, more complex genetic models (e.g., epistatic interactions of multiple variants/genes[100]) could play a role in genetic heterogeneity of CHD.[101] Clinical CHD phenotype could be influenced by susceptibility loci as well as genetic modifiers. Common genetic risk variants in multiple loci have been reported in CHD.[102,103] In a family with childhood–onset cardiomyopathy, three missense variants in *MKL2, MYH7*, and *NKX2-5* were required to cause disease.[104] Interaction between *DNAH6* and other primary ciliary dyskinesia genes was demonstrated to lead to CHD along with primary ciliary dyskinesia in a study by Li et al.[105] Furthermore, the severity of CHD phenotype may be associated with mosaicism, including the proportion of abnormal cell numbers in the disease-relevant tissues, allele frequencies of pathogenic variants, and types of variants.[106] The allele frequency of a pathogenic variant found in either blood or saliva specimen may not reflect the proportion of abnormal cell numbers/percentages in the patient's cardiac tissue. Various variants (such as missense, nonsense, frame-shift, splicing-site, indel, insertional variants) may have different impacts on the severity of CHD phenotype.

Identification of a fetus with left ventricular outflow tract obstruction (LVOTO) malformations should prompt cardiac evaluation for other family members, especially parents and siblings even when genetic testing is not pursued or the specific genetic cause in the family is not identified. This is because bicuspid aortic valve (BAV)/aneurysm syndrome may present with variations of left-sided defects, including: BAV with aneurysm, BAV without aneurysm, aortic aneurysm and normal aortic valve, as well as more severe defects including coarctation of the aorta or hypoplastic left heart in family members. BAV/aneurysm syndrome is inherited in an autosomal dominant pattern and can show decreased penetrance and therefore may appear to "skip generations" in affected families. Thus, all first-degree relatives in families with BAV/aneurysm syndrome should obtain echocardiograms to check for valve abnormalities as well as aortic root and ascending aortic aneurysms.[107] Deleterious variants in *NOTCH1* are associated with aortic valve disease 1 (OMIM #109730) which can present with BAV, aortic calcification/-stenosis, mitral valve atresia, ventricular septal defects, double-outlet right ventricle, and hypoplastic left heart.

3. Genetic testing

3.1. Karyotype and FISH for CHD

Conventional chromosome analysis/karyotype recognized chromosomal aneuploidies a few decades ago.[13] Karyotype visualizes the chromosomes during metaphase for diagnosing numerical chromosome abnormalities (aneuploidies, gross deletions and duplications) and structural chromosome abnormalities (balanced and unbalanced translocations, inversions, insertions, ring chromosomes, etc.) with a resolution of 5–10 Mb. It can detect balanced structural chromosome abnormalities, which are not detectable by CMA. Furthermore, it is important to perform parental karyotypes of children with chromosomal abnormalities (such as trisomy 13 or trisomy 21 caused by Robertsonian translocations, deletions, duplications, or balanced or unbalanced reciprocal translocations, etc.) for potential chromosomal abnormalities to assess risk of recurrence in future pregnancies. Inherited translocations have been reported to increase risk of aneuploidy recurrence and spontaneous abortion.[108]

FISH targets a specific chromosomal region using fluorescent probes, and can detect aneuploidies, deletions, and duplications with a resolution of 100 kilobase pairs. FISH can be performed on cells during any stage of the cell cycle without cell culture and is therefore a useful means of rapid diagnosis. Aneuploidy screening using FISH probes for chromosomes 13, 18, 21, X, and Y is used prenatally and postnatally to determine various trisomies or sex chromosome abnormalities in fetuses or newborn babies.[25] FISH is also used to detect pathogenic CNVs that result in well-known deletion/duplication syndromes such as chromosome 1p36 deletion syndrome, 7q11.23 deletion syndrome (Williams-Beuren syndrome) and 22q11.2 deletion syndrome (DiGeorge syndrome).[14,17] FISH is useful to reveal mosaicism when cells with chromosomal aneuploidies or CNVs are present along with normal cells. False negative results may occur when atypical or smaller deletions/duplications are present either proximal or distal to the FISH probes.[109] Therefore, a normal FISH result cannot definitively rule out the aforementioned deletions, and CMA remains the gold standard for diagnosis of CNVs.

3.2. Chromosomal microarray for CHD

Microarray-based comparative genomic hybridization (CGH) and single nucleotide polymorphism (SNP) microarrays can identify submicroscopic CNVs, which are too small to be seen by standard cytogenetic analysis via karyotype. Both array CGH and SNP microarrays can detect genome-wide CNVs; however, SNP microarray can also detect triploidy, loss of heterozygosity, uniparental disomy, and mosaicism.[110] The widespread use of CMA as a first-line test for the diagnosis of congenital anomalies, including cardiac defects, has led to the discovery of many CNVs associated with CHD. CMA is routinely used in both the prenatal and postnatal settings for patients with both syndromic and non-syndromic CHDs, and the American College of Obstetricians and Gynecologists (ACOG) states that CMA may be used for prenatal diagnosis in the setting of fetal anomalies, as well as for any person undergoing prenatal diagnosis, in light of its increased diagnostic yield as compared to standard karyotype.[111] Unlike aneuploidies, the incidence of CNVs is not dependent upon maternal age. In the general population (i.e. fetuses without sonographic anomalies or other risk factors), the incidence of CNVs is estimated to be approximately 0.4%.[111] In the setting of a fetal CHD, the yield of CMA is estimated to be increased to approximately 3%–25%, but varies greatly depending upon the type of CHD and the presence of other anomalies, soft markers, and/or growth restriction. Many recurrent CNVs are the result of flanking repeat sequences, which predispose to non-allelic homologous recombination and recurrent *de novo* deletions or duplications encompassing the same genomic interval. CNV-mediated CHDs are associated with a poorer prognosis, as compared to cases of CHD without CNVs. Specifically, the presence of pathogenic CNVs has been associated with significantly decreased transplant-free survival after surgery, worse linear growth, and worse neurocognitive outcomes.[28]

3.3. Targeted next-generation sequencing (pathogenic variants) for CHD

NGS is a massively parallel sequencing technology used to identify small genetic variation down to the nucleotide level. NGS panels are designed to include multiple genes of interest for a specific disorder (e.g., Noonan syndrome/RASopathy gene panels) or a group of related disorders (e.g., heterotaxy/primary ciliary dyskinesia panels) or may even be a broad CHD panel including several genes associated with non-syndromic and syndromic CHD. Some broad panels may additionally contain one or more heterotaxy/primary ciliary dyskinesia genes (Table 2), and even contain genes associated with other cardiac disorders such as cardiomyopathies, arrhythmias, etc. Test offerings can vary greatly among laboratories, not just in which condition(s) the panel targets, but also in the genes surveyed for the condition(s). Massively parallel sequencing

Table 2
Genes commonly included in targeted CHD panels.

CHD Genes	MIM	Disorder	Inheritance
CHD7[b]	214800	CHARGE syndrome[b]	AD
	N/A	Isolated CHD[52]	
ELN[a]	185500	Supravalvular aortic stenosis	AD
GATA4[a]	607941	Atrial septal defect 2	AD
	614430	Atrioventricular septal defect 4	
	187500	Tetralogy of Fallot	
	614429	Ventricular septal defect 1	
GATA6[b]	614475	Atrial septal defect 9	AD
	614474	Atrioventricular septal defect 5	
	600001	Pancreatic agenesis and congenital heart defects[b]	
	217095	Persistent truncus arteriosus	
	187500	Tetralogy of Fallot	
GDF1[b]	208530	Right atrial isomerism[b]	AR
	613854	Congenital heart defects, multiple types, 6	AD
JAG1[b]	118450	Alagille syndrome[b]	AD
	187500	Tetralogy of Fallot[66]	
NKX2-5[a]	108900	Atrial septal defect 7	AD
	217095	Conotruncal heart malformations, variable	
	614435	Hypoplastic left heart syndrome 2	
	187500	Tetralogy of Fallot	
	614432	Ventricular septal defect 3	
NKX2-6[a]	217095	Conotruncal heart malformations	AR
	217095	Persistent truncus arteriosus	
NOTCH1[b]	109730	Aortic valve disease 1	AD
	616028	Adams-Oliver syndrome 5[b]	
NR2F2[a]	615779	Congenital heart defects, multiple types, 4	AD
TBX1[b]	188400	DiGeorge syndrome[b]	AD
	192430	Velocardiofacial syndrome[b]	
	217095	Conotruncal anomaly face syndrome[b]	
	187500	Tetralogy of Fallot	
TBX5[b]	142900	Holt-Oram syndrome[b]	AD
	N/A	Atrioventricular septal defect, Atrial septal defect, Ventricular septal defect[61,62,67]	
ZIC3[b]	306955	Congenital heart defects, nonsyndromic, 1, X-linked	XLR
	306955	Heterotaxy, visceral, 1, X-linked[b]	
	314390	VACTERL association, X-linked[b]	

Table lists 13 genes common across targeted CHD panels offered by four different laboratories included in the Genetic Testing Registry, and available for prenatal testing. An additional 32 CHD genes common across panels from 3 laboratories, and 35 genes common across 2 laboratories are available for testing but not included in this table.

Note: AD: autosomal dominant; AR: autosomal recessive; XLR:X-linked recessive.

[a] Gene associated with NS-CHD (non-syndromic congenital heart disease) only.

[b] Gene associated with NS-CHD as well as extracardiac/syndromic presentation or heterotaxy.

involves simultaneous sequencing of all genes on the panel, precluding the need for consecutive testing of genes of interest. Sequencing is targeted to the coding regions and exon-intron boundaries (≤10 bp of the flanking intronic sequence on either side of each exon) in each gene, as these regions harbor the majority of monogenic disease-causing variants.[89] NGS panel testing can detect single nucleotide variants and small deletions or insertions (INDELs) in the targeted genes, which are not detectable using karyotyping and CMA. Additional technologies that enable detection of exon-level deletions and duplications are also typically incorporated.

NGS panel testing for CHD is sometimes utilized in the postnatal setting, more so for syndromic CHD, but in general, it is not the standard of care for sporadic non-syndromic CHD as the majority of these cases are expected to have multifactorial etiology with low yield on current genetic testing technologies.[112] Studies to determine and compare the utility and detection rate of NGS panel testing in specific CHD cohorts, i.e., non-syndromic vs. syndromic, adult vs. pediatric, and/or familial vs. sporadic have not yet been performed.

Two postnatal studies have utilized NGS panels to test families with seemingly non-syndromic CHD.[61,62] The NGS panel in both studies included 57 CHD-associated genes (the gene set included in each panel was not identical) and identified likely disease-causing variants in 5/16 families (31%) and 6/13 families (46%) respectively. *TBX5, TFAB2B, ELN, NOTCH1*, and *MYH6* were implicated in these two studies, with disease-causing variants in *NOTCH1* and *TBX5* being common to both studies. The yield from these two studies is likely inflated as they only investigated multiplex families, and they did not utilize the more stringent criteria of American College of Medical Genetics & Genomics (ACMG) for variant interpretation. Nevertheless, the two studies seem to demonstrate the utility of NGS panels for familial CHD.

Currently, NGS panel testing is only sometimes employed in the testing of fetuses with CHD.[113] While a family history of CHD is a consistent risk factor in the identification of fetal CHD, etiology is still more likely to be multifactorial rather than monogenic.[114] Prenatal studies to assess diagnostic yield of NGS panel testing in fetuses with familial CHD have not yet been performed. To-date, a single prenatal study assessing the utility of NGS panel testing in the diagnosis of fetuses with sporadic CHD has been conducted.[70] The study employed a panel of 77 CHD-associated genes to test 44 fetuses with either non-syndromic or syndromic CHD, after they had undergone karyotyping and CMA with negative results. Seven fetuses (15.9%) had a positive result, all of them attributable to *de novo* variants, while 79.5% had variants of uncertain significance (VUSs). Positive results in this cohort were attributable to pathogenic or likely pathogenic variants in the *CHD7* gene for CHARGE syndrome, *CITED2* (associated with atrial septal defect and ventral septal defect), *MYH6* (associated with atrial septal defect, dilated cardiomyopathy and hypertrophic cardiomyopathy), *JAG1* (associated with tetralogy of Fallot and Alagille syndrome), and in two fetuses, *KMT2D* -associated Kabuki syndrome. The authors noted that the detection rate in the prenatal cohort was lower compared to the two postnatal studies, possibly attributable to the inclusion of sporadic rather than familial CHD cases, differences in type of cardiac lesions tested for, differences in accuracy of diagnosis owing to limitations with *in utero* phenotyping, and the application of ACMG criteria for variant interpretation in the prenatal study.[61,62]

In 2018, the American Heart Association recognized the utility of gene panel testing in cases of suspected monogenic disease with a small differential diagnosis.[28] Following this, the ACMG in 2020 published a statement recommending single-gene testing or a phenotype-based gene panel test as the initial or first-line test to be performed when anomalies in the fetus strongly suggest a specific monogenic disorder.[115] When a phenotype is genetically heterogeneous (e.g., Noonan syndrome), panel testing has increased detection rate over single gene testing, is less expensive than an approach of reflexing to the next gene of interest after a negative single gene test, and is also less expensive than WES or WGS. Panel tests typically have a short turnaround time of 2–4 weeks, with the added advantage of identifying far fewer VUSs compared to WES/WGS, making result interpretation that much more manageable. Notably, the VUS rate increases incrementally with the inclusion of additional genes on a panel. It would be important for the ordering provider to be aware that the associated risk for CHD for each gene on a panel may vary, and that including low-risk genes or candidate genes will likely result in a greater number of VUSs being identified.

3.4. Whole exome sequencing and whole genome sequencing for CHD

Similar to NGS panels, WES and WGS both utilize massively parallel NGS technology, but in comparison with panels, interrogation by WES is more large-scale while WGS is the most comprehensive (Fig. 2). WES limits examination to the exons (protein-coding regions) and exon-intron boundaries of the approximately 20,000 genes that together comprise 1–2% of the nuclear genome yet harbor 85% of variants contributing to monogenic traits (Fig. 2).[116] WES can detect single nucleotide variants (SNVs), small INDELs, and CNVs involving one or more exons (Fig. 2).[117] WGS involves testing of most of the 3 billion base pairs in the nuclear

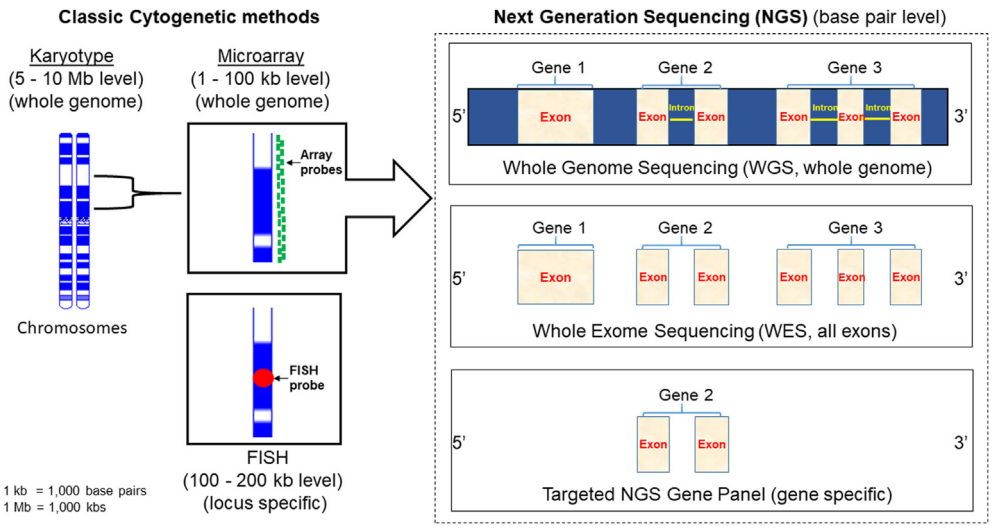

Fig. 2. Diagnostic capability of genetic tests.

genome, and in addition to all exons, also covers non-coding regions (deep intronic regions, untranslated regions or intergenic regions) that harbor an estimated 15% of variants contributing to monogenic disease (Fig. 2).[22] Another strength of WGS is the ability to identify copy number variants and gross chromosomal abnormalities or SVs not identifiable by other sequencing methods including panel tests and WES.[22,117,118]

WES has been offered as a clinical test since 2011. Some laboratories limit analysis to the coding sequence of known disease-associated genes alone (called a clinical exome), but others may include analysis of variants identified in candidate genes as well.[115] WGS is also available as a clinical test, but is not frequently utilized at this time mainly owing to increased cost and longer turnaround time.[115] However, based on the ability of WES and WGS to facilitate novel gene discovery, and the extra benefit of identifying variant types by WGS that are not identifiable by other sequencing tests, WES and WGS are commonly used in research.

WES has been utilized in the testing of fetuses with CHD, typically after karyotype and/or CMA have yielded negative results and has demonstrated incremental yield in non-syndromic CHD as well as syndromic CHD with extra-cardiac anomalies. The CODE study (COngenital heart disease and the Diagnostic yield with Exome sequencing) involved antenatal trio WES of a prospective CHD cohort of 197 fetuses, as well as systematic review of 18 published studies in which WES was performed on a total of 636 prenatally diagnosed CHD cases.[57] In the prospective cohort the overall detection rate was 12.7%, 11.5% in the isolated CHD prospective cohort, and 14.7% in the prospective cohort with CHD and extra-cardiac anomalies; the corresponding pooled yields from systematic literature review were 21%, 11% and 37% respectively. When sub-analysis of studies with at least 20 cases was performed, the incremental yields were similar except for CHD associated with extra-cardiac anomalies, which had higher yield at 49%. Higher WES detection rate for CHD with extra-cardiac anomalies over isolated CHD was demonstrated. The yield was highest for cardiac shunt lesions (41%), followed by right-sided lesions (26%), complex lesions (23%), and left-sided obstructive lesions (18%). Kabuki syndrome and CHARGE syndrome were the most frequently identified disorders in this study. The majority (approximately 70%) of the pathogenic variants identified occurred *de novo*, and in genes associated with autosomal dominant disease.

A recent study utilized trio WGS in 111 fetuses with structural or growth anomalies including cardiac defects, and was able to detect every pathogenic variant that was identified in the same cohort by concurrently performed CMA plus WES (22/111 cases; 19.8%).[117] Additionally, WGS was also able to detect a balanced translocation in a parent which resulted in CNVs in twin fetuses, another case with a dual diagnosis owing to presence of a CNV along with a heterozygous SNV, and a third case of intrauterine cytomegalovirus infection, which are genetic and non-genetic etiologies not detectable by CMA, WES or NGS panel testing. A subsequent prenatal WGS study by Wang et al. also resulted in a detection rate of 19%, identifying not only sequencing variants but also CNVs detected by CMA.[119] These results suggest a role for WGS as a first-tier test replacing multiple consecutive genetic tests such as microarray, gene panels, and WES, to provide the most comprehensive analysis in a timely manner essential in the prenatal setting.

Recognizing that diagnostic rate from WES and WGS are comparable to that of karyotyping and CMA, and even higher for certain indications, the International Society for Prenatal Diagnosis (ISPD), the Society for Maternal Fetal Medicine (SMFM), and the Perinatal Quality Foundation (PQF) published a joint position statement acknowledging the use of panel testing, WES and WGS for fetal diagnosis in certain situations. The position statement was recently updated and considers the presence of a single major fetal anomaly or involvement of multiple organ systems suspicious for genetic etiology as indications for prenatal sequencing.[71] They recommend a trio WES approach, and that it be performed for the above prenatal indications when CMA results are non-informative, or that CMA be run in parallel with WES as the latter cannot detect CNVs. The authors of the position statement consider WGS as still applicable in the research setting only for many reasons including difficulty in interpretation of non-coding variants and SVs. The position statement also recognizes a role for the application of sequencing methods in the testing of parents who present for preconception counseling with history of recurrent anomalies in more than one pregnancy, and a sample from the affected proband or fetus cannot be obtained. The ACMG in 2020 has also published a statement that trio WES be considered in the testing of fetuses with ultrasound anomalies for which karyotype and CMA do not provide informative results.[115] Further, the ACMG and ISPD publications also provide guidance regarding points to consider in the reporting of WES and WGS findings that may go beyond testing of known CHD-associated genes, to include candidate genes, secondary findings, and incidental findings.[71,115]

3.5. *Secondary genomic findings for CHD*

The ACMG has published recommendations for reporting incidental findings in clinical WES or WGS since 2013.[120] The most recent version is the ACMG SF v3.0 list published in 2021.[121] It includes 73 genes based on the medical actionability of the associated condition and maximizing the potential to reduce morbidity and mortality. Thirty-three genes related to cardiovascular phenotypes are in the list, which include aortopathy genes (*FBN1*, *TGFBR1, TGFBR2, SMAD3, ACTA2, MYH11*), arrhythmogenic

cardiomyopathy genes (*PKP2*, *DSP, DSC2, TMEM43, DSG2*), catecholaminergic polymorphic ventricular tachycardia genes (*RYR2*, *CASQ2, TRDN*), dilated cardiomyopathy genes (*TNNT2, LMNA, FLNC,TTN*), vascular type of Ehlers-Danlos syndrome (*COL3A1*), familial hypercholesterolemia genes (*LDLR, APOB, PCSK9*), hypertrophic cardiomyopathy genes (*MYH7, MYBPC3, TNNI3, TPM1, MYL3, ACTC1, PRKAG2, MYL2*), and genes for long QT syndrome types 1 to 3 (*KCNQ1, KCNH2, SCN5A*). It is recommended that pathogenic or likely pathogenic variants in these CHD-related genes be reported in clinical exome and genome sequencing, unrelated to the indication for testing, and with the patient's consent.

3.6. Noninvasive prenatal testing for CHD

Since its launch in 2011, circulating cell-free DNA screening (cfDNA) has revolutionized non-invasive screening for aneuploidy. cfDNA methodology analyzes extracellular DNA fragments in maternal circulation (typically 150–200 base pairs in length), released by the trophoblast cells of the placenta, to screen for chromosome abnormalities using various methodologies, such as massively parallel shotgun sequencing (MPSS) or SNP-based methods. cfDNA most commonly screens for the common viable aneuploidies: trisomy 21, trisomy 18, trisomy 13, and sometimes sex chromosomes aneuploidies. However, the scope of this screening continues to grow, and some laboratories now offer expanded panels which may include other autosomal aneuploidies (such as trisomy 16, trisomy 22, or even "all-chromosome" cfDNA), select microdeletion syndromes, single gene disorders, and fetal Rh status.

cfDNA screening is clinically available as early as ~9 weeks of gestation and is known to have high sensitivity and specificity for the common aneuploidies. Given this, cfDNA has rapidly become a first-line aneuploidy screening tool for both high- and average-risk pregnancies alike. High-risk indications for cfDNA screening include advanced maternal age, positive serum screening results, or abnormal fetal ultrasound findings such as soft markers or congenital anomalies. While prenatal diagnosis via chorionic villus sampling (CVS), amniocentesis, or fetal blood sampling remains the gold standard for the diagnosis of genetic disease in pregnancy, many patients may opt for cfDNA screening over diagnostic testing, given the potential risk for miscarriage associated with a diagnostic procedure. As such, cfDNA screening is an available option in pregnancies diagnosed with CHD when the pregnant person declines diagnostic testing and remains an excellent screen for some of the most common causes for CHD, including Down syndrome, monosomy X, trisomy 18, and trisomy 13. A study by Salzer-Sheelo et al., in 2021 found that, surprisingly, 44% (n = 24/55) of cases with a prenatal CHD would be detectable by cfDNA screening inclusive of 5 chromosomes: 21, 18, 13, X, and Y.[122] An additional 15% of cases attributable to 22q11.2 deletion syndrome would (theoretically) also be detectable by cfDNA screening. Of note, the performance of cfDNA screening for other conditions such as microdeletions and single-gene disorders is less well-established but is known to be less sensitive and specific. The positive predictive value is often significantly lower for sub-chromosomal copy number variants, given the lower incidence of these conditions. Screening for select, frequently *de* novo, autosomal dominant disorders associated with advanced paternal age or abnormal ultrasound findings is also a newly available option; though performance data of this type of screen is even sparser. Therefore, patients opting for cfDNA screening over diagnostic testing should be thoroughly counseled about the limitations of this screening, in that a normal cfDNA result cannot rule out all possible genetic etiologies associated with CHD. Table 3 highlights select disorders that are known to cause CHDs and may be detectable by cfDNA screening.

Patients should also be counseled on the availability and potential utility of carrier screening for the parents of a fetus with a CHD. Carrier screening uses various methodologies such as targeted genotyping and/or gene sequencing to attempt to identify individuals who are at increased risk of having a child with an inherited autosomal recessive or X-linked disease. This screening can be performed before or during a pregnancy to identify at-risk couples, who then have the option to pursue alternative reproductive options to reduce the risk of having an affected child, such as prenatal diagnosis, pre-implantation genetic testing of embryos, use of a sperm/oocyte donor, or adoption. Historically, ethnicity-based carrier screening was performed for a handful of the most common disorders in a given ethnic group. Advances in genetic testing technology and decreased cost now allow for high throughput testing of several hundred genes simultaneously with a rapid turnaround time, and many carrier screening laboratories can now test for several hundred diseases via a single blood and/or saliva sample. The ACMG now recommends an ethnicity-agnostic, tiered approach to carrier screening.[123] Tier 1 screening is defined as screening for cystic fibrosis, spinal muscular atrophy, and risk-based screening as determined by personal and family history, as well as other risk factors. Tier 2 screening is defined as screening for conditions with a carrier frequency of 1 in 100 or greater. Tier 3 screening is defined as screening for conditions with a carrier frequency of 1 in 200 or greater. Tier 4 screening is defined as screening for conditions with a carrier frequency of less than 1 in 200. For the general population, ACMG recommends offering tier 3 screening. Tier 4 screening should be considered in cases of parental consanguinity, or when a family or personal medical history warrants it.

While the yield of carrier screening for the purposes of identifying a diagnosis in a fetus with a prenatally detected CHD is often low, there are numerous autosomal recessive disorders which have been associated with CHD and, in the setting of non-specific fetal anomalies, large carrier screening panels inclusive of several hundreds of genes can provide a potentially cost-effective means of "casting a wide net" for fetal disease. Disorders that may be included on carrier screening panels that have also been implicated in CHD include Smith-Lemli-Opitz syndrome (OMIM 270400), Bardet-Biedl syndrome (OMIM PS209900), Barth syndrome (OMIM 302060), Ellis-van Creveld (OMIM 225500), Fanconi anemia (OMIM PS227650), some heterotaxy conditions such as *ZIC3* (OMIM 300265), and more. Identification of a couple at-risk for a disease on a carrier screening panel before/during early-stage of pregnancy, which later fits the fetal phenotype, can help direct targeted single gene testing on a prenatal specimen obtained via chorionic villus sampling (CVS) or amniocentesis.

4. Approach to genetic testing

An accurate diagnosis in individuals and families affected by CHD is important for guidance of medical, surgical, and palliative management, understanding of prognosis, estimation of recurrence risk for future pregnancies, and enabling of preimplantation genetic testing and prenatal diagnosis. A careful clinical evaluation and a thorough physical examination prior to genetic testing can narrow the diagnosis and tailor genetic testing using a more cost-effective approach in the postnatal period. However, *in utero* phenotyping may be inaccurate or incomplete, requiring a broader, more comprehensive genetic testing approach. We outlined an algorithm for the prenatal genetic workup of CHD (Fig. 3) based on recent guidelines from ACMG and ISPD.[71,115] Based on clinical phenotypes, CHD can be categorized as syndromic or non-syndromic CHD (the first decision branch in Fig. 3).

For syndromic CHD with neurodevelopmental delay or extra-cardiac anomalies, different genetic testing modalities (from karyotype, FISH, CMA, to sequencing) are recommended based on the disease(s) suspected (Fig. 3).[60] Non-syndromic CHD is further classified as familial or sporadic CHD based on family history (Fig. 3). CMA and sequencing assays including a CHD gene panel, WES, and WGS are recommended to reveal etiology of CHD (Fig. 3).

Postnatally, patients with CHD should be evaluated by professionals with specialized training to include cardiologists/CHD specialists and medical geneticists. Comprehensive evaluation includes dysmorphology assessment by medical geneticists with additional help of facial analysis

Table 3
Disorders known to cause CHDs that may be detectable by various cfDNA platforms.

Condition	Etiology	Commonly associated CHDs[a]	Estimated incidence	Reported performance (when available)
Down syndrome/Trisomy 21	Aneuploidy	• 50% have CHD[154] • Endocardial cushion defects, ventricular septal defects, atrial septal defects[154]	Dependent on the age of the oocyte	99.7% DR 0.04% FPR[155]
Trisomy 18	Aneuploidy	• >80% have CHD[154] • Complex CHD, polyvalvular dysplasia, ventricular septal defects, atrial septal defects, patent ductus arteriosus[154]	Dependent on the age of the oocyte	97.8% DR 0.04% FPR[155]
Trisomy 13	Aneuploidy	• >90% have CHD[154] • Complex CHD, single ventricle physiology, polyvalvular dysplasia, ventricular septal defects, atrial septal defects, patent ductus arteriosus, transposition of the great arteries[154]	Dependent on the age of the oocyte	99% DR 0.04% FPR[155]
Trisomy 22	Aneuploidy	• ≥75% have CHD[154] • Septal defects, complex CHD[154]	Dependent on the age of the oocyte	
Trisomy 16	Aneuploidy	• Ventricular septal defect[156]	Dependent on the age of the oocyte	
Turner syndrome/Monosomy X/ Other monosomy X variants	Aneuploidy	• 23%–50% have CHD[154] • Coarctation of the aorta, bicuspid aortic valve, aortic dilation, hypoplastic left heart, congenital coronary artery anomalies, persistent left superior vena cava[154]	1/568 at amniocentesis,[157] 1/2500 in newborns[12]	95.8% DR 0.14% FPR[155]
Other sex chromosome aneuploidies	Aneuploidy	• CHD uncommon but possible[154]	Dependent on the age of the oocyte for most (exceptions such as XYY do not demonstrate a maternal age effect)	100% DR[b] 0.004% FPR[155]
Triploidy	Aneuploidy	• Ventricular septal defect, atrial septal defect, truncus arteriosus, papillary muscle calcification[158] • Less common: Tetralogy of Fallot, two-chamber heart, overriding aorta, transposition of the great arteries[158]	2%–3% of all conceptions, 1/10000 at live birth[159]	
DiGeorge syndrome/22q11.2 deletion	Microdeletion	• Most commonly conotruncal: Tetralogy of Fallot, transposition of the great arteries, truncus arteriosus[154]	1/4000[160]	
1p36 deletion syndrome	Microdeletion	• Atrial septal defects, ventricular septal defects, patent ductus arteriosus, valvular anomalies. tetralogy of Fallot, coarctation of the aorta, cardio-myopathy[161]	1/4000-1/10000[161]	
Wolf-Hirschhorn syndrome (4p- syndrome)	Microdeletion	• ~50% have CHD[162] • Typically simple heart defects: atrial and ventricular septal defects, pulmonary stenosis, patent ductus arteriosus with aortic insufficiency[162]	1/20000-1/50,000[162]	
Jacobsen syndrome	Microdeletion	• 56% have CHD[163] • Ventricular septal defects and left heart obstructive malformations are common: aortic/mitral valve abnormalities, coarctation of the aorta, Shone's complex, hypoplastic left heart (present in 5%)[163]	1/100000[163]	
Noonan syndrome/RASopathies (Cardiofacialcutaneous syndrome, Costello, LEOPARD)	Single gene disorder	• 50%–80% have CHD[164] • 25%–71% have pulmonary valve stenosis[164] • 10%–29% have hypertrophic cardio-myopathy[164]	1/1000-1/2500[164]	
Craniosynostoses (Antley Bixler, Apert, Crouzon, Jackson Weiss, Meunke, Pfeiffer)	Single gene disorder	• Apert: 10% have CHD[165] • Crouzon & Pfeiffer: CHD is uncommon[165] • Patent ductus arteriosus, coarctation of the aorta, transposition of the great vessels, pulmonary stenosis, atrial septal defects, tetralogy of Fallot[166]	• Antley Bixler: unknown • Apert: 1/65000-1/68000 • Crouzon: 16 per million • Jackson Weiss: unknown • Meunke: 1/30000 • Pfeiffer: 1/100000	
Alagille syndrome	Single gene disorder	• 90%–97% have CHD, typically involving pulmonary valve, pulmonary artery, and its branches[167] • Pulmonary stenosis (67%), tetralogy of Fallot (7%–16%), ventricular septal defect, atrial septal defect, aortic stenosis, coarctation of the aorta[167]	1/70000	
Cornelia de Lange syndrome	Single gene disorder	• 30% have CHD[168] • Pulmonary stenosis, ventricular septal defects, atrial septal defects, coarctation of the aorta, aortic valve anomalies, tetralogy of Fallot, double-outlet right ventricle[168]	1/10000-1/30000	
CHARGE syndrome	Single gene disorder	• Conotruncal defects (tetralogy of Fallot), atrioventricular canal defects, aortic arch anomalies[169]	1/8500-1/10000	

Note: DR: detection rate; FPR: false positive rate; PPV: positive predictive value.
[a] Not an exhaustive list.
[b] Number of reported cases too small for accurate assessment of performance.[155]

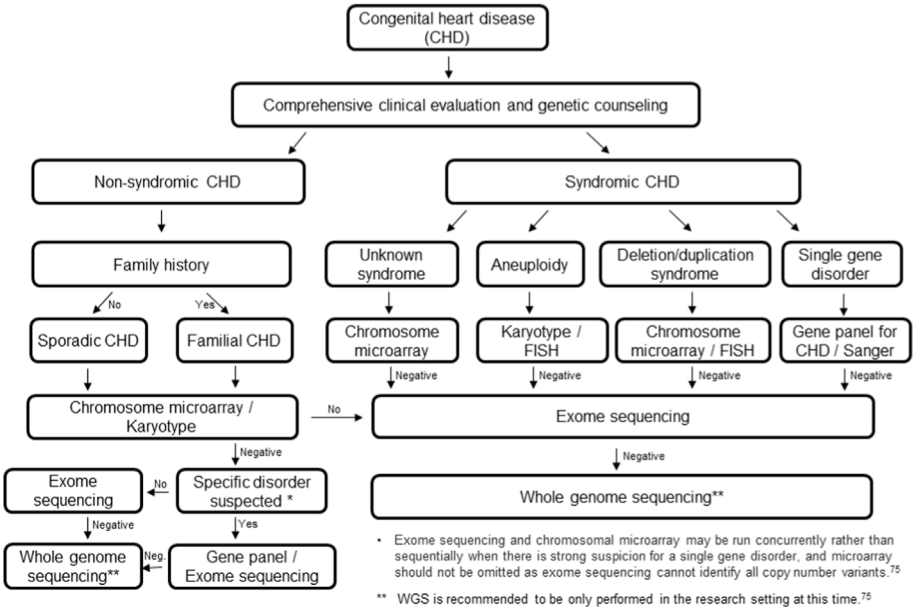

Fig. 3. The genetic workup for congenital heart disease (prenatal diagnosis).

technology, growth, and developmental charts (pediatric patients), imaging workup (such as echocardiogram, cardiac catheterization, renal ultrasound, skeletal X-ray or brain MRI), past medical history and family history. Involving a medical geneticist increases the CHD diagnosis by 7%–13%, after excluding Down syndrome.[124]

5. Best practices for NGS test in CHD

The current "best practice" for variant calling in clinical sequencing for germline analysis is the use of family trios (Fig. 4). Joint (simultaneous) variant calling in a proband and both parents enable the phasing of variants by parent of origin. A key advantage of joint variant calling for family trios is the ability to distinguish *de novo* alterations in the proband (Fig. 4), which account for a significant proportion of positive CHD diagnoses from clinical genetic testing (Fig. 1). *De novo* alterations should be queried against public databases of genome variation to rule out a known germline polymorphism, and manually review to exclude artifactual calls. Both *de novo* and inherited alterations need to be carefully classified into five specific categories including 'pathogenic', 'likely pathogenic', 'uncertain significance', 'likely benign', and 'benign' to describe variants identified in Mendelian disorders to determine potential pathogenesis in CHD.[125–127] Family trio NGS testing detects more genetic causes of CHD compared to patient-only NGS testing.[27,128] Periodic reclassification of alterations with uncertain significance identified on NGS testing is important and may become a component of a genetic testing program.[129]

6. Importance of genomic medicine in a multidisciplinary team approach for CHD

Optimal, patient-centered, comprehensive management of CHD requires a collaborative multidisciplinary team approach, including providers from Maternal-Fetal-Medicine, Pediatric Cardiology, Pediatric Cardiac Surgery, Neonatology, and Genomic Medicine (Genetics).

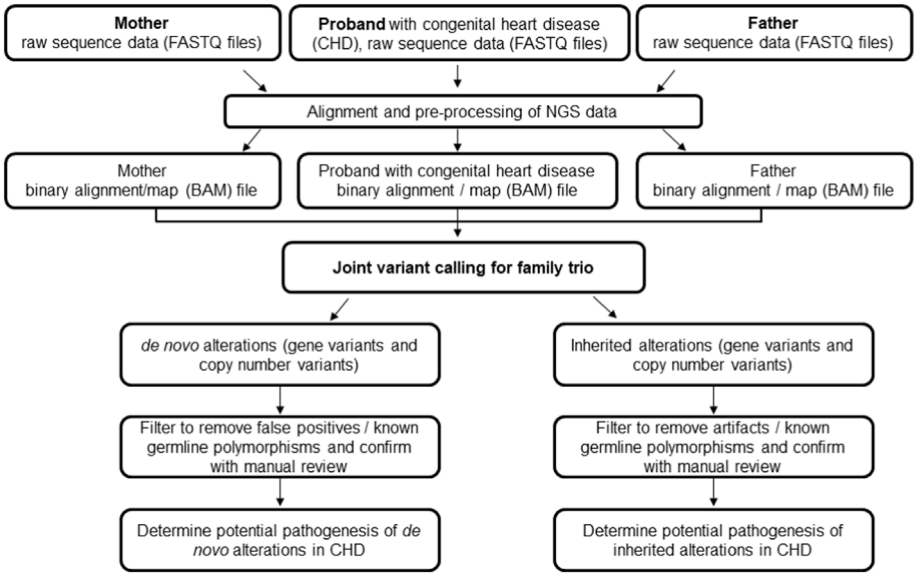

Fig. 4. Variant calling in family trio next-generation sequencing(NGS).

Genetic counseling is a key component of the diagnostic evaluation for fetuses with ultrasound anomalies, including CHD. Given the clinical and etiologic heterogeneity of CHDs, the rapidly expanding knowledge base of the genetic underpinnings of CHDs, and the intricacies of cytogenetic and molecular analyses, genetic counseling for CHDs can be complex. Medical geneticists and genetic counselors can obtain a complete medical and pregnancy history (including possible teratogenic exposures), construct a three-generation family history, develop a differential diagnosis, determine an appropriate testing strategy, facilitate decision-making, coordinate genetic testing, interpret results, provide recurrence risk counseling, and discuss available screening and testing for future pregnancies. In addition to training in clinical medical genetics, genetic counselors are also trained in psychosocial counseling and therefore can also provide support, referrals, and resources to families facing a new diagnosis of a CHD. Identifying an underlying genetic diagnosis, if present, can greatly influence prognostication, medical management of both the pregnancy and other potentially affected family members, recurrence risk, reproductive options, and even postnatal surgical intervention. Involving medical geneticists and genetic counselors in the care of families with CHD can help ensure comprehensive pre- and post-test counseling, informed consent, the provision of comprehensive, balanced information about genetic diagnoses, and non-coercive decision-making.

7. Towards emerging technologies in CHD

In terms of NGS processing pipelines for the detection of CNVs, besides institution developed CNV calling tools,[130,131] various CNV calling tools have been commercially available.[132–134] Using a combination of multiple CNV calling tools may achieve potential ability to detect CNV of various sizes (from exon-level to gene/chromosome-level). With further advances of CNV calling tools, WGS may gradually become an appealing replacement to CMA. Given a high diagnostic yield[22] and potential to identify SVs, WGS may grow into a first-tier genetic test in CHD.

SVs encompass deletions, duplications, insertions, inversions and translocations involving >50bp of DNA,[135] and tend to occur in repetitive regions of the genome.[136] While next-generation short-read sequencing tests (i.e. WES and WGS) and microarray can effectively identify large SVs with copy number changes involving >10 kb, smaller SVs such as transposon insertions or SVs without copy number changes (e.g. balanced translocations, inversions) are hard to identify, and breakpoints in complex SVs hard to define using these conventional techniques.[136] Currently, the full scope of genome-wide SVs in CHD is overlooked, and technologies including long-read DNA sequencing (LRS) and optical genome mapping (OGM) bring new opportunities to study SVs in CHD. These technologies may be useful to include in research and clinical diagnosis of CHD with unknown etiology. Indeed, studies applying LRS to uncover genetic etiology in cases of CHD with and without additional phenotypic features exist. One study using LRS implicated a large deletion involving *MYH6* and *MYH7* in atrial septal defect,[135] and another identified complex genomic rearrangements due to chromothripsis in a patient with multiple anomalies.[137] The ability of OGM to identify SVs in syndromic CHD has also been demonstrated.[138] Further detailed characterization of SVs in large-scale studies will be essential to unveiling the complex architecture of SVs and determining accurate frequencies of SVs in CHD.

Future refinements in serum-based screening methods (such as cell-based prenatal diagnosis, capillary-based cfDNA screening,[139] and others) may provide additional avenues for non-invasive screening for select CHD-associated disorders during pregnancy.

Genome-wide association studies (GWAS) can identify multiple DNA sequence polymorphisms across the genome to investigate whether the cumulative effect of many variants is associated with disease penetrance or severity. Polygenic risk scores (PRS) combine the effects of many GWAS variants into a single score to attempt to provide a more personalized risk estimate. PRS have been shown to have predictive value for several common diseases, including cancer, diabetes, and psychiatric disease[140,141] among others.[142,143] PRS have also been applied to cardiac disorders, including coronary heart disease,[144,145] atrial fibrillation,[146] and CHD susceptibility and phenotype.[147,148] Polygenic models have also been observed to modify or contribute to cardiac phenotypes in syndromic disorders, including both Down syndrome[149] and Marfan syndrome.[150] However, polygenic models for CHD etiology are still being explored. While there is promise for the future clinical use of PRS, there are several limitations, including difficulty in replicating GWAS results, as well as limited applicability across diverse patient populations.[151] The practical applications of PRS for stratified screening or for guiding medical interventions in CHD patients remain to be defined in further studies.

8. Conclusions and future directions

Genetic testing to understand the etiology of CHD is important not only for the diagnosis, classification of CHD and clinical care of patients with CHD, but also for the prenatal care of CHD patients who are now of reproductive age and are at increased risk of having children with CHD. NGS has dramatically propelled the discovery of the genetic etiology of CHD, which includes single gene variants, copy number and structural variants. Given its increasing availability and decreasing cost, NGS is quickly replacing Sanger sequencing and CMA as a method of evaluating SNVs and CNVs in CHD. Because isolated CHD etiologies are likely to be more complex with multigenic causes, somatic mosaicism, epigenetic effects, and gene–environment interactions contributing, WES or WGS approaches beyond single gene effects will increase diagnostic yield.

Despite our current understanding of the genetic etiology of CHD, there is still much work to be done to reveal a complete picture of etiologies involved in CHD because etiologies remain unknown in more than half of patients with CHD. Novel CHD genes are highly likely to be discovered by prioritizing diverse patient recruitment or by focusing on population-specific CHD genotypes, as CHD phenotypes are unique between ancestrally diverse groups. Functional genomic studies using single-cell RNA sequencing, ChIP-seq, ATAC-seq, methylation (epigenomics) profiles, and protein biomarker profiles are revealing more complex genetic networks with effects in specific cardiac lineages in CHD, and are comprehensively assessing non-coding variants and oligogenic variants in etiologies of CHD. Deciphering the effect of environmental exposures in terms of aberrant gene expression from direct analyses of human CHD tissues may lead to understanding environmental-gene relationships in CHD. Finally, accumulation of in vitro/in vivo data linked with patients' phenotypic data are starting to provide clinicians with resources to apply human genetic findings toward the clinical care of CHD patients. These technologic advances will improve clinical care of CHD patients and enrich the fundamental knowledge in human heart developmental and genome biology.

Author contribution

All authors designed the review, wrote the manuscript, and produced the final revision of the manuscript to be published.

Declaration of competing interest

All authors declare that there is no conflict of interest.

References

1. Bakker MK, Bergman JEH, Krikov S, et al. Prenatal diagnosis and prevalence of critical congenital heart defects: an international retrospective cohort study. *BMJ Open.* 2019;9(7), e028139. https://doi.org/10.1136/bmjopen-2018-028139.

2. Marelli AJ, Ionescu-Ittu R, Mackie AS, et al. Lifetime prevalence of congenital heart disease in the general population from 2000 to 2010. *Circulation*. 2014;130(9):749–756. https://doi.org/10.1161/CIRCULATIONAHA.113.008396.
3. van der Linde D, Konings EE, 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. https://doi.org/10.1016/j.jacc.2011.08.025.
4. McCracken C, Spector LG, Menk JS, et al. Mortality following pediatric congenital heart surgery: an analysis of the causes of death derived from the national death index. *J Am Heart Assoc*. 2018;7(22), e010624. https://doi.org/10.1161/JAHA.118.010624.
5. Egbe A, Lee S, Ho D, et al. Prevalence of congenital anomalies in newborns with congenital heart disease diagnosis. *Ann Pediatr Cardiol*. 2014;7(2):86–91. https://doi.org/10.4103/0974-2069.132474.
6. Homsy J, Zaidi S, Shen Y, et al. De novo mutations in congenital heart disease with neurodevelopmental and other congenital anomalies. *Science*. 2015;350(6265):1262–1266. https://doi.org/10.1126/science.aac9396.
7. Cowan JR, Ware SM. Genetics and genetic testing in congenital heart disease. *Clin Perinatol*. 2015;42(2):373–393. https://doi.org/10.1016/j.clp.2015.02.009. ix.
8. Jin SC, Homsy J, Zaidi S, et al. Contribution of rare inherited and de novo variants in 2,871 congenital heart disease probands. *Nat Genet*. 2017;49(11):1593–1601. https://doi.org/10.1038/ng.3970.
9. Zhang TN, Wu QJ, Liu YS, et al. Environmental risk factors and congenital heart disease: an umbrella review of 165 systematic reviews and meta-analyses with more than 120 million participants. *Front Cardiovasc Med*. 2021;8, 640729. https://doi.org/10.3389/fcvm.2021.640729.
10. Watkins WS, Hernandez EJ, Wesolowski S, et al. De novo and recessive forms of congenital heart disease have distinct genetic and phenotypic landscapes. *Nat Commun*. 2019;10(1):4722. https://doi.org/10.1038/s41467-019-12582-y.
11. Bull MJ. Down syndrome. *N Engl J Med*. 2020;382(24):2344–2352. https://doi.org/10.1056/NEJMra1706537.
12. Sybert VP, McCauley E. Turner's syndrome. *N Engl J Med*. 2004;351(12):1227–1238. https://doi.org/10.1056/NEJMra030360.
13. Hartman RJ, Rasmussen SA, Botto LD, et al. The contribution of chromosomal abnormalities to congenital heart defects: a population-based study. *Pediatr Cardiol*. 2011;32(8):1147–1157. https://doi.org/10.1007/s00246-011-0034-5.
14. Mlynarski EE, Xie M, Taylor D, et al. Rare copy number variants and congenital heart defects in the 22q11.2 deletion syndrome. *Hum Genet*. 2016;135(3):273–285. https://doi.org/10.1007/s00439-015-1623-9.
15. Bi W, Borgan C, Pursley AN, et al. Comparison of chromosome analysis and chromosomal microarray analysis: what is the value of chromosome analysis in today's genomic array era? *Genet Med*. 2013;15(6):450–457. https://doi.org/10.1038/gim.2012.152.
16. Wapner RJ, Martin CL, Levy B, et al. Chromosomal microarray versus karyotyping for prenatal diagnosis. *N Engl J Med*. 2012;367(23):2175–2184. https://doi.org/10.1056/NEJMoa1203382.
17. Costain G, Silversides CK, Bassett AS. The importance of copy number variation in congenital heart disease. *NPJ Genom Med*. 2016;1, 16031. https://doi.org/10.1038/npjgenmed.2016.31.
18. Sifrim A, Hitz MP, Wilsdon A, et al. Distinct genetic architectures for syndromic and nonsyndromic congenital heart defects identified by exome sequencing. *Nat Genet*. 2016;48(9):1060–1065. https://doi.org/10.1038/ng.3627.
19. van der Bom T, Zomer AC, Zwinderman AH, et al. The changing epidemiology of congenital heart disease. *Nat Rev Cardiol*. 2011;8(1):50–60. https://doi.org/10.1038/nrcardio.2010.166.
20. Fotiou E, Williams S, Martin-Geary A, et al. Integration of large-scale genomic data sources with evolutionary history reveals novel genetic loci for congenital heart disease. *Circ Genom Precis Med*. 2019;12(10):442–451. https://doi.org/10.1161/CIRCGEN.119.002694.
21. Li AH, Hanchard NA, Azamian M, et al. Genetic architecture of laterality defects revealed by whole exome sequencing. *Eur J Hum Genet*. 2019;27(4):563–573. https://doi.org/10.1038/s41431-018-0307-z.
22. Lionel AC, Costain G, Monfared N, et al. Improved diagnostic yield compared with targeted gene sequencing panels suggests a role for whole-genome sequencing as a first-tier genetic test. *Genet Med*. 2018;20(4):435–443. https://doi.org/10.1038/gim.2017.119.
23. Jegathisawaran J, Tsiplova K, Hayeems RZ, et al. Trio genome sequencing for developmental delay and pediatric heart conditions: a comparative microcost analysis. *Genet Med*. 2022;24(5):1027–1036. https://doi.org/10.1016/j.gim.2022.01.020.
24. Bianchi DW, Chiu RWK. Sequencing of circulating cell-free DNA during pregnancy. *N Engl J Med*. 2018;379(5):464–473. https://doi.org/10.1056/NEJMra1705345.
25. Bianchi DW, Parker RL, Wentworth J, et al. DNA sequencing versus standard prenatal aneuploidy screening. *N Engl J Med*. 2014;370(9):799–808. https://doi.org/10.1056/NEJMoa1311037.
26. International Society of Ultrasound in O, Gynecology. Cardiac screening examination of the fetus: guidelines for performing the 'basic' and 'extended basic' cardiac scan. *Ultrasound Obstet Gynecol*. 2006;27(1):107–113. https://doi.org/10.1002/uog.2677.
27. Alankarage D, Ip E, JO Szot, et al. Identification of clinically actionable variants from genome sequencing of families with congenital heart disease. *Genet Med*. 2019;21(5):1111–1120. https://doi.org/10.1038/s41436-018-0296-x.
28. Pierpont ME, Brueckner M, Chung WK, et al. Genetic basis for congenital heart disease: revisited: a scientific statement from the American heart association. *Circulation*. 2018;138(21):e653–e711. https://doi.org/10.1161/CIR.0000000000000606.
29. Oyen N, Poulsen G, Boyd HA, et al. Recurrence of congenital heart defects in families. *Circulation*. 2009;120(4):295–301. https://doi.org/10.1161/CIRCULATIONAHA.109.857987, 28.
30. Shieh JT, Bittles AH, Hudgins L. Consanguinity and the risk of congenital heart disease. *Am J Med Genet A*. 2012;158A(5):1236–1241. https://doi.org/10.1002/ajmg.a.35272.
31. Nora JJ, Gilliland JC, Sommerville RJ, et al. Congenital heart disease in twins. *N Engl J Med*. 1967;277(11):568–571. https://doi.org/10.1056/NEJM196709142771104.
32. Glessner JT, Bick AG, Ito K, et al. Increased frequency of de novo copy number variants in congenital heart disease by integrative analysis of single nucleotide polymorphism array and exome sequence data. *Circ Res*. 2014;115(10):884–896. https://doi.org/10.1161/CIRCRESAHA.115.304458.
33. Evans PR. Cardiac anomalies in mongolism. *Br Heart J*. 1950;12(3):258–262. https://doi.org/10.1136/hrt.12.3.258.
34. Tennstedt C, Chaoui R, Korner H, et al. Spectrum of congenital heart defects and extracardiac malformations associated with chromosomal abnormalities: results of a seven year necropsy study. *Heart*. Jul 1999;82(1):34–39. https://doi.org/10.1136/hrt.82.1.34.
35. Wimalasundera RC, Gardiner HM. Congenital heart disease and aneuploidy. *Prenat Diagn*. 2004;24(13):1116–1122. https://doi.org/10.1002/pd.1068.
36. Hattori M, Fujiyama A, Taylor TD, et al. The DNA sequence of human chromosome 21. *Nature*. 2000;405(6784):311–319. https://doi.org/10.1038/35012518.
37. Donnelly JC, Platt LD, Rebarber A, et al. Association of copy number variants with specific ultrasonographically detected fetal anomalies. *Obstet Gynecol*. 2014;124(1):83–90. https://doi.org/10.1097/AOG.0000000000000336.
38. Wang Y, Cao L, Liang D, et al. Prenatal chromosomal microarray analysis in fetuses with congenital heart disease: a prospective cohort study. *Am J Obstet Gynecol*. 2018;218(2). https://doi.org/10.1016/j.ajog.2017.10.225, 244 e241-244 e217.
39. Kim DS, Kim JH, Burt AA, et al. Burden of potentially pathologic copy number variants is higher in children with isolated congenital heart disease and significantly impairs covariate-adjusted transplant-free survival. *J Thorac Cardiovasc Surg*. 2016;151(4):1147–1151. https://doi.org/10.1016/j.jtcvs.2015.09.136. e1144.
40. Lander JWS. Copy number variation in congenital heart defects. *Curr Genet Med Rep*. 2014;2:168–178.
41. Nickerson E, Greenberg F, Keating MT, et al. Deletions of the elastin gene at 7q11.23 occur in approximately 90% of patients with Williams syndrome. *Am J Hum Genet*. 1995;56(5):1156–1161. https://www.ncbi.nlm.nih.gov/pubmed/7726172.
42. Botto LD, May K, Fernhoff PM, et al. A population-based study of the 22q11.2 deletion: phenotype, incidence, and contribution to major birth defects in the population. *Pediatrics*. 2003;112(1 Pt 1):101–107. https://doi.org/10.1542/peds.112.1.101.
43. Goldmuntz E. 22q11.2 deletion syndrome and congenital heart disease. *Am J Med Genet C Semin Med Genet*. 2020;184(1):64–72. https://doi.org/10.1002/ajmg.c.31774.
44. Jerome LA, Papaioannou VE. DiGeorge syndrome phenotype in mice mutant for the T-box gene, Tbx1. *Nat Genet*. 2001;27(3):286–291. https://doi.org/10.1038/85845.
45. Lindsay EA, Vitelli F, Su H, et al. Tbx1 haploinsufficieny in the DiGeorge syndrome region causes aortic arch defects in mice. *Nature*. 2001;410(6824):97–101. https://doi.org/10.1038/35065105.
46. 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. https://doi.org/10.1016/s0092-8674(01)00247-1.
47. Fulcoli FG, Franzese M, Liu X, et al. Rebalancing gene haploinsufficiency in vivo by targeting chromatin. *Nat Commun*. 2016;7, 11688. https://doi.org/10.1038/ncomms11688.
48. Pehlivan T, Pober BR, Brueckner M, et al. GATA4 haploinsufficiency in patients with interstitial deletion of chromosome region 8p23.1 and congenital heart disease. *Am J Med Genet*. 1999;83(3):201–206. https://www.ncbi.nlm.nih.gov/pubmed/10096597.
49. Goldmuntz E, Paluru P, Glessner J, et al. Microdeletions and microduplications in patients with congenital heart disease and multiple congenital anomalies. *Congenit Heart Dis*. 2011;6(6):592–602. https://doi.org/10.1111/j.1747-0803.2011.00582.x.
50. Tomita-Mitchell A, Mahnke DK, Struble CA, et al. Human gene copy number spectra analysis in congenital heart malformations. *Physiol Genom*. 2012;44(9):518–541. https://doi.org/10.1152/physiolgenomics.00013.2012.
51. Sailani MR, Makrythanasis P, Valsesia A, et al. The complex SNP and CNV genetic architecture of the increased risk of congenital heart defects in Down syndrome. *Genome Res*. 2013;23(9):1410–1421. https://doi.org/10.1101/gr.147991.112.
52. 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. https://doi.org/10.1038/nature12141.
53. Morton SU, Quiat D, Seidman JG, et al. Genomic frontiers in congenital heart disease. *Nat Rev Cardiol*. 2022;19(1):26–42. https://doi.org/10.1038/s41569-021-00587-4.
54. Diab NS, Barish S, Dong W, et al. Molecular genetics and complex inheritance of congenital heart disease. *Genes (Basel)*. 2021;12(7). https://doi.org/10.3390/genes12071020.
55. Petracchi F, Sisterna S, Igarzabal L, et al. Fetal cardiac abnormalities: genetic etiologies to be considered. *Prenat Diagn*. 2019;39(9):758–780. https://doi.org/10.1002/pd.5480.

56. JO Szot, Cuny H, Blue GM, et al. A screening approach to identify clinically actionable variants causing congenital heart disease in exome data. *Circ Genom Precis Med*. 2018;11(3), e001978. https://doi.org/10.1161/CIRCGEN.117.001978.
57. Mone F, Eberhardt RY, Morris RK, et al. Congenital heart disease and the diagnostic yield with exome sequencing (CODE) study: prospective cohort study and systematic review. *Ultrasound Obstet Gynecol*. 2021;57(1):43–51. https://doi.org/10.1002/uog.22072.
58. Tuuli MG, Dicke JM, Stamilio DM, et al. Prevalence and likelihood ratios for aneuploidy in fetuses diagnosed prenatally with isolated congenital cardiac defects. *Am J Obstet Gynecol*. 2009;201(4):390 e391–395. https://doi.org/10.1016/j.ajog.2009.06.035.
59. Jansen FA, Blumenfeld YJ, Fisher A, et al. Array comparative genomic hybridization and fetal congenital heart defects: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol*. 2015;45(1):27–35. https://doi.org/10.1002/uog.14695.
60. Blue GM, Kirk EP, Giannoulatou E, et al. Advances in the genetics of congenital heart disease: a clinician's guide. *J Am Coll Cardiol*. 2017;69(7):859–870. https://doi.org/10.1016/j.jacc.2016.11.060.
61. Blue GM, Kirk EP, Giannoulatou E, et al. Targeted next-generation sequencing identifies pathogenic variants in familial congenital heart disease. *J Am Coll Cardiol*. 2014;64(23):2498–2506. https://doi.org/10.1016/j.jacc.2014.09.048.
62. Jia Y, Louw JJ, Breckpot J, et al. The diagnostic value of next generation sequencing in familial nonsyndromic congenital heart defects. *Am J Med Genet A*. 2015;167A(8):1822–1829. https://doi.org/10.1002/ajmg.a.37108.
63. Yasuhara J, Garg V. Genetics of congenital heart disease: a narrative review of recent advances and clinical implications. *Transl Pediatr*. 2021;10(9):2366–2386. https://doi.org/10.21037/tp-21-297.
64. Reuter MS, Chaturvedi RR, Liston E, et al. The cardiac genome clinic: implementing genome sequencing in pediatric heart disease. *Genet Med*. 2020;22(6):1015–1024. https://doi.org/10.1038/s41436-020-0757-x.
65. Page DJ, Miossec MJ, Williams SG, et al. Whole exome sequencing reveals the major genetic contributors to nonsyndromic tetralogy of fallot. *Circ Res*. 2019;124(4):553–563. https://doi.org/10.1161/CIRCRESAHA.118.313250.
66. Bauer RC, Laney AO, Smith R, et al. Jagged 1 (JAG1) mutations in patients with tetralogy of Fallot or pulmonic stenosis. *Hum Mutat*. 2010;31(5):594–601. https://doi.org/10.1002/humu.21231.
67. Fahed AC, Gelb BD, Seidman JG, et al. Genetics of congenital heart disease: the glass half empty. *Circ Res*. 2013;112(4):707–720. https://doi.org/10.1161/CIRCRESAHA.112.300853.
68. Mills JA, Herrera PS, Kaur M, et al. NIPBL(+/-) haploinsufficiency reveals a constellation of transcriptome disruptions in the pluripotent and cardiac states. *Sci Rep*. 2018;8(1):1056. https://doi.org/10.1038/s41598-018-19173-9.
69. Basson CT, Cowley GS, Solomon SD, et al. The clinical and genetic spectrum of the Holt-Oram syndrome (heart-hand syndrome). *N Engl J Med*. 1994;330(13):885–891. https://doi.org/10.1056/NEJM199403313301302.
70. Hu P, Qiao F, Wang Y, et al. Clinical application of targeted next-generation sequencing in fetuses with congenital heart defect. *Ultrasound Obstet Gynecol*. 2018;52(2):205–211. https://doi.org/10.1002/uog.19042.
71. Van den Veyver IB, Chandler N, Wilkins-Haug LE, et al. International society for prenatal diagnosis updated position statement on the use of genome-wide sequencing for prenatal diagnosis. *Prenat Diagn*. 2022;42(6):796–803. https://doi.org/10.1002/pd.6157.
72. Richter F, Morton SU, Kim SW, et al. Genomic analyses implicate noncoding de novo variants in congenital heart disease. *Nat Genet*. 2020;52(8):769–777. https://doi.org/10.1038/s41588-020-0652-z.
73. Ameres SL, Zamore PD. Diversifying microRNA sequence and function. *Nat Rev Mol Cell Biol*. 2013;14(8):475–488. https://doi.org/10.1038/nrm3611.
74. Duenas A, Exposito A, Aranega A, et al. The role of non-coding RNA in congenital heart diseases. *J Cardiovasc Dev Dis*. 2019;6(2). https://doi.org/10.3390/jcdd6020015.
75. Wei JW, Huang K, Yang C, et al. Non-coding RNAs as regulators in epigenetics (Review). *Oncol Rep*. 2017;37(1):3–9. https://doi.org/10.3892/or.2016.5236.
76. Garcia-Padilla C, Dominguez JN, Aranega AE, et al. Differential chamber-specific expression and regulation of long non-coding RNAs during cardiac development. *Biochim Biophys Acta Gene Regul Mech*. 2019;1862(10), 194435. https://doi.org/10.1016/j.bbagrm.2019.194435.
77. Yan HL, Hua YM. [Research advances on role of microRNAs in congenital heart diseases]. *Zhong Guo Dang Dai Er Ke Za Zhi*. 2014;16(10):1070–1074. https://www.ncbi.nlm.nih.gov/pubmed/25344195.
78. Scheuermann JC, Boyer LA. Getting to the heart of the matter: long non-coding RNAs in cardiac development and disease. *EMBO J*. 2013;32(13):1805–1816. https://doi.org/10.1038/emboj.2013.134.
79. Pulignani S, Vecoli C, Sabina S, et al. 3'UTR SNPs and Haplotypes in the GATA4 gene contribute to the genetic risk of congenital heart disease. *Rev Esp Cardiol (Engl Ed)*. 2016;69(8):760–765. https://doi.org/10.1016/j.rec.2016.03.004.
80. Huang J, Li X, Li H, et al. Down-regulation of microRNA-184 contributes to the development of cyanotic congenital heart diseases. *Int J Clin Exp Pathol*. 2015;8(11):14221–14227. https://www.ncbi.nlm.nih.gov/pubmed/26823736.
81. Sucharov CC, Sucharov J, Karimpour-Fard A, et al. Micro-RNA expression in hypoplastic left heart syndrome. *J Card Fail*. 2015;21(1):83–88. https://doi.org/10.1016/j.cardfail.2014.09.013.
82. Guo R, Feng Z, Yang Y, et al. Association of a MiR-499 SNP and risk of congenital heart disease in a Chinese population. *Cell Mol Biol (Noisy-Le-Grand)*. 2018;64(10):108–112. https://www.ncbi.nlm.nih.gov/pubmed/30084801.
83. Tian J, An X, Niu L. Role of microRNAs in cardiac development and disease. *Exp Ther Med*. 2017;13(1):3–8. https://doi.org/10.3892/etm.2016.3932.
84. Song G, Shen Y, Zhu J, et al. Integrated analysis of dysregulated lncRNA expression in fetal cardiac tissues with ventricular septal defect. *PLoS One*. 2013;8(10), e77492. https://doi.org/10.1371/journal.pone.0077492.
85. Gu M, Zheng A, Tu W, et al. Circulating LncRNAs as novel, non-invasive biomarkers for prenatal fetection of fetal congenital heart defects. *Cell Physiol Biochem*. 2016;38(4):1459–1471. https://doi.org/10.1159/000443088.
86. Oldereid NB, Wennerholm UB, Pinborg A, et al. The effect of paternal factors on perinatal and paediatric outcomes: a systematic review and meta-analysis. *Hum Reprod Update*. 2018;24(3):320–389. https://doi.org/10.1093/humupd/dmy005.
87. Zhang R, Huo C, Wang X, et al. Two common MTHFR gene polymorphisms (C677T and A1298C) and fetal congenital heart disease risk: an updated meta-analysis with trial sequential analysis. *Cell Physiol Biochem*. 2018;45(6):2483–2496. https://doi.org/10.1159/000488267.
88. Cai B, Zhang T, Zhong R, et al. Genetic variant in MTRR, but not MTR, is associated with risk of congenital heart disease: an integrated meta-analysis. *PLoS One*. 2014;9(3), e89609. https://doi.org/10.1371/journal.pone.0089609.
89. Zhang Y, Ai F, Zheng J, et al. Associations of GATA4 genetic mutations with the risk of congenital heart disease: a meta-analysis. *Medicine (Baltimore)*. 2017;96(18), e6857. https://doi.org/10.1097/MD.0000000000006857.
90. Wang Z, Zou L, Zhong R, et al. Associations between two genetic variants in NKX2-5 and risk of congenital heart disease in Chinese population: a meta-analysis. *PLoS One*. 2013;8(8), e70979. https://doi.org/10.1371/journal.pone.0070979.
91. Hedermann G, Hedley PL, Thagaard IN, et al. Maternal obesity and metabolic disorders associate with congenital heart defects in the offspring: a systematic review. *PLoS One*. 2021;16(5), e0252343. https://doi.org/10.1371/journal.pone.0252343.
92. Cavadino A, Sandberg L, Ohman I, et al. Signal detection in EUROmediCAT: identification and evaluation of medication-congenital anomaly associations and use of vigiBase as a complementary source of reference. *Drug Saf*. 2021;44(7):765–785. https://doi.org/10.1007/s40264-021-01073-z.
93. Kalisch-Smith JI, Ved N, Sparrow DB. Environmental risk factors for congenital heart disease. *Cold Spring Harbor Perspect Biol*. 2020;12(3):a037234. https://doi.org/10.1101/cshperspect.a037234.
94. Jenkins KJ, Correa A, Feinstein JA, et al. 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. https://doi.org/10.1161/CIRCULATIONAHA.106.183216.
95. Feng Y, Wang S, Chen R, et al. Maternal folic acid supplementation and the risk of congenital heart defects in offspring: a meta-analysis of epidemiological observational studies. *Sci Rep*. 2015;5:8506. https://doi.org/10.1038/srep08506.
96. Chapman G, Moreau JLM, I PE, et al. Functional genomics and gene-environment interaction highlight the complexity of congenital heart disease caused by Notch pathway variants. *Hum Mol Genet*. 2020;29(4):566–579. https://doi.org/10.1093/hmg/ddz270.
97. Roifman M, Chung BHY, Reid DM, et al. Heterozygous NOTCH1 deletion associated with variable congenital heart defects. *Clin Genet*. 2021;99(6):836–841. https://doi.org/10.1111/cge.13948.
98. Gould RA, Aziz H, Woods CE, et al. ROBO4 variants predispose individuals to bicuspid aortic valve and thoracic aortic aneurysm. *Nat Genet*. 2019;51(1):42–50. https://doi.org/10.1038/s41588-018-0265-y.
99. Tan HL, Glen E, Topf A, et al. Nonsynonymous variants in the SMAD6 gene predispose to congenital cardiovascular malformation. *Hum Mutat*. 2012;33(4):720–727. https://doi.org/10.1002/humu.22030.
100. Mackay TF. Epistasis and quantitative traits: using model organisms to study gene-gene interactions. *Nat Rev Genet*. 2014;15(1):22–33. https://doi.org/10.1038/nrg3627.
101. Del Monte-Nieto G, Ramialison M, Adam AAS, et al. Control of cardiac jelly dynamics by NOTCH1 and NRG1 defines the building plan for trabeculation. *Nature*. 2018;557(7705):439–445. https://doi.org/10.1038/s41586-018-0110-6.
102. Cordell HJ, Bentham J, Topf A, et al. Genome-wide association study of multiple congenital heart disease phenotypes identifies a susceptibility locus for atrial septal defect at chromosome 4p16. *Nat Genet*. 2013;45(7):822–824. https://doi.org/10.1038/ng.2637.
103. Hu Z, Shi Y, Mo X, et al. A genome-wide association study identifies two risk loci for congenital heart malformations in Han Chinese populations. *Nat Genet*. 2013;45(7):818–821. https://doi.org/10.1038/ng.2636.
104. Gifford CA, Ranade SS, Samarakoon R, et al. Oligogenic inheritance of a human heart disease involving a genetic modifier. *Science*. 2019;364(6443):865–870. https://doi.org/10.1126/science.aat5056.
105. Li Y, Yagi H, Onuoha EO, et al. DNAH6 and its Interactions with PCD Genes in heterotaxy and primary ciliary dyskinesia. *PLoS Genet*. 2016;12(2), e1005821. https://doi.org/10.1371/journal.pgen.1005821.
106. Hsieh A, Morton SU, Willcox JAL, et al. EM-mosaic detects mosaic point mutations that contribute to congenital heart disease. *Genome Med*. 2020;12(1):42. https://doi.org/10.1186/s13073-020-00738-1.
107. Freeze SL, Landis BJ, Ware SM, et al. Bicuspid aortic valve: a review with recommendations for genetic counseling. *J Genet Counsel*. 2016;25(6):1171–1178. https://doi.org/10.1007/s10897-016-0002-6.
108. Alberman E, Mutton D, Morris JK. Cytological and epidemiological findings in trisomies 13, 18, and 21: england and Wales 2004-2009. *Am J Med Genet A*. 2012;158A(5):1145–1150. https://doi.org/10.1002/ajmg.a.35337.
109. Beaujard MP, Chantot S, Dubois M, et al. Atypical deletion of 22q11.2: detection using the FISH TBX1 probe and molecular characterization with high-density SNP arrays. *Eur J Med Genet*. 2009;52(5):321–327. https://doi.org/10.1016/j.ejmg.2009.05.010.

110. Wu XL, Li R, Fu F, et al. Chromosome microarray analysis in the investigation of children with congenital heart disease. *BMC Pediatr*. 2017;17(1):117. https://doi.org/10.1186/s12887-017-0863-3.
111. American College of O, Gynecologists' Committee on Practice B-O, Committee on G, Society for Maternal-Fetal M. Screening for fetal chromosomal abnormalities: ACOG practice bulletin, number 226. *Obstet Gynecol*. 2020;136(4):e48–e69. https://doi.org/10.1097/AOG.0000000000004084.
112. Ison HE, Griffin EL, Parrott A, et al. Genetic counseling for congenital heart disease - practice resource of the National Society of Genetic Counselors. *J Genet Counsel*. 2022;31(1):9–33. https://doi.org/10.1002/jgc4.1498.
113. Qiao F, Wang Y, Zhang C, et al. Comprehensive evaluation of genetic variants using chromosomal microarray analysis and exome sequencing in fetuses with congenital heart defect. *Ultrasound Obstet Gynecol*. 2021;58(3):377–387. https://doi.org/10.1002/uog.23532.
114. Landis BJ, Ware SM. The current landscape of genetic testing in cardiovascular malformations: opportunities and Challenges. *Front Cardiovasc Med*. 2016;3:22. https://doi.org/10.3389/fcvm.2016.00022.
115. Monaghan KG, Leach NT, Pekarek D, et al. The use of fetal exome sequencing in prenatal diagnosis: a points to consider document of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2020;22(4):675–680. https://doi.org/10.1038/s41436-019-0731-7.
116. Choi M, Scholl UI, Ji W, et al. Genetic diagnosis by whole exome capture and massively parallel DNA sequencing. *Proc Natl Acad Sci U S A*. 2009;106(45):19096–19101. https://doi.org/10.1073/pnas.0910672106.
117. Zhou J, Yang Z, Sun J, et al. Whole genome sequencing in the evaluation of fetal structural anomalies: a parallel test with chromosomal microarray plus whole exome sequencing. *Genes (Basel)*. 2021;12(3). https://doi.org/10.3390/genes12030376.
118. Scocchia A, Wigby KM, Masser-Frye D, et al. Clinical whole genome sequencing as a first-tier test at a resource-limited dysmorphology clinic in Mexico. *NPJ Genom Med*. 2019;4:5. https://doi.org/10.1038/s41525-018-0076-1.
119. Wang Y, Greenfeld E, Watkins N, et al. Diagnostic yield of genome sequencing for prenatal diagnosis of fetal structural anomalies. *Prenat Diagn*. 2022;42(7):822–830. https://doi.org/10.1002/pd.6108.
120. Green RC, Berg JS, Grody WW, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genet Med*. 2013;15(7):565–574. https://doi.org/10.1038/gim.2013.73.
121. Miller DT, Lee K, Chung WK, et al. ACMG SF v3.0 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2021;23(8):1381–1390. https://doi.org/10.1038/s41436-021-01172-3.
122. Salzer-Sheelo L, Polak U, Barg A, et al. Prenatal and postnatal chromosomal microarray analysis in 885 cases of various congenital heart defects. *Arch Gynecol Obstet*. 2022. https://doi.org/10.1007/s00404-021-06366-3.
123. Gregg AR, Aarabi M, Klugman S, et al. Screening for autosomal recessive and X-linked conditions during pregnancy and preconception: a practice resource of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2021;23(10):1793–1806. https://doi.org/10.1038/s41436-021-01203-z.
124. Geddes GC, Earing MG. Genetic evaluation of patients with congenital heart disease. *Curr Opin Pediatr*. 2018;30(6):707–713. https://doi.org/10.1097/MOP.0000000000000682.
125. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of medical genetics and genomics and the association for molecular pathology. *Genet Med*. 2015;17(5):405–424. https://doi.org/10.1038/gim.2015.30.
126. Koboldt DC. Best practices for variant calling in clinical sequencing. *Genome Med*. 2020;12(1):91. https://doi.org/10.1186/s13073-020-00791-w.
127. Rehder C, Bean LJH, Bick D, et al. Next-generation sequencing for constitutional variants in the clinical laboratory, 2021 revision: a technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2021;23(8):1399–1415. https://doi.org/10.1038/s41436-021-01139-4.
128. Liu Y, Chang X, Glessner J, et al. Association of rare recurrent copy number variants with congenital heart defects based on next-generation sequencing data from family trios. *Front Genet*. 2019;10:819. https://doi.org/10.3389/fgene.2019.00819.
129. Quiat D, Witkowski L, Zouk H, et al. Retrospective analysis of clinical genetic testing in pediatric primary dilated cardiomyopathy: testing outcomes and the effects of variant reclassification. *J Am Heart Assoc*. 2020;9(11), e016195. https://doi.org/10.1161/JAHA.120.016195.
130. Pallavajjala A, Haley L, Stinnett V, et al. Utility of targeted next-generation sequencing assay to detect 1p/19q co-deletion in formalin-fixed paraffin-embedded glioma specimens. *Hum Pathol*. 2022;126:63–76. https://doi.org/10.1016/j.humpath.2022.05.001.
131. Jiang L, Pallavajjala A, Huang J, et al. Clinical utility of targeted next-generation sequencing assay to detect copy number variants associated with myelodysplastic syndrome in myeloid malignancies. *J Mol Diagn*. 2021;23(4):467–483. https://doi.org/10.1016/j.jmoldx.2021.01.011.
132. Roca I, Gonzalez-Castro L, Fernandez H, et al. Free-access copy-number variant detection tools for targeted next-generation sequencing data. *Mutat Res Rev Mutat Res*. 2019;779:114–125. https://doi.org/10.1016/j.mrrev.2019.02.005.
133. Gordeeva V, Sharova E, Babalyan K, et al. Benchmarking germline CNV calling tools from exome sequencing data. *Sci Rep*. 2021;11(1), 14416. https://doi.org/10.1038/s41598-021-93878-2.
134. Gabrielaite M, Torp MH, Rasmussen MS, et al. A comparison of tools for copy-number variation detection in germline whole exome and whole genome sequencing data. *Cancers (Basel)*. 2021;13(24). https://doi.org/10.3390/cancers13246283.
135. Sonoda K, Ishihara H, Sakazaki H, et al. Long-read sequence confirmed a large deletion including MYH6 and MYH7 in an infant of atrial septal defect and atrial arrhythmias. *Circ Genom Precis Med*. 2021;14(4), e003223. https://doi.org/10.1161/CIRCGEN.120.003223.
136. Mitsuhashi S, Matsumoto N. Long-read sequencing for rare human genetic diseases. *J Hum Genet*. 2020;65(1):11–19. https://doi.org/10.1038/s10038-019-0671-8.
137. Lei M, Liang D, Yang Y, et al. Long-read DNA sequencing fully characterized chromothripsis in a patient with Langer-Giedion syndrome and Cornelia de Lange syndrome-4. *J Hum Genet*. 2020;65(8):667–674. https://doi.org/10.1038/s10038-020-0754-6.
138. Sahajpal NS, Barseghyan H, Kolhe R, et al. Optical genome mapping as a next-generation cytogenomic tool for detection of structural and copy number variations for prenatal genomic analyses. *Genes (Basel)*. 2021;12(3). https://doi.org/10.3390/genes12030398.
139. Kazachkova N, Gontar J, Verlinsky O, et al. Successful early fetal sex determination using cell-free fetal DNA isolated from maternal capillary blood: a pilot study. *Eur J Obstet Gynecol Reprod Biol X*. 2019;3, 100038. https://doi.org/10.1016/j.eurox.2019.100038.
140. Khera AV, Chaffin M, Aragam KG, et al. Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. *Nat Genet*. Sep 2018;50(9):1219–1224. https://doi.org/10.1038/s41588-018-0183-z.
141. Eeltink E, van der Horst MZ, Zinkstok JR, et al. Polygenic risk scores for genetic counseling in psychiatry: lessons learned from other fields of medicine. *Neurosci Biobehav Rev*. 2021;121:119–127. https://doi.org/10.1016/j.neubiorev.2020.11.021.
142. Mars N, Koskela JT, Ripatti P, et al. Polygenic and clinical risk scores and their impact on age at onset and prediction of cardiometabolic diseases and common cancers. *Nat Med*. 2020;26(4):549–557. https://doi.org/10.1038/s41591-020-0800-0.
143. Ritchie SC, Lambert SA, Arnold M, et al. Integrative analysis of the plasma proteome and polygenic risk of cardiometabolic diseases. *Nat Med*. 2021;3(11):1476–1483. https://doi.org/10.1038/s42255-021-00478-5.
144. Khera AV, Emdin CA, Drake I, et al. Genetic risk, adherence to a healthy lifestyle, and coronary disease. *N Engl J Med*. 2016;375(24):2349–2358. https://doi.org/10.1056/NEJMoa1605086.
145. Mosley JD, Gupta DK, Tan J, et al. Predictive accuracy of a polygenic risk score compared with a clinical risk score for incident coronary heart disease. *JAMA*. 2020;323(7):627–635. https://doi.org/10.1001/jama.2019.21782.
146. Lupo PJ, Mitchell LE, Jenkins MM. Genome-wide association studies of structural birth defects: a review and commentary. *Birth Defects Res*. 2019;111(18):1329–1342. https://doi.org/10.1002/bdr2.1606.
147. Lahrouchi N, Tadros R, Crotti L, et al. Transethnic genome-wide association study provides insights in the genetic architecture and heritability of long QT syndrome. *Circulation*. 2020;142(4):324–338. https://doi.org/10.1161/CIRCULATIONAHA.120.045956.
148. Spendlove SJ, Bondhus L, Lluri G, et al. Polygenic risk scores of endo-phenotypes identify the effect of genetic background in congenital heart disease. *HGG Adv*. 2022;3(3), 100112. https://doi.org/10.1016/j.xhgg.2022.100112.
149. Trevino CE, Holleman AM, Corbitt H, et al. Identifying genetic factors that contribute to the increased risk of congenital heart defects in infants with Down syndrome. *Sci Rep*. 2020;10(1), 18051. https://doi.org/10.1038/s41598-020-74650-4.
150. Aubart M, Gazal S, Arnaud P, et al. Association of modifiers and other genetic factors explain Marfan syndrome clinical variability. *Eur J Hum Genet*. 2018;26(12):1759–1772. https://doi.org/10.1038/s41431-018-0164-9.
151. Lewis CM, Vassos E. Polygenic risk scores: from research tools to clinical instruments. *Genome Med*. 2020;12(1):44. https://doi.org/10.1186/s13073-020-00742-5.
152. Lynch TA, Abel DE. Teratogens and congenital heart disease. *J Diagn Med Sonogr*. 2015;31:301–305.
153. Patil AS, Nguyen C, Groff K, et al. Severity of congenital heart defects associated with assisted reproductive technologies: case series and review of the literature. *Birth Defects Res*. 2018;110(8):654–661. https://doi.org/10.1002/bdr2.1228.
154. Lin AE, Santoro S, High FA, et al. Congenital heart defects associated with aneuploidy syndromes: new insights into familiar associations. *Am J Med Genet C Semin Med Genet*. 2020;184(1):53–63. https://doi.org/10.1002/ajmg.c.31760.
155. Gil MM, Accurti V, Santacruz B, et al. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: updated meta-analysis. *Ultrasound Obstet Gynecol*. 2017;50(3):302–314. https://doi.org/10.1002/uog.17484.
156. Armstrong AA, Gaw SL, Platt LD. Mosaic trisomies 8, 9, and 16. In: Copel Joshua A, Mary E D'Alton, Helen Feltovich, et al., eds. *Obstetric Imaging: Fetal Diagnosis and Care*. second ed. Elsevier; 2018:617–620.
157. Gravholt CH, Juul S, Naeraa RW, et al. Prenatal and postnatal prevalence of Turner's syndrome: a registry study. *BMJ*. 1996;312(7022):16–21. https://doi.org/10.1136/bmj.312.7022.16.
158. Toufaily MH, Roberts DJ, Westgate MN, et al. Triploidy: variation of phenotype. *Am J Clin Pathol*. 2016;145(1):86–95. https://doi.org/10.1093/ajcp/aqv012.
159. Kolarski M, Ahmetovic B, Beres M, et al. Genetic counseling and prenatal diagnosis of triploidy during the second trimester of pregnancy. *Med Arch*. 2017;71(2):144–147. https://doi.org/10.5455/medarh.2017.71.144-147.
160. Oskarsdottir S, Vujic M, Fasth A. Incidence and prevalence of the 22q11 deletion syndrome: a population-based study in Western Sweden. *Arch Dis Child*. 2004;89(2):148–151. https://doi.org/10.1136/adc.2003.026880.
161. Lodato V, Orlando V, Alesi V, et al. 1p36 deletion syndrome and the aorta: a report of three new patients and a literature review. *J Cardiovasc Dev Dis*. 2021;(11):8. https://doi.org/10.3390/jcdd8110159.

162. Battaglia A, Carey JC, South ST. Wolf-Hirschhorn syndrome: a review and update. *Am J Med Genet C Semin Med Genet.* 2015;169(3):216–223. https://doi.org/10.1002/ajmg.c.31449.
163. Mattina T, Perrotta CS, Grossfeld P. Jacobsen syndrome. *Orphanet J Rare Dis.* 2009; 4:9. https://doi.org/10.1186/1750-1172-4-9.
164. Roberts AE. Noonan syndrome. In: Adam MP, Mirzaa GM, Pagon RA, et al., eds. *GeneReviews® [Internet] Seattle (WA)*. Seattle: University of Washington; 2001: 1993–2022.
165. Wenger TL, Hing AV, Evans KN, et al. Apert syndrome. In: Adam MP, Mirzaa GM, Pagon RA, et al., eds. *GeneReviews® [Internet] Seattle (WA)*. Seattle: University of Washington; 2019:1993–2022.
166. Sawh-Martinez R, Steinbacher DM. Syndromic craniosynostosis. *Clin Plast Surg.* 2019;46(2):141–155. https://doi.org/10.1016/j.cps.2018.11.009.
167. Spinner NB, Gilbert MA, Loomes KM, Krantz ID. Alagille syndrome. In: Adam MP, Mirzaa GM, Pagon RA, et al., eds. *GeneReviews® [Internet] Seattle (WA)*. Seattle: University of Washington; 2000:1993–2022.
168. Deardorff MA, Noon SE, Krantz ID. Cornelia de Lange syndrome. In: Adam MP, Mirzaa GM, Pagon RA, et al., eds. *GeneReviews® [Internet] Seattle (WA)*. Seattle: University of Washington; 2015:1993–2022.
169. van Ravenswaaij-Arts CM, Hefner Meg, Blake K, Martin DM. CHD7 disorder. In: Adam MP, Mirzaa GM, Pagon RA, et al., eds. *GeneReviews® [Internet] Seattle (WA)*. Seattle: University of Washington; 2006:1993–2022.