Circulation

Genetic Basis for Congenital Heart Disease: Revisited

A Scientific Statement From the American Heart Association

Endorsed by the American Academy of Pediatrics

ABSTRACT: This review provides an updated summary of the state of our knowledge of the genetic contributions to the pathogenesis of congenital heart disease. Since 2007, when the initial American Heart Association scientific statement on the genetic basis of congenital heart disease was published, new genomic techniques have become widely available that have dramatically changed our understanding of the causes of congenital heart disease and, clinically, have allowed more accurate definition of the pathogeneses of congenital heart disease in patients of all ages and even prenatally. Information is presented on new molecular testing techniques and their application to congenital heart disease, both isolated and associated with other congenital anomalies or syndromes. Recent advances in the understanding of copy number variants, syndromes, RASopathies, and heterotaxy/ciliopathies are provided. Insights into new research with congenital heart disease models, including genetically manipulated animals such as mice, chicks, and zebrafish, as well as human induced pluripotent stem cell–based approaches are provided to allow an understanding of how future research breakthroughs for congenital heart disease are likely to happen. It is anticipated that this review will provide a large range of health care–related personnel, including pediatric cardiologists, pediatricians, adult cardiologists, thoracic surgeons, obstetricians, geneticists, genetic counselors, and other related clinicians, timely information on the genetic aspects of congenital heart disease. The objective is to provide a comprehensive basis for interdisciplinary care for those with congenital heart disease.

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This review has been compiled to provide information for clinicians about new developments in our understanding of the genetic contributions to the pathogenesis of congenital heart disease (HD), providing an update of the 2007 American Heart Association scientific statement on this subject.[1] Not included in this review that is intended to cover genetic aspects of structural heart defects are the aortopathies, arrhythmia/channelopathies, and isolated cardiomyopathies for which there are recent reviews.[2–5] At the time the previous scientific statement was published, genetic testing techniques such as chromosomal microarray and next-generation sequencing (NGS) were not in wide use. Since the rapid dissemination of these testing modalities and others described in this review, discoveries of numerous pathogenic copy number variants (CNVs) and gene mutations have significantly advanced our understanding of the causes of congenital HD. Because of the availability of new genomic technologies, the pace of discovery of new genes for congenital HD is now very rapid.

CONGENITAL HD EPIDEMIOLOGY AND IMPORTANCE OF IDENTIFYING A GENETIC BASIS FOR CONGENITAL HD

Current research indicates that congenital HD is the most common birth defect, affecting nearly 10 to 12 per 1000 liveborn infants (1%–1.2%).[6–8] Not all individuals with congenital HD are diagnosed early, so the actual prevalence has been difficult to determine, but one estimate from Canada suggested that the overall prevalence is 13.1 per 1000 children and 6.1 per 1000 adults.[9] Their data also suggested that congenital HD prevalence increased 11% in children and 57% in adults from 2000 to 2010. The impact of successful medical and surgical management of congenital HD on the survival of individuals with congenital HD is likely contributing to a large extent to its increased prevalence among older children and adults. More and more patients with severe types of congenital HD are surviving into their 30s and beyond. Of note, estimates of congenital HD incidence and prevalence have not included cases of isolated bicuspid aortic valve (BAV), unarguably a form of congenital HD. Because the population prevalence of BAV is 1% to 2% (based on studies at autopsy and of liveborn infants and healthy adolescents), the total prevalence of congenital HD is closer to 2% to 3%.[10–12]

Epidemiological studies have suggested that a genetic or environmental cause can be identified in 20% to 30% of congenital HD cases.[13] Single-gene disorders are found in 3% to 5%, gross chromosomal anomalies/aneuploidy in 8% to 10%, and pathogenic CNVs in 3% to 25% of those with congenital HD as part of a syndrome, and in 3% to 10% among those with isolated congenital HD. The largest genetic study of congenital HD with NGS suggested that 8% and 2% of cases are attributable to de novo autosomal dominant and inherited autosomal recessive variation, respectively.[14] Environmental causes are identifiable in 2% of congenital HD cases. The unexplained remainder of congenital HD is presumed to be multifactorial (oligogenetic or some combination of genetic and environmental factors).[13]

Uncovering a genetic pathogenesis for congenital HD is increasingly clinically relevant, in part because of the aforementioned improved survival. For the clinician caring for a child or adult with congenital HD, important reasons for determining the genetic cause can include (1) assessing recurrence risks for the offspring of the congenital HD survivor, additional offspring of the parents, or other close relatives; (2) evaluating for associated extracardiac involvement; (3) assessing risk for neurodevelopmental delays for newborns and infants; and (4) providing more accurate prognosis for the congenital HD and outcomes for congenital HD–related interventions.

MOLECULAR TECHNIQUES AND DIAGNOSIS

Human Genetic Variation

In addition to aneuploidies and large chromosomal rearrangements, the past 10 years of genetics research has advanced a contemporary understanding of normal and pathogenic human genetic variation based on the concept of detecting individual differences relative to a reference sequence defined as normal. The human reference sequence for both medical and research use was released by the Human Genome Project in 2000 and has been corrected and refined in subsequent years.[15,16] Multiple individuals were included in the creation of the reference, which can be thought of not as the genome of a single person but as being composed of genetic information from as many as 20 to 25 individuals.[17] A single-nucleotide polymorphism (SNP) is a change in a single nucleotide of DNA (eg, reference GGT**C**TC, alternative GGT**G**TC). An insertion or deletion (INDEL) is a change in multiple nucleotides that results in a difference in length relative to the reference sequence (eg, reference GGT**C**TC, alternatives GGT**GCG**TC or GGTTC). CNVs constitute large insertions or deletions of DNA, frequently defined as >1000 nucleotides in length, and can occur anywhere throughout the genome (these lesions can also be referred to as microdeletions or microduplications). Each of these types of genetic variation has well-described causal roles in a variety of different diseases, including congenital HD, to be discussed in later sections.

Table 1. Clinical Tests

	Genomic vs Targeted	Aneuploidies and Chromosomal Rearrangements	Copy Number Variation	SNPs and INDELS	Example of Clinical Use
Karyotype	Genomic	+++	+	−	Confirmation of trisomy 21
Array CGH	Genomic	++	+++	−	Multiple congenital anomalies without obvious syndromic association
FISH	Targeted	+	+	−	Suspected 22q11.2 deletion syndrome
Gene panel testing	Targeted	−	+	+++	Suspected monogenic disease with a small differential diagnosis
Exome sequencing	Genomic	−	−	+++	Broad genetic differential diagnosis without obvious syndromic association, or previous negative panel testing
Genome sequencing	Genomic	+	+	+++	Broad genetic differential diagnosis without obvious syndromic association, or previous negative panel testing and need for rapid turnaround time

Sensitivity of tests for the types of genetic variation are indicated as not detected (-), low (+), medium (++), or high (+++). Array CGH indicates comparative genomic hybridization using arrays; FISH, fluorescence in situ hybridization; INDEL, insertion or deletion; and SNP, single-nucleotide polymorphism.

Technologies and Testing Paradigms

Genetic testing can be divided into 2 categories; genomic tests capture ≥1 types of variation at all locations within the human genome, whereas targeted tests capture information about ≥1 select genetic locations (Table 1). There is now significant overlap between the types of variation detectable by different testing technologies. Genomic tests can offer an unbiased approach to detecting clinically relevant genetic variation, whereas targeted assays test a specific hypothesis about ≥1 genes or loci involved in disease.[18] Many tests use, in some form, the principle of DNA hybridization by which a sequence of DNA can be separated into 2 complementary strands (eg, ATCGGTC binding to TAGCCAG), and these individual strands will bind very specifically to a synthetic complementary strand (for a specific region of the genome, genetic sequence, or series of genes) under appropriately controlled chemical conditions.

Large Genetic Variation

Karyotyping, a genomic test, is the gold standard for detecting aneuploidies and large chromosomal rearrangements that occur throughout the genome; it is performed on metaphase chromosomes in an automated or semiautomated process before review by a cytogeneticist. In comparative genomic hybridization (CGH), the copy number of DNA sequences from a subject is compared to those DNA from a control or reference by hybridizing both to DNA probes spaced throughout the human genome. Most commercially available CGH tests (which use arrays and thus are called *array CGH*) also test for common (not disease-related) SNPs to provide additional information regarding unusual conditions, such as uniparental disomy. As indicated by the name, an array CGH is a genomic test and is used to detect CNVs; depending on the specifics

of the commercial platform used, it can detect a lower size limit of ≈100 000 nucleotides.[19] Fluorescence in situ hybridization (FISH) is a targeted test in which a probe for a specific region of the genome is hybridized against metaphase chromosomes from the patient to detect for CNVs at a specific genetic locus. At many institutions, FISH and karyotypes are falling out of common usage, being largely supplanted by array CGH or SNP genotyping arrays (collectively referred to as chromosome microarrays or CMAs).[20]

Small Genetic Variation

The detection of SNPs and INDELs for clinical genetic testing now almost universally uses NGS technologies, also known as sequencing by synthesis or short-read sequencing. Gene panel tests are targeted tests and rely on either hybridization or polymerase chain reaction to capture the regions of genes (typically 1 to 100 genes) that encode for protein sequences. Whole exome sequencing (WES) is a genomic test that captures (by hybridization) the protein coding regions of all 18 000 human genes (≈1.5% of the entire genome), followed by NGS. Whole genome sequencing is a genomic test that obtains genetic information from the entire human genome without a complex capture process followed by NGS, although functionally the analysis of the data obtained is usually limited to the protein-coding regions of the genome.[21] After generation of the sequencing information by NGS, gene panel tests, exome sequencing, and genome sequencing all require a subsequent bioinformatic analysis to detect and classify SNPs and INDELs in genes relevant to the disease under consideration.[22,23] Most gene panel tests now offer concurrent detection of deletion or duplication (arising from a CNV) of the small number of genes on the panel test,[24] and this is an emerging offering from some providers of clinical exome-sequencing services.

Practical Considerations

In contemporary practice, genetic counselors and other qualified clinicians are essential for the appropriate and ethical application of any genetic test in the clinical setting. Genetic counselors with extensive disease- and gene-specific domain knowledge are often the primary interpreters of genetic information detected on panel, exome, and genome tests in subspecialty clinics that do not include a medical geneticist.[25,26] Just as with all other medical tests, each technique in modern genetic testing can display false-positive and false-negative results for specific types of genetic variation, which can often be linked to the fundamental technical aspects of the sequencing chemistries and bioinformatics used in processing a specific test.[27,28] In addition, the genetic testing will not always yield a "yes" or "no" result; variants of unknown significance are commonly identified, and the communication of results to the family is best accomplished by genetic counselors, geneticists, and qualified clinicians with expertise in congenital HD.

Emerging Technologies

There are a number of additional technologies that have entered or will shortly enter clinical use, of which 2 will be discussed here. The sequencing of fetal cell-free DNA (fcfDNA) is commonly used in the prenatal setting as a screening tool for aneuploidies. Whole blood from a pregnant woman contains DNA from the fetal trophoblastic cells that can be separated by centrifugation. Once the fcfDNA is separated, the ratio of genetic information can be sampled and compared between different chromosomes to detect aneuploidies,[29] and in the future, clinical testing could also be expanded to include detection of specific subchromosomal deletions or duplications such as the 22q11.2 deletion. However, current implementations of fcfDNA technology display measurably lower sensitivity and specificity for aneuploidy relative to gold standard tests such as FISH testing of amniotic fluid.[30]

Additional advances in sequencing technology that effectively increase the length of sampling for DNA (current NGS sample size is ≈100–250 nucleotides; long-read sequencing is often >10 000 nucleotides) are more robust for detecting structural variation. Once perfected and when cost-effective, long-read sequencing will allow for the robust detection of SNPs/INDELs and CNVs simultaneously with a single clinical test.[31]

CHROMOSOMAL ANEUPLOIDIES AND CNVs ASSOCIATED WITH CONGENITAL HD

Aneuploidies

Aneuploidy is an abnormal number of chromosomes, and aneuploidies that most commonly survive to term include trisomy 21, 18, and 13 and sex chromosome aneuploidies such as Turner syndrome (Appendix). There is an increased risk of many aneuploidies with increasing maternal age. Increasingly, aneuploidies are detected prenatally with noninvasive prenatal diagnostic screening, and in this section, aneuploidies commonly associated with congenital HD such as Down syndrome and Turner syndrome are presented. Information on other aneuploidies is present in the Appendix. Less common aneuploidies such as trisomy 8 and 9 survive to term only when they are mosaic. Fetal echocardiograms allow for early and accurate diagnosis of the cardiac anatomy when aneuploidies are detected.

Down Syndrome

Down syndrome is the most common aneuploidy and is usually caused by trisomy 21. It is also the most common chromosome abnormality associated with congenital HD.[32]

Common Features

The common features of Down syndrome include characteristic facial features; short stature; hypotonia; intellectual disability ranging from mild to moderate; behavioral issues including problems with attention, obsessive/compulsive behavior, and tantrums; and a range of congenital anomalies, including ≈40% to 50% with congenital HD. Individuals with Down syndrome often experience a gradual decline in cognition and have an increased risk of Alzheimer disease. Health supervision guidelines are available and treatment is based on specific clinical manifestations.[33]

Cardiac Features

Congenital HD is frequently diagnosed in infants and children with Down syndrome (40%–50%). The most common congenital HDs include atrioventricular septal defect (AVSD), ventricular septal defect (VSD), atrial septal defect (ASD), patent ductus arteriosus (PDA), and tetralogy of Fallot.[34] Congenital HD and cardiac complications are common causes of mortality in patients with Down syndrome, contributing to 13% of deaths in childhood and 23% of deaths in adulthood.[35] Individuals with Down syndrome have increased risk of pulmonary hypertension, as well as congenital respiratory tract anomalies, pulmonary abnormalities, and hypotonia, each of which can lead to worse outcomes after surgery.[36] One subset of patients with Down syndrome that has a higher surgical risk is those undergoing single-ventricle palliation. Among those patients undergoing staged single-ventricle palliation, individuals with Down syndrome had higher in-hospital mortality rates.[37]

Prevalence

Down syndrome occurs in ≈1 in 800 newborns. Approximately 5300 babies with Down syndrome are born

in the United States each year, and ≈200 000 people in the United States have Down syndrome. The risk of having a child with Down syndrome increases with advanced maternal age.

Molecular Genetics

Most individuals with Down syndrome have trisomy 21, but rarely, Down syndrome results from a translocation of chromosome 21 with another chromosome (commonly 21, 14, or 13) or mosaicism in a subset of cells.

Cardiovascular Genotype/Phenotype Correlations

The vast majority of individuals with Down syndrome have trisomy 21, so there is little genotype/phenotype correlation. However, in general for individuals with mosaicism, the lower the level of mosaicism for trisomy 21, the less severe the cognitive deficits are.

Turner Syndrome

Turner syndrome is another common chromosomal condition, caused by loss of part or all of an X chromosome in females.

Common Features

The most common features of Turner syndrome include short stature, early loss of ovarian function manifesting as delayed puberty and delayed menarche (and in adult women, anovulation and infertility), lymphedema, webbed neck, low posterior hairline, cubitus valgus, congenital HD, skeletal anomalies, renal anomalies, and developmental delays, nonverbal learning disabilities, and behavioral problems in some girls.

Treatment with growth hormone is often beneficial, ideally beginning in early childhood, and can increase final adult height by 8 to 10 cm. Estrogen replacement therapy is usually started at the time of normal puberty, around 12 years of age, to initiate normal timing of breast development and to help prevent osteoporosis. Estrogen and progesterone are given to support menstruation.

Cardiac Features

Cardiac structural anomalies usually involve the left side of the heart and most commonly include BAV and coarctation of the aorta and less commonly partial anomalous pulmonary venous return and hypoplastic left heart syndrome (HLHS). All patients with Turner syndrome should have a baseline echocardiogram and cardiac evaluation and follow-up as necessary based on the baseline evaluation. Aortic root dilatation is present in 3% to 8% and can lead to dissecting aneurysms and rupture. BAV, coarctation of the aorta, and systemic hypertension are associated with aortic dilatation and dissection.[38–40] Serial aortic arch imaging by echocardiogram or magnetic resonance imaging for

adolescents should be performed every 5 years if there is no history of aortic dilation, BAV, or hypertension, and more frequent screening can be beneficial for individuals with risk factors for aortic dissection.[41] Up to 40% of girls with Turner syndrome have hypertension, which should be treated aggressively.[38] A cardiovascular and renal evaluation should be completed when hypertension is identified.

Prevalence

Turner syndrome occurs in ≈1 in 2000 to 1 in 2500 live female births.[42]

Molecular Genetics

The exact genetic abnormality found on karyotype analysis varies and can include classic 45,X but also individuals who are mosaic 45,X with another cell line, including 46,XX, 47,XXX, or 46,XY, as well as individuals with structural abnormalities of the X chromosome, including deletions and translocations of the X chromosome. Array CGH is useful to define precisely the extent of the deletions or translocation. For mosaic individuals, the phenotype is generally less severe as the percentage of 45,X cells decreases. It is important to determine whether there are any cells with a Y chromosome using an *SRY* polymerase chain reaction test, because this can be associated with gonadal dysgenesis that might require surgical removal of gonadal tissue to prevent the increased risk of cancer.

Cardiovascular Genotype/Phenotype Correlations

The prevalence of cardiovascular abnormalities in individuals with Turner syndrome varies between 20% and 40% and is higher with monosomy X relative to those with structural abnormalities of the X chromosome and in girls with a more pronounced clinical phenotype.[38,39,43,44]

COPY NUMBER VARIANTS

Review of Types of CNVs

CNVs range widely in size from single genes to large segmental deletions or duplications of millions of base pairs. In general, deletions are more deleterious than duplications because of the sensitivity in gene dosage for many genes that do not tolerate haploinsufficiency. CNVs that encompass multiple genes can have a wide range of phenotypic effects because of the additive impact of individual genes on individual phenotypes or the pleiotropic effects of single genes on multiple phenotypes. Identification of the relevant gene for congenital HD within a CNV interval requires mapping of multiple patients with overlapping CNVs to identify a critical interval and ultimately a single gene within the critical interval that is associated consistently with con-

genital HD. Additional supportive evidence for the congenital HD gene is provided by examples of patients with point mutations within that single gene within the critical region who have congenital HD.

Association of Pathogenic CNVs as a Class With Clinical Outcome

On average as a group, children with pathogenic CNVs associated with congenital HD have poorer outcomes than children without pathogenic CNVs. At least part of the explanation for the worse outcome could be an association with extracardiac manifestations that impact medical care. In one series of 58 patients with congenital HD and other dysmorphic features or other anomalies, 20.7% of the patients had potentially pathogenic CNVs that ranged in size from 240 kb to 9.6 Mb.[45] In another series of 422 children with nonsyndromic, isolated congenital HD followed up prospectively from before their first surgery, there was an increased frequency of potentially pathogenic CNVs in 12.1% of congenital HD subjects compared with 5% of control subjects, and in this series, the presence of a CNV was associated with significantly decreased transplant-free survival after surgery, with an adjusted 2.6-fold increased risk of death or transplantation.[46] Beyond survival, putatively pathogenic CNVs that were more frequent in congenital HD patients with single-ventricle physiology (13.9% of 223 affected individuals compared with 4.4% of control subjects) were associated with worse linear growth and worse neurocognitive outcomes.[47] There is undoubtedly heterogeneity in outcomes across CNVs, and future studies will require refined analyses specific to the individual CNV to determine which ones are associated with differential prognosis when controlling for the cardiac anatomy, as well as studying the other associated anomalies, ventricular function, and arrhythmias that could account for differential outcomes.

Many new CNVs associated with congenital HD have been identified over the past 10 years and now have been observed in sufficient numbers of patients to define the clinical features associated with them.[48] Most of the CNVs are flanked by repeat sequences that lead to nonallelic homologous recombination and recurrent de novo deletions or duplications of the same interval, although a minority of patients have smaller or larger CNVs associated with less or more severe phenotypes, respectively. There are several common principles that apply across the CNVs. Each of the CNVs includes contiguous gene deletions or duplications, and generally deletions are associated with greater severity of neurocognitive phenotype. Because each of the CNVs includes multiple genes, it is not always clear whether the overall phenotype is caused by the effects of multiple genes on multiple aspects of the phenotype or whether

certain single genes within the interval have pleiotropic effects on multiple aspects of the phenotype. For each of the CNVs discussed below, the associated congenital HD is incompletely penetrant. It is usually unclear what the other determinants of congenital HD are, but it is likely that they are interacting with genetic factors either on the opposite allele or genetic variants in *cis* on the same chromosome or in *trans* on other chromosomes, as well as nongenetic factors. Most CNVs are associated with effects on behavior and cognition, and many are associated with growth effects that are independent of the congenital HD and are important to appreciate when assessing clinical outcomes.

DESCRIPTIONS OF SPECIFIC CNVs ASSOCIATED WITH CONGENITAL HD

In this section, several CNVs are highlighted. The Appendix provides information on other less frequent CNVs.

22q11.2 Deletion Syndrome

The 22q11.2 deletion syndrome (22q11.2DS) is the most common microdeletion syndrome, with a prevalence estimated at 1 per 5950 live births.[49] The clinical features of the 22q11.2DS are those described for the DiGeorge and velocardiofacial syndromes and the Takao conotruncal anomaly face syndrome, although the phenotype can vary, even within a family.[50] Although highly overlapping, the 22q11.2DS and DiGeorge and velocardiofacial syndromes are not synonymous, because nearly 10% of patients with DiGeorge and velocardiofacial syndromes do not have a 22q11.2 deletion, and not all patients with 22q11.2 deletion will demonstrate classic features of DiGeorge and velocardiofacial syndromes.

Common Features

Frequent clinical features include dysmorphic facies, congenital HD (especially conotruncal malformations and aortic arch anomalies), palatal malformations, learning difficulties, and immunodeficiency. Facial features are characteristic but can be relatively subtle, especially in infants. Facial dysmorphisms include myopathic facies, tubular nose with bulbous nasal tip, hypoplastic alae nasi, and low-set or dysplastic ears. Additional findings include hypocalcemia, significant feeding and swallowing problems (including regurgitation through the nose), constipation, renal anomalies, hearing loss, laryngotracheoesophageal anomalies, growth hormone deficiency, autoimmune disorders, seizures, central nervous system anomalies, skeletal abnormalities, ophthalmologic abnormalities, enamel hypoplasia, and malignancies (rare). Behavioral and learning disabilities become more evident in school-aged children, whereas

Table 2. Common Clinical Features of 22q11.2DS and Most Common Age at Presentation

Clinical Feature	Infancy	Toddler/ School Age	Adolescent/ Adult
Congenital HD (conotruncal defects and interrupted aortic arch)	X	X	
Characteristic facial features	X	X	
Palatal abnormalities (velopharyngeal insufficiency, hypernasal speech)	X	X	
Feeding problems/nasal regurgitation of feeds	X		
Hypocalcemia	X		
Immune deficiency/thymus anomalies	X	X	
Learning difficulties (nonverbal learning disability)		X	
Psychiatric disease (autism, schizophrenia in adults)			X

22q11.2DS indicates 22q11.2 deletion syndrome; and HD, heart disease.

Table 3. Suggested Patients With Congenital HD to Test for a 22q11.2 Deletion

All fetuses with interrupted aortic arch, truncus arteriosus, tetralogy of Fallot, VSD,* or aortic arch anomaly (if amniocentesis performed for diagnostic purposes)
All newborns/infants/children/adolescents/adults with:
Interruption of aortic arch
Truncus arteriosus
Tetralogy of Fallot
VSD* with aortic arch anomaly
Isolated aortic arch anomaly
Congenital HD and additional feature of 22q11.2DS

22q11.2DS indicates 22q11.2 deletion syndrome; HD, heart disease; and VSD, ventricular septal defect.

*Malalignment, conoseptal hypoplasia, perimembranous.

psychiatric disorders often become manifest in adolescence and adulthood (Table 2). Delays in emergence of language, intellectual disability, and learning differences (nonverbal learning disability with verbal IQ significantly greater than the performance IQ) are common. Autism or autism spectrum disorder is found in ≈20% of children, and psychiatric illness (schizophrenia) is present in 25% of adults. Attention deficit disorder, anxiety, perseveration, and difficulty with social interactions are also common.[51]

Cardiovascular Features
Conotruncal malformations account for 70% of the heart defects associated with a 22q11.2 deletion.[52] The most common cardiovascular defects include tetralogy of Fallot (20%), truncus arteriosus (6%), conoventricular VSD (14%), type B interruption of the aortic arch (IAA), and other aortic arch anomalies (13%).[53–56] ASDs, pulmonary valve stenosis (PVS), HLHS, double-outlet right ventricle, transposition of the great arteries (TGA), vascular rings, and heterotaxy syndrome are less common but have also been reported.

Prevalence
The estimated prevalence of the 22q11.2 deletion among various cardiovascular malformations is 1.9%.[52]

Molecular Genetics
The majority of 22q11.2 deletions are de novo, but they are inherited from a parent in an autosomal dominant fashion in 6% to 28% of cases.[50] It is not unusual in familial cases for one of the parents to be diagnosed with the 22q11.2 deletion only after their child is diagnosed.[50,57]

It is important to identify the cardiac patient with a 22q11.2 deletion by CMA to offer accurate genetic counseling and familial screening and to allow for identification of associated noncardiac features that require specific management. For example, there is a higher operative mortality in some patients with 22q11.2 deletion.[58,59] It is important for clinicians to be aware of a 22q11.2 deletion to plan surgery and postoperative care, particularly with respect to immunologic issues and calcium metabolism. Affected individuals should receive leukocyte-depleted and cytomegalovirus-negative blood products to prevent serious graft-versus-host disease or overwhelming infection.

Given the frequency of 22q11.2 deletions in the congenital HD population, it is reasonable to test all individuals with IAA type B, truncus arteriosus, tetralogy of Fallot, VSD (malalignment, conoseptal hypoplasia, or perimembranous) with aortic arch anomaly, or isolated aortic arch anomaly (Table 3). Clinical assessment for syndromic features alone might not consistently identify the infant carrying a 22q11 deletion, because facial features can evolve with time. Therefore, routine screening of individuals with selected types of congenital HD using CMA is warranted either prenatally or postnatally.

Cardiovascular Genotype/Phenotype Correlations
The estimated 22q11.2 deletion frequency is particularly high for IAA (22%–48%),[42,60] truncus arteriosus (12%–35%), tetralogy of Fallot (8%–13%), and isolated aortic arch anomalies (24%).[42,60,61] In patients with VSDs, the deletion frequency is low overall[42] (2%) but higher when associated with aortic arch anomaly (right aortic arch, cervical location or abnormal branching pattern, and discontinuous branch pulmonary arteries).[60] For IAA, 22q11.2 deletions are specifically associated with type B (accounting for more than half of the cases of type B interruption) and not commonly associated with type A. Among those with tetralogy of Fallot, the strongest association with 22q11.2 deletions is for those with pulmonary atresia.[60] A 22q11.2

deletion is infrequent in children with double-outlet right ventricle (2%)[42] and those with TGA. Aortic root dilation has been described with 22q11.2 deletions either in association with a conotruncal defect or other cardiac defect or as an isolated finding.[62,63] Tetralogy of Fallot with aortic arch abnormalities is the most frequent congenital HD with aortic dilation in 22q11.2DS.[62]

Phenotypic Variability and Related Conditions

22q11.2DS is a contiguous gene deletion syndrome, and >40 genes are deleted in the most common deletion. Deletion of several genes within this region contributes to the cardiac and noncardiac features. The size of the deletion can be precisely determined by CMA. The vast majority (97%) of affected individuals will have either a common recurrent ≈3-Mb deletion or a smaller, less common ≈1.5-Mb nested deletion. Smaller or larger deletions can contribute to atypical clinical phenotypes. Mutations outside the interval or on the nondeleted 22q11.2 allele are also known to modify the phenotype. An example of this is Bernard-Soulier syndrome, an autosomal recessive trait, which includes giant platelets, thrombocytopenia, and a prolonged bleeding time. One cause of Bernard-Soulier syndrome is biallelic loss-of-function mutations in the gene encoding the β-subunit of the platelet glycoprotein GPIb (GPIBB), which resides in the 22q11.2 critical region. Several cases of Bernard-Soulier syndrome have been reported in which a 22q11.2del was combined with a loss-of-function GP1BB mutation on the nondeleted allele. Although rare, the occurrence of these 2 conditions together can potentially place the 22q11.2 individual at risk for life-threatening bleeding in conjunction with surgeries and procedures.[64]

Duplication of the same 22q11.2 CNV region causes an extremely variable disorder with a phenotype that ranges from normal to learning disability and, infrequently, congenital defects including heart defects. Generally, the duplication is associated with milder and more variable manifestations than the deletion. The duplication can be either de novo or inherited from a phenotypically normal parent.[65] Congenital HD occurs in 15% with similar defects as 22q11.2DS.[66] The associated features are largely neurobehavioral and range from apparently normal to intellectual disability/learning disability, delayed development, or hypotonia. Many of the reported series likely suffer from ascertainment bias compared with phenotypes in unselected population-based cohorts.[65,67]

A small number of individuals have distal deletions of 1.4 to 2.1 Mb of 22q11.2 that do not overlap with the DiGeorge proximal 22q11.2 deletion. Patients with the distal deletion share some overlapping neurobehavioral features, including speech delay and learning disabilities, with proximal 22q11.2DS, but this represents

a distinct genomic disorder.[68] Other clinical features include prematurity, problems with growth, cleft palate, skeletal anomalies, and congenital HD including truncus arteriosus and BAV.[68] CRKL and MAPK1 are the genes in this region that might play a role in cardiac development.[69]

A recent study compared rare CNVs outside the common 22q11.2 deletion region in 607 22q11.2DS subjects with congenital HD compared with 339 22q11.2DS subjects with normal cardiac anatomy. Although there was no significant difference in the overall burden of rare CNVs, an overabundance of CNVs affecting cardiac-related genes was detected in 22q11.2DS individuals with congenital HDs, which suggests that CNVs outside the 22q11.2 region might contain genes that modify risk for congenital HDs in some 22q11.2DS patients.[70] Finally, another recent study has shown that the phenotypic variability observed in a subset of individuals with 22q11.2DS is attributable to other mutations on the nondeleted chromosome.[71]

Williams-Beuren Syndrome

Williams-Beuren syndrome or Williams syndrome (WS) is a contiguous gene deletion syndrome caused by deletion at 7q11.23.

Common Features

Clinical manifestations (Table 4) include dysmorphic features, characteristic cardiovascular defects (vascular stenoses, elastin arteriopathy), a specific cognitive profile, unique personality characteristics ("social personality"), growth abnormalities, connective tissue and skeletal abnormalities, and endocrine abnormalities (infantile hypercalcemia, hypercalciuria, hypothyroidism, and early puberty). Feeding difficulties during infancy often lead to poor weight gain. Adults have short stature (less than third percentile) and tend to be overweight or obese and to have complications of systemic hypertension, diabetes mellitus, and diverticulosis.[72]

Cardiovascular Features

Frequent cardiovascular anomalies include supravalvular aortic stenosis (SVAS), often in combination with supravalvular pulmonary artery stenosis and branch pulmonary artery stenosis. The SVAS can progress during childhood and is the most common abnormality requiring surgical intervention. In contrast, the branch pulmonary artery stenosis often regresses with time.[73,74] These arterial abnormalities constitute an elastin arteriopathy or vasculopathy caused by deletion of the ELN gene. Any artery can be narrowed, including the ascending aorta, aortic arch, and descending thoracic and abdominal aorta, as well as central and peripheral arteries including the coronary arteries, carotid and cerebral arteries, mesenteric arteries, renal arteries, and pulmo-

Table 4. Clinical Features of Williams Syndrome

Cardiovascular
Elastin arteriopathy: any artery may be narrowed; can be multiple
Supravalvular aortic stenosis: most common (75%) and most clinically significant
Pulmonary arterial stenosis: common in infancy
Aortic/mitral valve defects
Systemic hypertension
Distinctive facies
Stellate iris pattern
Broad forehead, bitemporal narrowing
Periorbital fullness
Malar flattening
Short nose, broad nasal tip, long philtrum
Full lips/wide mouth/small, widely spaced teeth
Craniofacial
Recurrent otitis media
Malocclusion
Ophthalmologic
Strabismus
Hyperopia
Neurological
Cognitive disability: strength in language; extreme weakness in visuospatial
Unique personality: overfriendliness, empathy, generalized anxiety, specific phobias
Hyperacusis
Connective tissue abnormalities
Hoarse voice
Inguinal/umbilical hernia
Bowel/bladder diverticulae
Rectal prolapse
Joint limitation or laxity/soft, lax skin
Growth abnormalities
Prenatal growth deficiency
Infantile failure to thrive
Constipation
Adult height less than third percentile
Overweight/obese
Endocrine
Hypercalcemia/hypercalciuria
Hypothyroidism
Adult diabetes mellitus
Renal/bladder disorders
Structural anomalies
Nephrocalcinosis
Chronic urinary tract infections

nary arteries. Affected arteries typically have thickened walls and narrowed lumens. There is an increased risk of anesthesia-related complications and sudden cardiac death. Risk factors include myocardial ischemia attributable to coronary stenosis or severe biventricular outflow tract obstruction, but the causative mechanisms have not been fully delineated.[75–79]

Prevalence
WS occurs in 1 per 7500 to 20 000 births.

Molecular Genetics
The vast majority of affected individuals with a clinical diagnosis of WS have been found by FISH or deletion/duplication testing to have a microdeletion at chromosome 7q11.23, typically a recurrent 1.5- to 1.8-Mb deletion of the Williams-Beuren syndrome critical region that encompasses *ELN*, the gene encoding elastin.[80] Most cases arise de novo, although parent-to-child transmission with an autosomal dominant pattern of inheritance has been reported. As with other contiguous gene deletion syndromes, WS has a broad range of phenotypic variability. The size of the deletion can be precisely determined by CMA. Although there is wide phenotypic variability even among individuals with the typical deletion, smaller or larger deletions might contribute to atypical clinical phenotypes.

Given the clinical variability of WS and the fact that the physical and developmental signs can be relatively subtle during infancy, it is not unusual for the diagnosis to be confirmed only after identification of a characteristic cardiovascular defect such as SVAS. The severity of SVAS and other vascular defects tends to be greater in males, and infants and children with more severe vascular involvement tend to be diagnosed with WS at younger ages than those with trivial or no cardiovascular involvement.[81,82]

Because SVAS is very common in WS and uncommon in the general population, it is appropriate to consider testing all patients with SVAS at the time of diagnosis of the cardiovascular defect. Furthermore, if peripheral pulmonary artery stenosis persists beyond infancy, it is also appropriate to consider testing for WS. Similarly, if any of the defects associated with the elastin arteriopathy, including coronary artery ostial stenosis, renal artery stenosis, and middle aortic syndrome (abdominal coarctation), are diagnosed at any age, testing for WS should be considered.

Cardiovascular Genotype/Phenotype Correlations
Point mutations or small intragenic deletions of *ELN* have been found in the autosomal dominant disorder familial SVAS without other characteristics of WS. The vascular disease in the nonsyndromic familial SVAS is indistinguishable from that seen in WS. Of note, CNVs in the 7q11.23 region have been found to be associated with autism in a study of >4000 individuals who did not have WS,[83] and dilation of the ascending aorta occurs in almost half of individuals with 7q11.23 duplication syndrome.[84,85]

General Clinical Recommendations

Early diagnosis of WS is important to optimize management of other potential medical problems (Table 4).[86] Renal anomalies are common, and a renal ultrasound is recommended at baseline and as needed.[87] Endocrine abnormalities include idiopathic hypercalcemia, hypercalciuria, hypothyroidism, subclinical hypothyroidism, and early puberty.[72] Hypercalcemia and hypercalciuria can be treated with appropriate diet and medication. Hypercalcemia occurs most commonly in the first year of life, whereas hypercalciuria can persist and occur at any age. Hypercalcemia can lead to nephrocalcinosis and renal failure. Obesity, abnormal oral glucose tolerance tests, and diabetes mellitus are common, especially in adults.[72] Systemic hypertension is also common and often presents during childhood or adolescence.[88] About half of adults with WS will have high blood pressure. Intellectual disability is common and usually mild, but with a specific cognitive profile with strengths in verbal short-term memory and language and extreme weakness in visuospatial constructive cognition.[89] An early diagnosis of WS allows for enhancement of learning and development in children with WS. Attention deficit disorder and anxiety are common. Whereas deletion of *ELN* accounts for the cardiovascular and connective tissue abnormalities in WS, deletion of additional genes in the Williams-Beuren syndrome critical region (*LIMK1*, *GTF2I*, *STX1A*, *BAZ1B*, *CLIP2*, *GTF2IRD1*, *NCF1*) has been implicated in other manifestations of WS.[72]

Jacobsen Syndrome (11q Terminal Deletion Disorder)

Since Jacobsen's initial report in 1973,[90] >200 patients with Jacobsen syndrome (JS) have been reported. JS is a clinically recognizable contiguous gene deletion syndrome involving deletions from subband 11q23 to the telomere, ranging in size from 7 to 16 Mb.

Common Features

Clinical manifestations include dysmorphic features, growth retardation sometimes associated with IGF-1 (insulin-like growth factor 1) deficiency, cognitive and behavioral dysfunction, congenital HD, thrombocytopenia and platelet dysfunction (Paris-Trousseau syndrome), recurrent infections, immune deficiency, and ophthalmologic, gastrointestinal, and genitourinary problems.[91,92]

A prospective study of 110 patients with the 11q terminal deletion disorder, diagnosed by karyotype rather than CMA, provided detailed delineation of the clinical manifestations, as well as a comprehensive set of recommendations for the clinical management of patients with this disorder. Molecular analysis of the deletion breakpoints in 65 patients defined genetic "critical regions" for 14 clinical phenotypes, as well as for the neuropsychiatric profiles.[91] Subsequent studies have implicated caus-

ative genes for a number of these clinical phenotypes, including heart defects (see Molecular Genetics).[92]

Cardiovascular Features

More than half of affected individuals have congenital HD, most of whom require surgical intervention. About one-third of patients with heart defects have a membranous VSD, and another third have left ventricular outflow tract defects with various degrees of hypoplasia or obstruction of the mitral valve, left ventricle, aortic valve, or aorta. This spectrum includes mitral stenosis, BAV, aortic valve stenosis, coarctation of the aorta, Shone complex, and HLHS. HLHS is highly overrepresented in patients with JS (5%–10%), an estimated frequency that is 1000 to 2000 times that of the general population. The other one-third of children with congenital HD have a variety of heart defects including double-outlet right ventricle, TGA, AVSD, secundum ASD, dextrocardia, aberrant right subclavian artery, PDA, persistent left superior vena cava, tricuspid atresia, type B IAA, truncus arteriosus, and PVS.

Prevalence

The prevalence of JS is estimated to be 1 in 50 000 to 1 in 100 000 live births.

Molecular Genetics

Diagnosis of JS is currently accomplished by CMA. Through a combination of human genetic techniques and using genetically engineered animal models, *ETS1* has been identified as the causal gene for congenital HD in JS.[92] Homozygous deletion of *Ets1* caused VSDs and abnormal ventricular morphology with nearly 100% penetrance in mice in a C57/B6 background but not in an FVBN-1 background.[93] The fact that homozygous *Ets1* knockout mice do not have heart defects in at least 1 strain strongly implies the presence of a genetic modifier. Most recently, a patient with a complex congenital HD including mitral atresia and hypoplastic left ventricle was found to carry a de novo frameshift mutation in *ETS1*, likely a loss-of-function mutation, providing further confirmation that loss of *ETS1* is the cause of congenital HD in JS.[94]

Cardiovascular Genotype/Phenotype Correlations

There is no correlation between the size of the deletion and whether or not there is congenital HD or what the specific congenital HD is. Using FISH, Grossfeld and colleagues[91] found that the smallest terminal deletion associated with a congenital HD (HLHS) was ≈7 Mb (cardiac critical region).

Thombocytopenia and Platelet Dysfunction

Nearly all patients with JS have Paris-Trousseau syndrome, characterized by thrombocytopenia and platelet dysfunction, and heterozygous loss of the *FLI1* gene has been identified as the cause.[92] The thrombocytopenia presents in the neonatal period. Platelet dysfunction persists in older individuals, despite normal platelet

counts. Risk for bleeding is one of the most common causes of mortality in JS and likely places these patients at increased risk for the development of brain hemorrhages.[91,92,95] Platelet transfusion or desmopressin may be necessary for bleeding and high-risk procedures.

Cognitive Function and Behavior

Cognitive function ranges from normal intelligence to moderate cognitive disability. Nearly half of the patients have mild cognitive disability, with a characteristic neuropsychiatric profile demonstrating near-normal receptive language ability but mild to moderate impairment in expressive language, with full-scale IQs typically in the 60s to 70s.[91] The severity of intellectual disability correlates with the size of the deletion; those with the largest deletions (>12 Mb) have the most severe intellectual disability (IQ <50).[96] Approximately one-half of all JS patients fulfill the diagnostic criteria for autism, and candidate genes for autism (RICS) and intellectual disability (BSX-1) have been identified.[96,97]

General Clinical Recommendations

Individuals with suspected or confirmed JS should have a thorough genetics evaluation. The extent of the deletion can be precisely delineated by CMA in the proband. Most deletions are de novo, with only 8% resulting from a parental translocation, or in rare cases from a relatively mildly affected parent carrying an 11q deletion.[91] Parents should be screened for a translocation or deletion. Patients with JS require coordinated multisystem care. A cardiac evaluation including an echocardiogram is recommended at baseline and as needed. Careful monitoring of the platelet count is necessary in infancy and early childhood, and once the platelet count normalizes, platelet function studies should be evaluated periodically. Neurocognitive and behavioral difficulties are common. Baseline and ongoing evaluations by a neuropsychologist and behavioral specialist are recommended, as well as brain imaging at baseline and as needed. Patients should be screened for ophthalmologic issues including exotropia, amblyopia, refractive errors, ptosis, and retinal artery tortuosity. Common gastrointestinal issues include failure to thrive, constipation, and pyloric stenosis. Genitourinary anomalies include cryptorchidism and renal anomalies. A baseline renal ultrasound is recommended.

1p36 Deletion

The 1p36 deletion syndrome is the second most common deletion syndrome.

Common Features

Clinical features include dysmorphic facies, intellectual disability ranging from mild to severe, hypotonia, seizures, structural brain abnormalities, congenital heart defects, ophthalmologic and vision issues, hearing loss, skeletal abnormalities, and genitourinary anomalies.[98]

Among individuals with the 1p36 deletion, there is a range of neurocognitive disability, but ≈90% of individuals have severe to profound intellectual disability, and 75% are nonverbal. Behavioral disorders include autism, tantrums, self-mutilation, stereotypies, and hyperphagia. Structural brain abnormalities include dilation of the lateral ventricles, cortical atrophy, and hypoplasia or agenesis of the corpus callosum. Seizures are present in approximately half of the individuals with the 1p36 deletion.

Cardiac Features

Structural heart defects include ASD, VSD, valvular abnormalities, PDA, tetralogy of Fallot, coarctation of the aorta, infundibular stenosis of the right ventricle, and Ebstein's anomaly.[99,100] In addition, 27% of individuals with the 1p36 deletion have cardiomyopathy, 23% with left ventricular noncompaction and 4% with dilated cardiomyopathy.[99]

Prevalence

The prevalence of 1q36 deletion is 1 in 5000 to 10 000 births, with a 2:1 female-to-male ratio.[99–101]

Molecular Genetics

This condition is identified by CMA testing. The deletion varies in size and can extend to >5 Mb.

1q21.1 Deletion

Common Features

Recurrent 1.35-Mb deletions of 1q21.1 are typically associated with microcephaly, mild intellectual disability, mildly dysmorphic features, short stature, eye abnormalities (strabismus, chorioretinal and iris colobomas, microphthalmia, hypermetropia, Duane anomaly, and cataracts), and sensorineural hearing loss.[102,103] Less commonly, there are other associated findings, including congenital HD, genitourinary alterations, skeletal malformations (craniosynostosis, scoliosis), and seizures. The associated psychiatric and behavioral anomalies include autistic spectrum disorder, attention deficit hyperactivity disorder, mood disorder, and sleep disturbances.[104] Neurocognitive issues are generally global, with generalized learning disabilities and challenges with gross motor development and coordination.

Cardiac Features

The types of congenital HD include PDA, truncus arteriosus, VSD, ASD, tetralogy of Fallot, BAV, dilation of the ascending aorta, aortic insufficiency, coarctation of the aorta, IAA, anomalous origin of the right coronary artery, PVS, and TGA.[105]

Prevalence

The prevalence of the 1q21.1 microdeletion is ≈0.2% of individuals with developmental delays, intellectual disabilities, or congenital abnormalities.[106]

Molecular Genetics

The condition is identified by CMA. The gene responsible for congenital HD within the interval is possibly *GJA5,* which encodes for a cardiac gap junction protein connexin 40.[107]

1q21.1 Duplication

The reciprocal duplication of 1q21.1 is also associated with congenital HD, more commonly tetralogy of Fallot but also including VSD, TGA, and PVS.[106,108,109] Again, the gene responsible for congenital HD within the interval is possibly *GJA5.*[107] Other congenital malformations associated with the CNV include hypospadias, clubbed feet, hemivertebrae, and hip dysplasia. There is a tendency toward larger head size. Some individuals have neurobehavioral manifestations, including intellectual disabilities, developmental delay, expressive language delay, learning disabilities, features of autism, or attention deficit hyperactivity disorder, but others have no neurobehavioral problems.[104,110]

8p23.1 Deletion

Deletions of 8p23.1 are associated with congenital HD, congenital diaphragmatic hernia, growth impairment, microcephaly, behavioral problems including hyperactivity and impulsivity, mild to moderate intellectual disability, and developmental delays.[111,112] Types of congenital HD typically associated include ASD, AVSD, and PVS. There are also several reported cases of more complex cardiac anatomy including hypoplastic right ventricle, double-outlet right ventricle, and double-inlet left ventricle. The gene most likely responsible for the congenital HD is GATA binding protein 4 (*GATA4*) encoding a zinc finger transcription factor.[113]

WELL-CHARACTERIZED SYNDROMES CAUSED BY SINGLE-GENE VARIATION

During the past 10 to 15 years, a period of active gene discovery, the molecular basis of many syndromes has been identified. Numerous syndromes caused by single-gene variants (traditionally referred to as mutations) have additionally been found to be genetically heterogeneous, which means that an individual variant in >1 gene is capable of causing a similar condition (Table 5). Several selected syndromes are discussed in more detail with regard to their cardiac malformations, including Alagille, Holt-Oram, Char, Ellis-van Creveld, Adams-Oliver, Kabuki, and CHARGE syndromes. Table 5 can be consulted for details of multiple other genetic syndromes.

Alagille Syndrome

Alagille syndrome (ALGS) is an autosomal dominant syndromic disorder characterized by cardiovascular, hepatic, orthopedic, and ophthalmologic complications.

Common Features
Recognizable Facial Features
Children with ALGS have a prominent forehead, deeply set eyes, hypertelorism, straight nose with a bulbous tip, and pointed chin.

Development
Mild gross motor delays are reported in 16% of individuals,[159] and mild intellectual disability is seen in only 2%. Neurovascular accidents, likely secondary to prestenotic vessel aneurysms, occur in up to 15% of cases and can cause significant neurological compromise.[160]

Hepatic
There is considerable intrafamilial and interfamilial variability in the hepatic complications of ALGS, and some individuals have no detectable liver disease. The most common complications are chronic cholestasis, elevated liver enzymes, hypercholesterolemia, or liver failure.[161] Infants with ALGS can present with jaundice, cholestasis, and pruritis. The typical pathological finding is paucity of the bile ducts on liver biopsy. It is estimated that ≈15% of affected individuals will require liver transplantation.[160]

Ophthalmologic
More than 80% will have posterior embryotoxon, an anterior chamber defect, by slit-lamp examination.[160,162] Although not of any functional significance to vision, it is a useful marker for diagnosis. Additional complications include Axenfeld anomaly, Rieger anomaly, optic disk drusen, and retinal pigmentary changes.[163]

Orthopedic
It is estimated that 30% to 90% of individuals with ALGS have butterfly vertebrae by radiography.[159] Butterfly vertebrae are typically not of any clinical consequence but are a useful marker for diagnosis. Less commonly reported skeletal features include hemivertebrae, spina bifida occulta, and rib anomalies.

Renal
Kidney complications including small hyperechoic kidneys, uteropelvic obstruction, renal tubular acidosis, hypertension, and renal artery stenosis are reported in ≈40%.[164]

Cardiovascular Features
Two-thirds of those with ALGS have peripheral or branch pulmonary stenosis or other arterial narrowing (aortic coarctation, renal artery, middle aortic syndrome, Moya-moya, basilar, and middle cerebral arteries).[159] Structural cardiac defects are also reported, including tetralogy of Fallot (7%–15% of cases), aortic stenosis, ASD, and VSD.[160]

Table 5. Genes and Loci Associated With Congenital HD

Syndrome	Gene(s)	Loci	Cardiac Disease	% Congenital HD	Other Clinical Findings	References
Very commonly associated						
Alagille	JAG 1 NOTCH2	20p12.2 1p12-p11	PPS, TOF, PA	>90	Bile duct paucity, posterior embryotoxon, butterfly vertebrae, renal defects	114, 115
CFC	BRAF KRAS MAP2K1 MAP2K2	7q34 12p12.1 15q22.31 19p13.3	PVS, ASD, HCM	75	Curly hair, sparse eyebrows, feeding problems, developmental delay, intellectual disability	116
Cantu	ABCC9	12p12.1	PDA, BAV, HCM, CoA, PE, AS	75	Hypertrichosis at birth, macrocephaly, narrow thorax, coarse facies, macroglossia, broad hands, advanced bone age	117, 118
Char	TFAP2B	6p12.3	PDA, VSD	58	Wide-set eyes, down-slanting palpebral fissures, thick lips, hand anomalies	119, 120
CHARGE	CHD7	8q12	TOF, PDA, DORV, AVSD, VSD	75–85	Coloboma, choanal atresia, genital hypoplasia, ear anomalies, hearing loss, developmental delay, growth retardation, intellectual disability	121
Costello	HRAS	11p15.5	PVS, ASD, VSD, HCM, arrhythmias	44–52	Short stature, feeding problems, broad facies, bitemporal narrowing, redundant skin, intellectual disability	122
22q11.2DS	TBX1	22q11.2 deletion	Conotruncal defects, VSD, IAA, ASD, VR	74–85	Cleft palate, bifid uvula, velopharyngeal insufficiency, microcephaly, hypocalcemia, immune deficit, psychiatric disorder, learning disability	54
Ellis-van Creveld	EVC EVC2	4p16.2 4p16.2	Common atrium	60	Skeletal dysplasia, short limbs, polydactyly, short ribs, dysplastic nails, respiratory insufficiency	123, 124
Holt-Oram	TBX5	12q24.1	VSD, ASD, AVSD, conduction defects	50	Absent, hypoplastic, or triphalangeal thumbs; phocomelia; defects of radius; limb defects more prominent on left	125
Kabuki	KMT2D KDM6A	12q13 Xp11.3	CoA, BAV, VSD, TOF, TGA, HLHS	50	Growth deficiency, wide palpebral fissures, large protuberant ears, fetal finger pads, intellectual disability, clinodactyly	126, 127
Noonan	PTPN11 SOS1 RAF1 KRAS NRAS RIT1 SHOC2 SOS2 BRAF	12q24.13 2p22.1 3p25.2 12p12.1 1p13.2 1q22 10q25.2 14q21.3 7q34	Dysplastic PVS, ASD, TOF, AVSD, HCM, VSD, PDA	75	Short stature, hypertelorism, down-slanting palpebral fissures, ptosis, low posterior hairline, pectus deformity, bleeding disorder, chylothorax, cryptorchidism	128
VACTERL association	Unknown		VSD, ASD, HLHS, PDA, TGA, TOF, TA	53–80	Vertebral anomalies, anal atresia, tracheoesophageal fistula, renal anomalies, radial dysplasia, thumb hypoplasia, single umbilical artery	129
Williams-Beuren	7q11.23 deletion (ELN)	7q11.23	SVAS, PAS, VSD, ASD	80	Unusual facies, thick lips, strabismus, stellate iris pattern, intellectual disability	130
Frequently associated						
Carpenter	RAB23	6p11.2	VSD, ASD, PDA, PS, TOF, TGA	50	Craniosynostosis, brachydactyly, syndactyly, polydactyly, obesity	131
Coffin-Siris	ARID1B SMARCB1 ARID1A SMARCB1 SMARCA4 SMARCE1	6q25 22q11 1p36.1 22q11.23 19p13.2 17q21.2	ASD, AVSD, VSD, MR, PDA, PS, DEX, AS	20–44	Developmental delay, coarse facies, hypoplastic distal phalanges, short stature, intellectual disability	132, 133

(Continued)

Table 5. Continued

Syndrome	Gene(s)	Loci	Cardiac Disease	% Congenital HD	Other Clinical Findings	References
Frequently associated (Continued)						
Cornelia deLange	NIPBL SMC1L1 SMC3	5p13 Xp11.22 10q25	PVS, VSD, ASD, PDA	33	Microbrachycephaly, synophrys, arching eyebrows, growth retardation, intellectual disability, micromelia	134
Goldenhar	Unknown		VSD, PDA, TOF, CoA, conotruncal defects	32	Hemifacial microsomia, epibulbar dermoids, microtia, hemivertebrae	135
Mowat-Wilson	ZEB2	2q22.3	VSD, CoA, ASD, PDA, PAS	54	Short stature, microcephaly, Hirschsprung disease, intellectual disability, seizures	136, 137
Rubinstein-Taybi	CBP EP300	16p13.3 22q13.2	PDA, VSD, ASD, HLHS, BAV	33	Microcephaly, growth retardation, down-slanting palpebral fissures, low-set malformed ears, prominent or beaked nose, intellectual disability, broad thumbs and toes	138
Smith-Lemli-Opitz	DHCR7	11q12-13	AVSD, HLHS, ASD, PDA, VSD	50	Microcephaly, ptosis, genital anomalies, renal anomalies, broad nasal tip with anteverted nostrils, intellectual disability, syndactyly	139
Occasionally associated						
Adams-Oliver	ARHGAP31 DOCK6 RBPJ EOGT NOTCH1 DLL4	3q13 19p13.2 4p15.2 3p14.1 9q34.3 15q15.1	ASD, VSD, CoA, HLHS, DORV	20	Aplasia cutis congenita, terminal transverse defects of hands, fingers, toes, feet	140, 141
Baller-Gerold	RECQL4	8q24.3	VSD, TOF, subaortic disease	25	Craniosynostosis, micrognathia, small mouth, radial aplasia/hypoplasia, imperforate anus, renal anomalies	142
Beckwith-Wiedemann	CDKNIC	11p15.4	VSD, HLHS, PS	6.5	Macrosomia, macroglossia, omphalocele, risk of malignancy	143
Coffin-Lowry	RSK2	Xp22.2	LVNC, MVP, AVA	5–14	Growth deficiency, coarse facies, everted lower lip, hypodontia, intellectual disability	144
Duane-radial ray (Okihiro)	SALL4	20q13.2	ASD, PVS, VSD	<10	Unilateral or bilateral Duane anomaly, hypoplasia of thumbs, hypoplastic radius and ulna, renal malformations, ear anomalies	145
Fragile X	FMR1	Xq27.3	MVP, aortic dilation	<10	Macrocephaly, intellectual disability, hand flapping, speech abnormality, autism spectrum disorder, macroorchidism, seizures, prominent forehead, large ears	146
Nance-Horan	NHS	Xp22.13	TOF, VSD, PDA	<10	Congenital cataracts, strabismus, peg-shaped supernumerary teeth, other dental anomalies, prominent ears, brachymetacarpalia	147
Peter's Plus	B3GALTL	13q12.3	ASD, VSD, PVS, BPV, subvalvular AS	<30	Short limb growth deficiency, intellectual disability, autism spectrum disorder, prominent forehead, cupid's bow upper lip, cleft lip ± cleft palate, Peter's anomaly, cataracts, hydronephrosis	148,149
Roberts	ESC02	8p21.1	ASD, AS	<20	Growth deficiency of prenatal onset, cleft lip ± cleft palate, hypertelorism, sparse hair, hypomelia with variable limb reduction defects, cryptorchidism	150
Robinow	RDR2 (AR) WNT5A (AD)	9q22	RVOTO	29 AD 13 AR	Macrocephaly, frontal bossing, prominent eyes, small upturned nose, short forearms, hemivertebrae, hypoplastic phalanges of hands and toes, hypoplastic genitalia	151, 152

(Continued)

Table 5. Continued

Syndrome	Gene(s)	Loci	Cardiac Disease	% Congenital HD	Other Clinical Findings	References
Occasionally associated (Continued)						
Saethre-Chotzen	TWIST	7p21p22	VSD	<10	Craniosynostosis, brachycephaly, high flat forehead, hypertelorism, ptosis, partial cutaneous syndactyly, broad great toes, strabismus	153
Short rib polydactyly type I	DYNC2H1	11q22.3	TGA, DORV, DOLV, AVSD, HRH	<25	Short stature, postaxial polydactyly of hands or feet, short horizontal ribs, small iliac bones, polycystic kidneys, early death from respiratory insufficiency	154
Simpson-Golabi-Behmel	GPC3	Xq26.2	TGA, VSD, PVS, CoA, AS, PDA, BAV, CM	26	Macrosomia, coarse face, macroglossia, hepatosplenomegaly, nephromegaly, variable cognitive disability	155, 156
Sotos	NSD1	5q35.3	ASD, PDA, VSD	21	Excessive size, large hands and feet, prominent forehead, hypotonia, variable intellectual disability, scoliosis, advanced bone age	157
Townes-Brocks	SALL1	16p12.1	ASD, TOF, VSD, TA, PA, PDA	14–25	Auricular anomalies, preauricular tags, hearing loss, thumb hypoplasia/polydactyly, imperforate anus, renal agenesis, multicystic kidney, microphthalmia	158

22q11.2DS indicates 22q11.2 deletion syndrome; AD, autosomal dominant; AR, autosomal recessive; AS, aortic stenosis; ASD, atrial septal defect; AVA, aortic valve anomaly; AVSD, atrioventricular septal defect; BAV, bicuspid aortic valve; BPV, bicuspid pulmonary valve; CFC, cardiofaciocutaneous; CHARGE, coloboma, heart defects, choanal atresia, retarded growth and development, genital anomalies, and ear anomalies; CM, cardiomyopathy; CoA, coarctation of the aorta; DEX, dextrocardia; DOLV, double-outlet left ventricle; DORV, double-outlet right ventricle; HCM, hypertrophic cardiomyopathy; HD, heart disease; HLHS, hypoplastic left heart; HRH, hypoplastic right heart; IAA, interruption of aortic arch; LVNC, left ventricular noncompaction; MR, mitral regurgitation; MVP, mitral valve prolapse; OFD, oral-facial-digital; PA, pulmonary atresia; PAS, pulmonary artery stenosis; PDA, patent ductus arteriosus; PE, pericardial effusion; PPS, peripheral pulmonary stenosis; PS, pulmonary stenosis; PVS, pulmonary stenosis; RVOTO, right ventricular outflow tract obstruction; SVAS, supravalvular aortic stenosis; TA, truncus arteriosus; TGA, transposition of great arteries; TOF, tetralogy of Fallot; VACTERL, association of vertebral defects, anal atresia, cardiac defects, tracheoesophageal fistula, renal and limb anomalies; VR, vascular ring; and VSD, ventricular septal defect.

Prevalence
There is no known racial or ethnic predilection for ALGS. There is an estimated incidence of 1:30 000 to 1:50 000 live births.[159,165]

Molecular Genetics
Pathogenic variants in JAG1 cause >90% of ALGS, with 89% attributable to sequence variants and 5% to 7% attributable to partial or complete gene deletions.[159] An estimated 1% to 2% of individuals who meet clinical criteria for ALGS and do not have a JAG1 mutation will have a NOTCH2 sequence variant.[115] The Jagged1 and Notch proteins are part of the Notch signaling pathway, which is important to regulation of cell fate in many cell types during development.[159]

Cardiovascular Genotype/Phenotype Correlations
Overall, there are no differences in the cardiovascular phenotype based on causative gene or mutation type (sequence variant versus deletion). However, there have been 2 variants reported for which affected individuals had cardiac but not liver disease, and further analysis demonstrated that the amount of JAG1 protein produced was more than in other ALGS variants but less than in wild type.[166] Because the characteristic facial features can be subtle and the presentation variable, it is important to consider a diagnosis of ALGS in those with characteristic cardiovascular findings, even in the absence of overt liver disease.

Holt-Oram Syndrome
Holt-Oram syndrome (HOS) is an autosomal dominant disorder often referred to as heart-hand syndrome because of the 2 most common features: congenital HD and radial ray defects.

Common Features
Orthopedic
Radial ray abnormalities can be unilateral or bilateral and, when bilateral, can be symmetrical or asymmetrical. The penetrance of upper limb anomalies in HOS is complete but ranges from subtle carpal abnormalities without functional consequence only seen by radiogram to complete phocomelia (the hand attached close to the trunk). Other reported abnormalities include triphalangeal thumb, absent thumb, radius hypoplasia or aplasia, and radioulnar synostosis.[167,168]

Family History
Because there is considerable intrafamilial phenotypic variability, a family history of a first-degree relative with a septal defect, cardiac conduction disease, or radial ray abnormality can provide a clue to the diagnosis.

Cardiac Features
Three quarters of those with HOS have congenital HD, most commonly involving the atrial or ventricular septum. ASDs can present as common atrium, often with

atrial isomerism.[169] Cardiac conduction disease is seen in those with or without congenital HD. Sinus bradycardia, first-degree atrioventricular heart block, and complete heart block with or without atrial fibrillation are all reported coincident with or subsequent to the time of congenital HD diagnosis (if present).[167] This has led to the recommendation that all individuals with HOS have an annual screening ECG.

Prevalence
HOS has an estimated prevalence of between 0.7 and 1 per 100 000.[169]

Molecular Genetics
Seventy percent of cases are caused by a heterozygous pathogenic variant in *TBX5,* <1% by a partial or complete gene deletion.[125] Most variants result in a null allele and haploinsufficiency. *TBX5* is a transcription factor and is an essential regulator of limb and cardiac development, particularly the cardiac septum and conduction system.[168]

Cardiovascular Genotype/Phenotype Correlations
Pathogenic missense variants at the 5′end of the T-box are associated with more serious cardiac defects.[170]

Char Syndrome
Char syndrome is an autosomal dominant familial PDA syndrome.

Common Features
Recognizable Facial Features
Flat midface, flat nasal bridge, broad nasal tip, hypertelorism, down-slanting palpebral fissures, mild ptosis, short philtrum, and everted lips are among the recognizable facial features.[171]

Orthopedic
Aplasia or hypoplasia of the middle phalanges of the fifth fingers is part of the diagnostic triad (along with typical facial features and PDA) of Char syndrome.[172]

Other
Case reports indicate a number of additional features can be seen in Char syndrome, including hypodontia, foot anomalies (joint fusion, clinodactyly, polydactyly, syndactyly), strabismus, and other hand anomalies (interstitial polydactyly, distal symphalangism of the fifth fingers, and third finger hypoplasia).[172]

Cardiac Features
The primary cardiac finding is PDA. Other heart defects, including VSD and more complex congenital HDs, have been reported.[172]

Prevalence
The prevalence has not been determined, but it is thought to be quite rare.

Molecular Genetics
Approximately half of families who have the diagnostic triad of Char syndrome (recognizable facial features, aplasia or hypoplasia of the middle phalanges of the fifth fingers, and PDA) will have a heterozygous pathogenic variant in *TFAP2B*.[119] The majority of mutations affect the highly conserved C-terminal half of the protein's basic domain that is essential for DNA binding.

Cardiovascular Genotype/Phenotype Correlations
There are no known genotype-phenotype correlations with regard to cardiovascular complications. There have been reports of *TFAP2B* pathogenic variants in cases of PDA without the facial or orthopedic findings of Char syndrome.[173]

Ellis-van Creveld Syndrome
Ellis-van Creveld syndrome (EVC) is an autosomal recessive skeletal dysplasia associated with a characteristic cardiac finding of a primary atrial septation defect resulting in a common atrium.[123]

Common Features
Recognizable Facial Features
Individuals with EVC can have a characteristic appearance of the mouth, with a short upper lip bound by frenula to the alveolar ridge.

Dental
A variety of dental abnormalities are reported, including natal teeth, partial adontia, small teeth, delayed tooth eruption, conical teeth, and enamel hypoplasia.[174,175]

Hair and Nails
The nails are often hypoplastic, and hair can be scant or fine.[176]

Growth
There is prenatal-onset short stature; adult stature is in the range of 43 to 60 inches.[177]

Orthopedic
The characteristic skeletal findings include postaxial polydactyly, usually of the hands, short limbs (with increasing severity from the proximal to distal portions of the limbs), and short ribs. Hand radiographs often show short, broad middle phalanges and hypoplastic distal phalanges, and sometimes carpal bone abnormalities.[175] Bone age is usually delayed.

Cardiac Features
It has been estimated that 50% to 60% of cases have congenital HD, characteristically common atrium. Abnormalities of the mitral and tricuspid valves, PDA, VSD, and HLHS are also reported.[178] The severity of the congenital HD is the main determinant of morbidity and mortality.[175]

Prevalence

The worldwide prevalence is not known. There is a founder mutation among the Amish, and large kindreds from Mexico, Ecuador, and Brazil that have also been reported.[123]

Molecular Genetics

Two-thirds of cases of EVC are caused by homozygous or compound heterozygous mutations in *EVC* or *EVC2*.[123,179] These 2 genes are in a 5′ to 5′ (head-to-head) orientation in close proximity and are thought to share a common, bidirectional promoter.[180]

Cardiovascular Genotype/Phenotype Correlations

No cardiovascular genotype-phenotype correlations have been reported.

Adams-Oliver Syndrome

Adams-Oliver syndrome (AOS) is an inherited malformation syndrome in which cardiac, scalp, and limb defects are present. There is genetic heterogeneity, with both autosomal dominant and autosomal recessive forms.

Common Features

Skin and Scalp Defects

There is considerable variability in the extent of aplasia cutis congenita in affected individuals, ranging from total absence of an area of scalp skin and skull to vertex hairless patches.

Orthopedic

Limb defects can include terminal transverse reduction defects of hands or feet, short distal phalanges, syndactyly, ectrodactyly, and polydactyly.

Central Nervous System

Brain anomalies occur in 35% of affected individuals, including microcephaly, encephalocele, neuronal migration anomalies, thin or absent corpus callosum, and enlarged ventricles. Some evidence of vascular sequelae in the brain has been documented, with calcification, periventricular leukomalacia, and stroke/thrombosis.[140]

Ophthalmologic

Ophthalmologic findings are uncommon in AOS but do occur, including retinal folds/detachment, cataract, and optic nerve hypoplasia.[140]

Cardiovascular Features

One-fourth of individuals with AOS have congenital HD, often left-sided obstructive malformations such as BAV, aortic stenosis, HLHS, coarctation of the aorta, and parachute mitral valve. Other defects include VSD, ASD, tetralogy of Fallot, and other conotruncal defects.[181] Vascular lesions such as hepatic thrombosis, PVS,[141,182] intracranial vascular lesions, and limb vascular anomalies have also been described.

Prevalence

There is an estimated incidence of 0.44 per 100 000 live births.[183] This could be a significant underestimate, because not all clinical features are present in all affected individuals.

Molecular Genetics

Six genes are currently involved in the pathogenesis of AOS. Two genes, *ARHGAP31* (autosomal dominant gain-of-function mutations) and *DOCK6* (homozygous loss-of-function mutations), are part of the CDC42/RAC1 pathway. The other 4 genes (*RBPJ*, *EGOT*, *NOTCH1*, and *DLL4*) are part of the Notch signaling pathway, which regulates cell fate in many cell types.[140]

Cardiovascular Genotype/Phenotype Correlation

Pathogenic variants of *NOTCH1* are associated with a 42% occurrence of congenital HD and vasculopathy in AOS.[141,184] The other 5 genes associated with AOS do not have as frequent vasculopathies or cardiac malformations. Additionally, *NOTCH1* gene variants have been associated with autosomal dominant left ventricular outflow defects (most commonly BAV and calcific aortic stenosis) without evidence of AOS[185] and familial BAV without evidence of AOS.[186]

Kabuki Syndrome

Kabuki syndrome (KS) has both X-linked and autosomal dominant pathogeneses. The syndrome is characterized by specific facial features, skeletal anomalies, congenital HD, renal anomalies, intellectual disability, and growth deficiency.

Common Features

Recognizable Facial Features

Children with KS have long palpebral fissures, eversion of lateral one-third of the lower eyelid, arched eyebrows with sparse lateral third, large dysplastic ears, cleft palate, and depressed nasal tip.[187]

Development

Mild to moderate intellectual disability is seen in 82% to 90% of individuals, with one-third having expressive and receptive language difficulties. There can be associated autism spectrum disorder, communication difficulties, and repetitive behavior.

Orthopedic

More than 80% have skeletal findings such as vertebral anomalies, scoliosis, hip dislocation, short incurved fifth fingers, brachydactyly, and hyperextensible joints.[188]

Urogenital Anomalies

Cryptorchidism, duplicated collecting system, single fused kidney, and hypospadias have been reported.[188]

Other

Growth deficiency is present in 55% and hearing loss in 28% to 40%, with immunodeficiency and persistent fetal finger pads also common.[189]

Cardiovascular Features

Congenital HD occurs in 40% to 70% of individuals with KS.[126,127] There is a predominance of left-sided obstructive defects, including coarctation of the aorta, BAV, and HLHS.[190] The most common cardiac malformations are coarctation of the aorta, ASD, and VSD. Other defects include double-outlet right ventricle, pulmonary stenosis, mitral atresia/stenosis, and partial anomalous pulmonary venous return.[191–193]

Prevalence

The prevalence in Japan was estimated at 1:32 000,[194] as the initial patients described were Japanese. A minimum birth incidence of 1:86 000 in Australia and New Zealand has been calculated.[195]

Molecular Genetics

KS is caused mainly by pathogenic variants in the *KM-T2D* (*MLL2*) and *KDM6A* genes. Only a few cases with a Kabuki-like phenotype have been described with *RAP1A* and *RAP1B* and *HNKRNPK* variants.[196] Germline dominant, usually truncating, variants in *KMT2D* cause most cases of KS (75%).[197] Pathogenic variants in the X-linked gene *KDM6A* are found in ≈5% of individuals with KS, which are also generally truncating variants but with a few whole-gene deletions described.[198] It has been noticed that KS and CHARGE have some overlapping clinical features. Additionally, there are a number of patients with a Kabuki-like syndrome who are under investigation for related genes in the CHARGE/Kabuki spectrum, as well as heterogeneous nuclear ribonucleoprotein K (*HNRNPK*) haploinsufficiency.[199] Furthermore, a *KMT2D* novel variant has been found in 2 generations of a family with choanal atresia, which also suggests a connection to CHARGE syndrome.[200]

Cardiovascular Genotype/Phenotype Correlations

A preponderance of males compared with females with left-sided obstructive lesions has been described among *KMT2D* patients.[196] Those with *KDM6A* mutations have a frequency of congenital HD of 45%, with a higher prevalence of right-sided congenital HD.[196]

CHARGE Syndrome

The acronymic name of this condition includes C for coloboma, H for heart defects, A for choanal atresia, R for retarded growth and development, G for genital anomalies, and E for ear anomalies. CHARGE syndrome is inherited as an autosomal dominant condition, but it is usually sporadic. Diagnostic criteria are helpful in determining a clinical diagnosis for individuals suspected of having CHARGE syndrome.[121,201]

Common Features

Recognizable Facial Features

Characteristic facial features can include orofacial clefts, unilateral or bilateral facial palsy, and malformed protruding ear pinnae.

Development

Marked developmental delay is usual. Motor skills are delayed with hypotonia. Delayed language development can result from hearing loss and reduced vision. The intellectual outcome is variable, with up to 50% with good outcome.[202] If microcephaly, brain malformations, and extensive coloboma are present, these suggest a poorer intellectual outcome.

Ophthalmologic

More than 80% to 90% of individuals will have unilateral or bilateral colobomas variably affecting the iris, retina, choroid, or optic discs, sometimes associated with microphthalmia. Vision is variably affected by the colobomas.

Ears and Hearing

Malformed external ear pinnae, anomalies of the ossicles, Mondini cochlea defect, and absent or small semicircular canals are present in >90% of affected individuals. Hearing loss is extremely common and varies from mild to profound.[203,204]

Respiratory

Bilateral or unilateral choanal atresia is present in >50%, necessitating immediate evaluation and possibly tracheostomy.

Gastrointestinal

Individuals can have severe swallowing difficulties, aspiration problems, gastroesophageal reflux, and tracheoesophageal fistula. Feeding problems are very common, and gastrostomy feeding measures are often required.[204]

Genital/Renal

Males can have cryptorchidism and micropenis, and both males and females can have hypogonadotropic hypogonadism.[203] Occasional renal anomalies such as horseshoe kidney and renal dysgenesis are found.

Orthopedic

Hand anomalies including polydactyly and occasional scoliosis are possible.

Cardiovascular Features

Three-fourths of those with CHARGE have congenital HD, and these are often complex.[205] These can be conotruncal defects, including tetralogy of Fallot, IAA, truncus arteriosus, and double-outlet right ventricle. Multiple other abnormalities have been seen in CHARGE, including vascular rings, aortic arch anomalies, AVSD, septal defects, and PDA.[206]

Prevalence

CHARGE syndrome occurs in 1 in 8500 births.[207]

Molecular Genetics

CHARGE syndrome is caused by pathogenic variants in the *CHD7* gene in the majority of individuals suspected clinically of having the syndrome.[203] A few rare instances of exon, whole gene, or large contiguous deletions have been found.[208] Most affected individuals are the only family member with clinical findings; however, some familial cases of CHARGE syndrome have been described.[209] Many individuals with familial *CHD7* variants are mildly affected and do not completely fulfill the diagnostic criteria for CHARGE syndrome.[210] Some of these more mildly affected individuals were only recognized after a more seriously affected family member was found to have a *CHD7* pathogenic variant. Some families in which there are affected siblings and unaffected parents are likely examples of gonadal mosaicism. In recent years, marked overlap in clinical features with CHARGE syndrome has been seen in individuals eventually discovered to have KS, 22q11.2 deletion, or Kallmann syndrome.[200,210]

Cardiovascular Genotype/Phenotype Correlations

Congenital HD is more commonly found in individuals with truncating variants of *CHD7* (80%) than with missense or splice-site variants (58%).[205]

THE RASOPATHIES

The RASopathies are a group of autosomal dominant disorders with overlapping cardiac, growth, facial, and neurodevelopmental features. They are so named because they are caused by pathogenic variants in genes that encode proteins in or with close interaction with the RAS/mitogen-activated protein kinase pathway, which plays an important role in cellular programs, including apoptosis, development, differentiation, proliferation, and transformation. Somatic mutations in genes in the pathway have long been known to cause hematologic cancers and solid tumors. More recently, germline sequence variants have been found to cause Noonan syndrome (NS) and other uncommon phenotypically related disorders, including cardiofaciocutaneous syndrome (CFC), Costello syndrome (CS), and Noonan syndrome with multiple lentigines (NSML). Collectively, these disorders are termed the *RASopathies*. Although it has more unique features than it has overlapping features to NS, CFC, CS, and NSML, neurofibromatosis type 1 is often included as a RASopathy. Because it infrequently presents with significant cardiac complications (2% of cases), neurofibromatosis type 1 will not be further discussed here.[211]

Noonan Syndrome

Individuals with NS have characteristic facial features and structural and functional abnormalities involving multiple organ syndromes and a high incidence of cardiac abnormalities.

Common Features

Recognizable Facial Features

The facial features of NS change with age.[212] They can be subtle during infancy (tall forehead, widely spaced prominent eyes that slant down, depressed nasal bridge, bulbous nasal tip, and low-set ears), more obvious during childhood (ptosis and neck webbing also seen), and change again during adolescence (eyes less prominent, nasal root pinched with a thin bridge, and the shape of the face is that of an inverted triangle). In adulthood, the features are most often mild, although some adults retain significant, readily recognizable dysmorphisms.

Eye and Ear

An estimated 80% of those with NS have a structural eye abnormality, including ptosis (50%), strabismus (40%), refractive error (60%), posterior segment abnormalities (6%), and anterior segment abnormalities (60%).[213] A minority have conductive hearing loss attributable to middle ear effusion (20%) or sensorineural hearing loss (10%).[214]

Gastrointestinal

Early feeding problems related to hypotonia and delayed gastrointestinal motor development, gastroesophageal reflux, chronic constipation, and intestinal malrotation respond well to medical management and feeding therapy.[215] It is not uncommon for early feeding issues to be significant enough to require temporary placement of a gastrostomy feeding tube.

Growth and Endocrine

The most common endocrine complications include hypothyroidism, pubertal delay, and short stature. The pathogenesis for the short stature can be nutritional, attributable to growth hormone deficiency, or attributable to growth hormone insensitivity.[216] Short stature in NS is a Food and Drug Administration–approved indication for growth hormone therapy. Looking across studies, there is a mean gain in height of 9.5 to 13 cm for boys and 9 to 9.8 cm for girls with growth hormone therapy.[128] Treatment outcomes are best with earlier initiation and longer duration.[217] To date, there is no evidence that treatment with growth hormone exacerbates the cardiac complications of NS.[128]

Hematology

Coagulation factor deficiencies, thrombocytopenia, and platelet aggregation abnormalities have all been reported[218]; however, only a small proportion of those with abnormalities on coagulation testing have functional bleeding problems. The observed rate of postoperative bleeding complications in a cohort of 142 individuals with NS was <2% (half of the cohort had been screened preoperatively for a coagulopathy and half had not).[219]

NS attributable to *PTPN11* mutations is associated with an increased risk of hematologic malignancies, in-

cluding acute lymphoblastic leukemia and juvenile myelomonocytic leukemia. Myeloproliferative disorders are also more common in NS than in the general pediatric population and are associated with a benign course in 40% and an aggressive course in 15%.[220]

Lymphatic

Lymphatic abnormalities are thought to affect ≈20% of individuals.[221] Peripheral edema can be seen during infancy and usually regresses during the first year. It can occur or recur in adolescence or adulthood. Chylous effusion is a regularly reported complication of cardiac surgery. Less commonly, pulmonary, intestinal, and testicular lymphangiectasia are reported.[222]

Neurological, Cognitive, and Behavioral

Seizures are reported in a minority of cases (10%–13%) and include generalized, temporal lobe, and febrile seizures.[223] Structural brain abnormalities are rare, but there are multiple case reports of symptomatic Chiari I malformation.[224] There are highly variable neurocognitive and behavioral outcomes that depend, in part, on the causative gene. Gross and fine motor development are often delayed because of hypotonia, congenital HD, or orthopedic issues.[225] Although many school-aged children require individualized education plans or special education instruction, intellectual disability is uncommon (6%–23% across studies).[226]

Orthopedic

The most commonly reported orthopedic complications include radioulnar synostosis, pectus carinatum and excavatum, scoliosis, and pes planus.[227]

Renal and Genitourinary

Renal anomalies, including vesicoureteral reflux, hydronephrosis, and dysplastic kidney, are seen in 10% to 20% of individuals.[128] The majority of males have cryptorchidism (80%).

Cardiac Features

Cardiovascular involvement is observed in 80% to 90% of affected individuals, comprising congenital HD and hypertrophic cardiomyopathy (HCM).[222,228] The most common congenital HD is PVS, seen in roughly 40% of patients and often with dysplastic valve leaflets, but other prevalent lesions include aortic coarctation, mitral valve anomalies, ASDs, and tetralogy of Fallot. HCM, seen in up to 20% of patients, can vary from a mild, stable form, typically presenting in toddlers, to a severe, rapidly progressive form that presents in early infancy and is often life-threatening.[229] Arterial defects such as aneurysms (coronary, aortic, pulmonary, intracranial) and coronary atresia have also been observed.[230,231]

Prevalence

Systematic epidemiological studies have not been completed, but a prevalence of 1:1000 to 1:2500 has been estimated.[232]

Molecular Genetics

NS is an autosomal dominant disorder with complete penetrance and variable expressivity. Fifty percent of cases are explained by heterozygous *PTPN11* missense pathologic variants.[233] *PTPN11* encodes SHP2, a phosphatase that has an active and inactive conformation. Pathogenic variants alter residues that stabilize the inactive state, activating SHP2 and leading to increased RAS/ERK (extracellular signal-regulated kinase)/MAPK (mitogen-activated protein kinase) activation.[234] It is estimated that an additional 30% of cases can be explained by a variant in one of multiple genes in the RAS MAPK pathway including *SOS1*, *RAF1*, *RIT1*, *KRAS*, *SHOC2*, *NRAS*, *SOS2*, and *BRAF*.[212] Case reports or small case series implicate other genes in the pathway, including *A2ML1*, *LZTR1*, *MYST4*, *RASA2*, *RRAS*, *SPRY1*, and *SYNGAP1*.[235] Approximately half of the cases are de novo, and the other half are inherited. In the nonfamilial cases, there is an association with advanced paternal age.[236]

Cardiovascular Genotype/Phenotype Correlations

PTPN11-associated NS is more likely to cause PVS and less likely to cause HCM.[234] Septal defects are more common in those with *SOS1*-associated NS.[237] Although only ≈20% of patients with NS have HCM, 95% of those with a *RAF1* mutation and 75% with a *RIT1* mutation have it.[238,239]

Other RASopathies

CFC, CS, and NSML are among the other RASopathies. They share common features, including developmental delays, short stature, ptosis, hypertelorism, macrocephaly, and cardiac involvement. There is such significant phenotypic overlap that it can be challenging, particularly during infancy, to make the diagnosis based on clinical features alone.

Common Features

Cardiofaciocutaneous Syndrome

In contrast to NS, CFC is characterized by more significant feeding issues (often requiring long-term gastrostomy tube use) and cognitive delays (with the majority of individuals in the mild to moderate intellectual disability range) and by a variety of cutaneous abnormalities, including hyperkeratosis, ichthyosis, keratosis pilaris, ulerythema ophryogenes, and xerosis.[240] CFC is considerably rarer than NS, with an estimated prevalence of 1 in 810 000.[241]

Costello Syndrome

Features most often seen in those with CS but not in NS include coarse facial features, loose and soft skin with deep creases of the palms and soles, bronzing of the skin during childhood, papillomata of the face and perianal region, and an increased risk for malignant tumors.[242] Like NS and CFC, feeding issues are common

and often require use of a gastrostomy tube. Intellectual disabilities are much more common than in NS but less severe than those seen in CFC. CS has an estimated birth prevalence of 1 in 300 000 to 1 in 1 200 000.[241]

NS With Multiple Lentigines

NSML (formerly known as LEOPARD syndrome) has the unique finding of multiple lentigines of the face, back, and upper trunk that number in the thousands by adulthood. There is a higher prevalence of sensorineural hearing loss and a lower prevalence of short stature than is reported in NS.[243] The overall prevalence of NSML is unknown. Mild learning issues are reported in ≈30% of individuals, but intellectual disability is rare.[244]

Cardiac Features

Three-fourths of individuals with a RASopathy have a cardiac abnormality, which is the second most common reason a child comes to medical attention (after admission to a neonatal intensive care unit).[245] Although there are a wide variety of cardiovascular diagnoses reported, PVS, HCM, and ASD are the most common complications reported in each of the RASopathies. The majority of individuals with NSML have HCM compared with only one-fifth of those with NS.[246] CS can be complicated by arrhythmia, usually supraventricular tachycardia, and most distinctive is chaotic atrial rhythm/multifocal atrial tachycardia.[242]

Molecular Genetics

The majority of cases of CFC are caused by a heterozygous pathogenic variant in *BRAF*, *MAP2K1*, *MAP2K2*, or *KRAS*. *HRAS* is the only gene in which pathogenic variants are known to cause CS, and >95% of variants affect amino acid p.Gly[12] or p.Gly[13].[247] Ninety percent of NSML cases are caused by loss-of-function variants in *PTPN11* that impair SHP2 catalytic activity.[248] Less than 5% of NSML cases have been ascribed to a heterozygous pathogenic variant in *RAF1*, *BRAF*, or *MAP2K1*.

Clinical Genetic Testing

Potential approaches include single-gene testing, multigene panel testing, and more comprehensive genomic approaches such as whole-exome or whole-genome sequencing. Because there is significant genetic heterogeneity for a given diagnosis and extensive phenotypic overlap between diagnoses, multigene panel testing that includes all of the RASopathy genes is likely the most cost-effective and clinically indicated approach. A molecular genetic diagnosis allows for specific prognosis, anticipatory guidance, and recurrence risk estimates for families.

Suggested Cardiac Follow-up

If not completed already, an evaluation with a cardiologist, including an echocardiogram and ECG, is indicated at the time of diagnosis. Follow-up is tailored to the individual findings, ideally informed by the spectrum of disease and natural history of cardiac abnormalities in the RASopathies. If no cardiac disease is detected, repeat evaluation with a cardiologist is indicated every 5 years throughout childhood and adulthood.[128,222]

HETEROTAXY AND CILIOPATHIES

Cilia Structure and Function

Cilia are ancient organelles with a broad range of biological functions that center on sending and receiving signals to and from the extracellular environment. Abnormal cilia structure and function result in diverse diseases, including syndromic ciliopathies, primary ciliary dyskinesia (PCD), and heterotaxy syndrome.[249] Finally, there is growing evidence that abnormal function of cilia can result in isolated congenital HD.[250,251]

All cilia extend from a basal body and contain doublet microtubules (Figure [C]). These doublets extend as the axoneme, a highly ordered arrangement that is recognizable by transmission electron microscopy. Motile cilia are primarily found on epithelial cells that line the respiratory tract, brain ventricles, and oviducts. These cilia function to propel cells or extracellular fluid and are characterized by 9 microtubule doublets surrounding a central pair, a "9+2" arrangement that is visible by electron microscopy of cross-sectional views (Figure [A]). Absence or dysfunction of motile cilia causes PCD. A special subtype of motile cilia are the cilia found on the left-right organizer (LRO) that have a "9+0" arrangement but are motile. In contrast, sensory cilia have a "9+0" arrangement of microtubules (Figure [B]) but are nonmotile cilia. These cilia extend from the surface of almost all cell types in the human body, including epithelial cells lining the kidney tubules and bile ducts, as well as nonepithelial cells such as chondrocytes and neurons.[252] These cilia are responsible for sensing the extracellular environment, are important for transducing signals, and play a role in intercellular signaling during developmental patterning.

Cilia in Heart Development

The best understood role for cilia in heart development is establishing left-right (LR) asymmetry. The heart has striking asymmetries along the LR axis, all of which depend on global LR positional cues that originate from a ciliated LRO early in development, before cardiac morphogenesis. The LRO is highly conserved among vertebrates[253] and functions through motile LRO cilia to generate directional fluid flow.[254] Sensing this flow requires functional polycystin channels localized to the LRO sensory cilia.[255,256] Activation of the sensory LRO cilia leads to LR asymmetrical gene expression of genes including *cerl2*, *nodal*, *lefty*, and *pitx2*. The precise mechanisms that connect asymmetrical signals to heart development remain unknown.
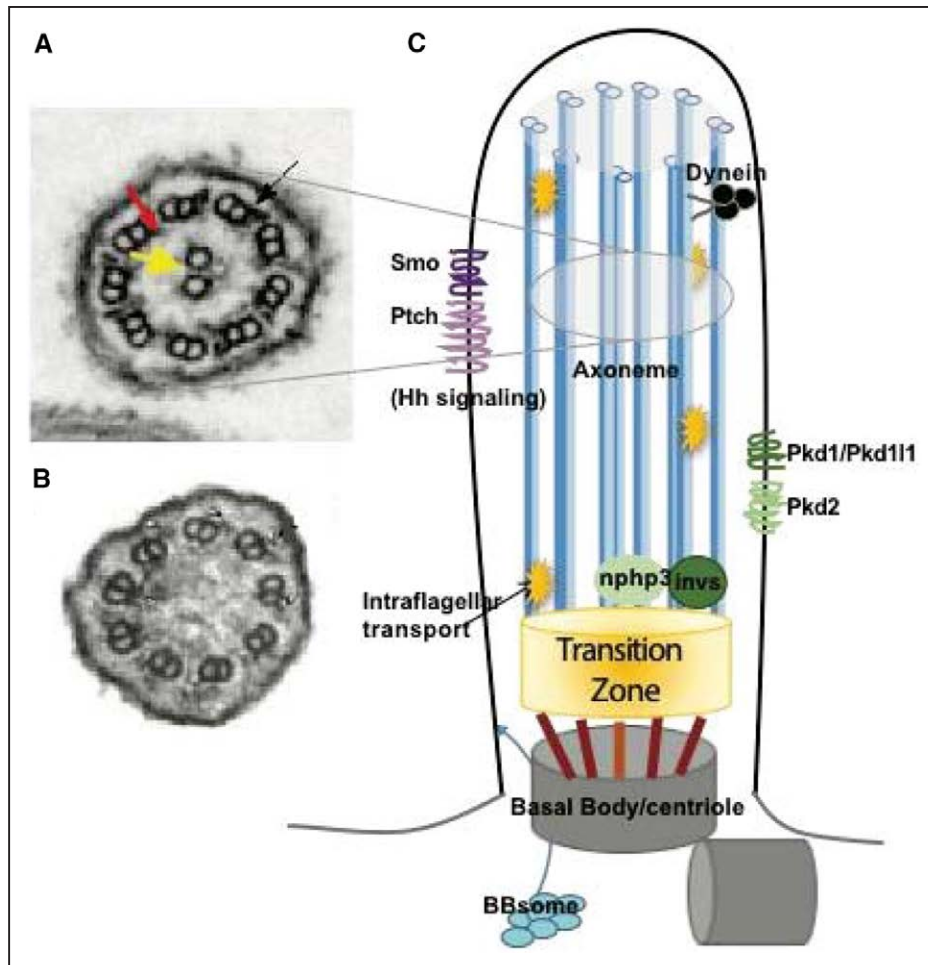
Figure. Ciliary structure.
A, Transmission electron microgram (TEM) cross section of a 9+2 motile cilium. The central pair is indicated by the yellow arrowhead. Outer dynein arms (black arrow) and inner dynein arms (red arrow) are shown linking the 9 sets of microtubule doublets. Location in the ciliary axoneme in (**C**) is indicated. **B**, TEM of a 9+0 sensory cilium; note the absence of central pair. **C,** Diagrammatic representation of a cilium indicating structures that have been linked to congenital heart disease. Hh indicates hedgehog.

Finally, asymmetrical left-sided signals are constrained by a midline barrier.[249,257] This framework predicts relationships between gene variants that result in abnormal cilia function and the specific associated cardiac laterality defects (Table 6).

Beyond their role at the LRO in establishing LR asymmetry, cilia are found in cardiac tissue, including the second heart field, where cilia are required for signaling via the sonic hedgehog pathway. Mouse and human mutations affecting ciliary hedgehog signaling, including genes important for syndromic ciliopathies such as *MKS1* (Meckel-Gruber syndrome type 1, Bardet-Biedl syndrome type 13),[250] *MKKS* (Bardet-Biedl syndrome type 6, McKusick-Kaufman syndrome),[258] and *EVC* and *EVC2* (Ellis-van Creveld syndrome)[123,259,260] lead to atrioventricular canal defects without accompanying laterality defects. Cilia are also found on embryonic myocardial cells, in mesenchymal cells in the developing AV valves, and in the developing vasculature.[261,262] It is possible that in these settings, they could function to integrate mechanical signals such as those generated by cardiac contraction or blood flow with cardiovascular development.

Heterotaxy Syndrome

Common Features

Heterotaxy, from the Greek *heteros* (different) and *taxis* (arrangement), refers to any placement of organs along the LR axis that deviates from complete situs solitus and complete situs inversus and includes left atrial isomerism (LAI) and right atrial isomerism (RAI). In LAI, there are 2 "left" sides with 2 left atrial appendages, absent sinus node, and multiple spleens; conversely, in RAI, there are 2 right atrial appendages, bilateral sinus nodes, and absent spleen. In both LAI and RAI, the liver is located at the midline, and abnormal positioning of the gall bladder and stomach is common. Abnormalities of spleen number (asplenia or polysplenia) can result in functional asplenia that requires management. Gut

Table 6.   Summary of Ciliopathies

Ciliopathy	Features	Gene(s)	Cardiac Defects
Primary ciliary dyskinesia	Bronchiectasis, sinusitis, otitis media, infertility, situs defects	AK7, ARMC4, C21orf59, CCDC103, CCDC114, CCDC151, CCDC39, CCDC40, CCDC65, CCNO, DNAAF1, DNAAF2, DNAAF3, DNAAF5, DNAH11, DNAH5, DNAH6, DNAI2, DNAL1, DNAJB13, DRC1, DYX1C1, GAS8, HEATR2, HYDIN, LRRC6, MCIDAS, NME8, PIH1D3, RPGR, TXNDC3, RSPH1, RSPH3, RSPH4A, RSPH9, SPAG1, TTC25, ZMYND10	Dextrocardia; heterotaxy spectrum heart defects in ≈12%; heterotaxy not thought to occur with genes associated with central pair or radial spoke
Polycystic kidney disease	Renal cysts; hepatic fibrosis; autosomal dominant and recessive forms	GANAB, PKHD1, PKD1, PKD2	Reported association with aortic dilation
Nephronophthisis	Renal cysts with or without extrarenal symptoms	NPHP1-4, IQCB1, CEP290, ANKS6, GLIS2, CEP83, CEP164, RPGRIP1L, NEK8, SDCCAG8, TMEM67, TTC21B, DCDC2, IFT172, WDR19, ZNF423	Reported in conjunction with allelic syndromes
Meckel-Gruber syndrome	Renal cysts, CNS anomalies (encephalocele), polydactyly, hepatic fibrosis, congenital heart defects	MKS1, TMEM216, TMEM67, CEP290, RPGRIP1L, CC2D2A, NPHP3, TCTN2, B9D1, B9D2, TMEM231, KIF14, TMEM107	Situs inversus; heterotaxy; HLHS
Joubert and related syndromes	Hypoplasia of the cerebellar vermis (molar tooth sign), dysregulated breathing pattern, retinal dystrophy, renal anomalies	AH1, C5ORF42, CC2D2A, CSPP1, TMEM216, NPHP1, CEP290, TMEM67, RPGRIP1L, INPP5E, TCTN2, MKS1, CEP104, CEP120, CEP41, KIAA0556, PDE6D, PIBF1, TCTN1, TCTN3, ARL13B, CEP41, KIAA0586, TMEM237, TMEM231, TMEM138, KIAA0753, TMEM107, KIF7, OFD1, C2CD3, IFT172, ARL13B, ZNF423, TTC21B, PDE60, POC18, B9D2, B9D1	Laterality defects; heart defects including septal defects, aortic valve anomalies, coarctation; in some cases, associated with features of OFD
Bardet-Biedl syndrome	Obesity, polydactyly, retinitis pigmentosa, anosmia, congenital heart defects	BBS1, 2, ARL6 (BBS3), 4, 5, MKKS (BBS6), 7, TTC8 (BBS8), 9, 10, TRIM32 (BBS11), 12. MKS1, CEP290, WDPCP, SDCCAG8, IFT27, IFT172, LZTFL1, BBIP1, IFT27; modifiers MKS3, CCDC28B	Heart defects with incomplete penetrance (7%–50%); AS, PDA, PS, ASD, VSD, cardiomyopathy
Oral-facial-digital syndromes (types I-XVI and unclassified)	Oral cavity, face, and digit anomalies; CNS abnormalities; cystic kidney disease	OFD1, TMEM216, C5orf42, TMEM107, TCTN3, TMEM231, TMEM138, KIAA0753, SCLT1, C2CD3, DDX59, WDPCP, INTU, TMEM231, IFT57	Mitral and tricuspid valve dysplasia, TOF, VSD, CoA, hypoplastic LV
Alström syndrome	Dilated cardiomyopathy, obesity, sensorineural hearing loss, retinitis pigmentosa, endocrine abnormalities, renal and hepatic disease	ALMS1	Dilated cardiomyopathy
McKusick-Kaufman syndrome	Urogenital anomalies including hydrometrocolpos, postaxial polydactyly, congenital heart defects	MKKS	AVC defects, ASD, VSD, TOF, PDA, hypoplastic LV, LSVC; defects in ≈14%
Ellis van Creveld syndrome	Skeletal dysplasia; congenital heart defects; polydactyly; ectodermal dysplasia	EVC, EVC2	AVC defects, APVR, septal defects
Short rib thoracic dysplasias including Jeune chondrodysplasia, Saldino-Mainzer	Skeletal dysplasia; thoracic deformities; polydactyly; renal cysts; retinitis pigmentosa	IFT80, DYNC2H1, TTC21B, WDR19, NEK1, WDR35, WDR60, IFT140, IFT172, WDR34, CEP120, KIAA0586, DYNC2LI1, IFT52, TCTEX1D2	Rare; septal defects, laterality defects
Cranioectodermal dysplasia (Sensenbrenner syndrome)	Cranioectodermal dysplasia; narrow thorax, dental anomalies, hepatic and renal involvement	IFT122, WDR35, IFT 43, WDR19	PDA, ASD, VSD, PS, LVH in 25%–50%
Carpenter syndrome	Acrocephaly; polysyndactyly, hypogenitalism, obesity, congenital heart defects	RAB23, MEGF8, RAB23	PDA, PS, VSD, situs inversus, heterotaxy

APVR indicates anomalous pulmonary vein return; AS, aortic stenosis; ASD, atrial septal defect; AVC, atrioventricular canal; CNS, central nervous system; CoA, coarctation of the aorta; HLHS, hypoplastic left heart syndrome; LSVC, left superior vena cava; LV, left ventricle; LVH, left ventricular hypertrophy; OFD, oral-facial-digital; PDA, patent ductus arteriosus; PS, pulmonary stenosis; TOF, tetralogy of Fallot; and VSD, ventricular septal defect.

malrotation poses a risk for volvulus. Extrahepatic biliary atresia is a severe extracardiac complication that affects prognosis and mortality rate. Central nervous system abnormalities can also be seen.[263–265] Heterotaxy is also associated with PCD, with one study finding that 37% of heterotaxy patients had features suggesting the possibility of PCD.[266] In a minority of cases, heterotaxy can be identified in patients with other genetic syndromes such as trisomy 13, 22q11.2DS, CHARGE syndrome, or a syndromic ciliopathy. However, the majority of cases of heterotaxy do not occur as part of a larger genetic syndromic condition, and intellectual development is usually normal.

Cardiac Features

Heterotaxy is associated with congenital HD in 50% to 95% of cases; the majority of cases with heterotaxy spectrum including RAI, levocardia with abdominal situs inversus (isolated levocardia), or dextrocardia with abdominal situs solitus (isolated dextrocardia) have significant congenital HD, which leads to major morbidity and mortality.[265] Notably, LAI is not always associated with significant intracardiac defects. Heterotaxy can be associated with almost all known congenital HD. The most prominent cardiac findings are atrioventricular canal defects that are frequently unbalanced and associated with other congenital HD such as malposed great vessels. Right ventricular obstruction and anomalous pulmonary venous return are more commonly observed in RAI, whereas left ventricular obstruction, interrupted inferior vena cava, and rhythm disturbances resulting from an absent sinus node are more commonly associated with LAI. The hallmark of congenital HD in heterotaxy, however, is that there is no absolutely defined pattern to the possible combination of cardiac and vascular defects.

Prevalence

Heterotaxy is estimated to occur in 1 per 10 000 live-births[265] and constitutes ≈3% of congenital HD cases. Its incidence could be underestimated because of subtle or clinically insignificant findings of laterality disorders, such as bilateral superior vena cava.

Molecular Genetics

Aneuploidies, complex chromosomal rearrangements, and microdeletions have all been identified in patients with heterotaxy.[263,267] Clinically relevant CNVs have been identified in 15% to 26% of patients with heterotaxy syndrome.[268–272] Heterotaxy has the highest relative risk among all classes of congenital HDs, which supports a strong genetic component.[273] Autosomal dominant, autosomal recessive, and X-linked inheritance patterns have all been described, but unlike other types of congenital HD, de novo sequence variants are not major contributors to heterotaxy.[14,274,275] Pathogenic variants in the X-linked transcription fac-

tor ZIC3, a zinc-finger transcription factor that is required to form a functional LRO[276] and is required to direct heart looping, causes heterotaxy in up to 5% of males and a smaller percentage of females with heterotaxy.[277–281] ZIC3 pathogenic variants are identified in ≈75% of pedigrees with possible X-linked inheritance. Although penetrance is high, at least 1 case of nonpenetrance has been identified.[282]

Although many genes linked to heterotaxy are associated with cilia and LRO structure and function, there are many additional genes, including SHROOM3, GRK5, and ANKS3, that have been reported to contribute to heterotaxy with functions distinct from cilia. Sequence variants in genes required for propagation of the asymmetrical signal at the left lateral plate mesoderm, including NODAL, CFC1, LEFTY2, GDF1, SMAD2, and ACVR2B, have been associated with human laterality disturbances.[283–285] Environmental modifiers such as maternal diabetes mellitus and monozygotic twinning have also been associated with heterotaxy spectrum defects.

Cardiovascular Genotype/Phenotype Correlations

One of the hallmarks of inherited laterality disorders is the broad range of both laterality and cardiac phenotypes that result from any given mutation. For example, loss-of-function variants in ZIC3 have been correlated with a range of phenotypes ranging from classic heterotaxy with variable extracardiac manifestations to isolated congenital HD.

Primary Ciliary Dyskinesia

The link between ciliary defects and congenital HD was first identified when Bjorn Afzelius identified defective ciliary structure in electron microscopic analysis of tracheal cilia with Kartagener's triad, a syndrome consisting of respiratory disease, male infertility, and situs inversus in 50% of affected patients.[286]

Common Features

Kartagener syndrome is a subset of PCD, a disorder defined by abnormal ciliary motility in the airway epithelia. PCD is a highly heterogeneous disorder with pathogenic variants in >39 genes identified (Table 6), of which 23 have been linked to cardiac abnormalities.[287] Not surprisingly, a retrospective study of patients diagnosed with PCD identified heterotaxy in a subset of the cohort.[288] Neonatal respiratory distress (not related to cardiovascular malformations) is a frequent manifestation of PCD. Chronic wet, productive cough, daily rhinitis, recurrent or chronic bacterial infections of the lower airways, recurrent sinusitis, and otitis media are common features. Bronchiectasis is seen in adults. The diagnosis of PCD is made through sequencing PCD genes, by either WES or dedicated PCD panels, combined with nasal ciliary bi-

opsy and analysis of ciliary structure and motility. Low nasal nitric oxide is a useful marker of PCD, but testing is not reliable in infants and young children.

Cardiac Features

Situs inversus totalis (mirror image reversal of all organs), the most common laterality phenotype associated with PCD, is part of the spectrum of laterality disorders resulting from ciliary dysfunction (Table 6) and occurs in 40% to 50% of cases. At least 12.1% of patients with classic PCD exhibit heterotaxy.[289] Because a diagnosis of PCD in patients with congenital HD is inherently challenging given the difficulty in differentiating whether respiratory symptoms are primary or secondary to the underlying cardiac pathology and the medical and surgical interventions required to manage the congenital HD, it is possible that some patients thought to have isolated congenital HD actually have congenital HD as part of PCD.

Prevalence

The prevalence of PCD is not known with certainty, but PCD is estimated to affect ≈1 per 20 000 individuals.

Molecular Genetics

PCD is most commonly inherited as an autosomal recessive condition, although a rare association of X-linked PCD with retinitis pigmentosa has been described and a new X-linked form of PCD has recently been identified.[290] There are at least 39 genes known to cause PCD, with additional candidate genes identified in animal models. The number of PCD-causing genes that can also cause isolated congenital HD is unknown, but recent work shows that predicted damaging variants are found in genes required for ciliary motility and function in patients with congenital HD.[14]

Cardiovascular Genotype/Phenotype Correlations

Absence of cilia motility in the setting of otherwise normal sensation and signal propagation results in random LR asymmetry: random movement of extraembryonic fluid is still able to trigger a "left identity" signal that is randomly distributed between the anatomic right and left side of the embryo. This leads to the characteristic phenotype observed in the setting of PCD caused by ciliary motility components such as the axonemal dynein DNAH5, DNAH11, and the dynein assembly factor DNAI1. Patients with PCD pathogenic variants most commonly present with randomization of situs (resulting in situs inversus or situs solitus) and only rarely with heterotaxy.[288]

Syndromic Sensory Ciliopathies

The sensory ciliopathies are a group of genetically and phenotypically heterogeneous disorders caused by abnormalities in the sensory or signaling functions of cilia. They are inherited in an autosomal dominant, autosomal recessive (most common), or X-linked pattern. Organs most commonly affected are those in which nonmotile sensory cilia play important roles, such as the eyes, ears, skeleton, brain, kidney, and liver.[291–296] Abnormal signal transduction via hedgehog, Hippo, and Wnt pathways underlies a variety of patterning defects and congenital anomalies identified in these syndromes, although other developmental pathways are also involved.

Common Features

Eye and Ear

Retinitis pigmentosa and cone-rod dystrophy are common eye findings. Sensorineural hearing loss occurs in a variety of ciliopathies.

Central Nervous System

Structural defects have been described, including the classic brain stem malformations (molar tooth sign) in Joubert syndrome, Dandy-Walker malformation, and neural tube defects, including encephalocele, holoprosencephaly, and agenesis of the corpus callosum.

Growth and Endocrine

Obesity is seen as a result of abnormal energy homeostasis/hypothalamic dysfunction and is common in Bardet-Biedl, Alstrom, and Carpenter syndromes. Diabetes mellitus is the most common endocrine abnormality.

Skeletal

Dwarfism, thoracic dysplasia, short limbs, and polydactyly characterize a variety of ciliopathies, some of which are perinatal lethal. Four groups with major skeletal involvement include the cranioectodermal dysplasias, the short-rib thoracic dysplasias, EVC, and the oral-facial-digital syndromes.

Hepatic

Prototypical features are hepatic fibrosis and hepatic cysts. Liver disease in ciliopathies is not a primary disease of the hepatocytes but rather is a developmental defect of the portobiliary system.

Renal

Polycystic kidneys are common features of many ciliopathies. Nephronophthisis is characterized by renal cysts, tubular basement membrane disruption, and tubulointerstitial fibrosis.

Skin

Ectodermal dysplasia affects hair, skin, teeth, and nails.

Cardiac Features

Congenital HDs and laterality defects occur in a subset of sensory ciliopathies (Tables 6 and 7). In addition to situs abnormalities of the heart, atrioventricular canal defects, septal defects, and valve defects can occur with reduced penetrance. The mechanistic basis of the congenital HDs has not yet been established for each syndrome. Find-

ings of laterality defects should reflect disruption of LRO function, whereas isolated congenital HDs might result from abnormalities of cilia within the heart. Cardiomyopathy is identified in Alstrom syndrome.

Prevalence

The prevalence varies by syndrome subtype, but all syndromes are quite rare, with estimates ranging from 1:50 000 to 1:1 000 000. There are examples of founder effects, with the incidence of Meckel-Gruber as high as 1:9000 in some populations (Finnish).[292]

Molecular Genetics

As seen in Table 7, several of the disorders have allelic overlap. For example, Joubert syndrome and Meckel-Gruber syndrome share many of the same genetic causes. More than 140 genes have been established as causative for syndromic sensory ciliopathies, along with >200 additional candidate genes.[293] Most cases are caused by loss of gene function.

Genotype-Phenotype Correlations

How the same allele causes disparate phenotypes for many of the ciliopathies is not fully apparent. For the multisystem disorders, specific organ involvement or severity can correlate with the particular gene involved. For example, in a patient with Joubert syndrome, pathogenic variants in *NPHP1*, a gene that can also cause nephronophthisis, are more likely to be found in association with renal involvement. Modifier alleles and digenic inheritance have been described, and these presumably affect phenotypic presentation. In some cases, the variant type (eg, loss of function versus missense) will dictate presentation. Genotype-phenotype correlations have not been described for cardiac presentations.

Isolated Congenital HD Related to Ciliary Defects

General

Studies of the mouse model predict that ciliary defects will be identified that cause recessively inherited isolated congenital HD in the absence of a syndromic ciliopathy or PCD,[251] and recent studies show an overrepresentation of rare, predicted damaging variants in recessive genes in patients with isolated congenital HD versus control subjects.[14]

Cardiac Features

Recent work on large cohorts of patients with severe mitral valve prolapse identified that mutations affecting *DCHS1* are linked to congenital mitral valve defects,[333] and DCHS1 localizes to the base of the ciliary apparatus.[334] In addition, patients with situs inversus with or without TGA have been identified with a deletion that affects *NPHP2* (inversin), and mutations that affect *GDF1* have been associated with RAI (Table 6). In the future, genomic analyses of large cohorts of congeni-

tal HD will likely yield additional cilia genes with a role in congenital HD and begin to establish more focused genotype-phenotype correlations.

Clinical Genetic Testing in Ciliopathies

Clinical genetic testing is directed on the basis of the differential established through medical history, including family history, and physical examination. All patients with heterotaxy should have CMA because of associations with chromosome abnormalities and pathogenic CNVs. In addition, strong consideration should be given to *ZIC3* testing, particularly in males with heterotaxy. Recurrence risk estimates are substantially impacted by test results. Although additional studies are necessary to further establish the prevalence of PCD in patients with heterotaxy, consideration should be given to evaluation for PCD, because respiratory and pulmonary management could be optimized to improve the higher than expected surgical morbidity and mortality in this patient group.[335] Genetic testing for PCD is available through multigene panels. Concern for syndromic ciliopathies should prompt molecular testing for these disorders via ciliopathy panels or exome sequencing.

The Impact of Ciliopathy Pathologic Variants on Clinical Outcomes in Patients With Congenital HD

One of the challenging aspects of caring for patients requiring surgical repair of congenital HD is the variation in postoperative outcomes even for patients with anatomically and physiologically identical congenital HD. Respiratory complications are one of the most important modulators of postoperative outcome that can be influenced by genetic pathogenesis of the congenital HD. If patients at increased risk for respiratory and other complications can be identified preoperatively, it might be possible to modify their care and improve clinical outcomes. Pathological variants in ciliary genes are known to cause heterotaxy, some types of nonheterotaxy congenital HD,[251,288] and PCD.[286,336] Poor mucociliary clearance leads to infection and inflammation that damage the airway, and it is especially important to note that patients with ciliary dysfunction depend entirely on cough for mucociliary clearance, a function that is compromised in patients on mechanical ventilatory support, such as postoperative congenital HD patients. With this in mind, it is not surprising that patients with congenital HD, heterotaxy, and associated airway ciliary dysfunction have a higher rate of respiratory complication postoperatively than similar patients without airway ciliary dysfunction.[335] These findings suggest that prospective knowledge of which patients might have

Table 7. The Relationship of Ciliary Components Shown in Figure 1 and the Associated Congenital HD

	Gene	Molecular Function	Cardiac Phenotype	Reference
LRO formation	GDF1	LRO formation	RAI, congenital HD	297
	GALNT11	LRO formation	HTX	271
Centriole	MKKS	Centriole	Congenital HD	258
	NEK2	Centriole	HTX	271
Transition zone/inversion compartment	NPHP2		SI, TGA	298–300
	NPHP3		VSD, HTX	301, 302
	ANKS6		SI, congenital HD	303
	NEK8		Congenital HD, HTX	304
Ciliary motility	DNAAF3	Dynein assembly	SI	305
	CCDC39	Dynein assembly	SI, HTX	306
	CCDC40	Dynein assembly	SI	307
	CCDC103	Dynein assembly	S1, HTX	308
	HEATR2	Dynein assembly	SI, HTX, congenital HD	309
	ARMC4	Dynein assembly	SI	310
	DYX1C1	Dynein assembly	SI, HTX, congenital HD	311
	C210RF59	Dynein assembly	SI, HTX	312
	SPAGI	Dynein assembly	SI	313
	CCDC151	Dynein assembly	SI, HTX, congenital HD	314
	PIHID3	Dynein assembly	SI	290
	DNAAF1	Dynein assembly	SI	315, 316
	DNAAF2	Dynein assembly	SI	317
	DNAH5	Dynein outer arm	SI, HTX, congenital HD	288, 318
	TXNDC3	Dynein outer arm	SI, HTX	319
	DNAH11	Dynein outer arm	SI	320
	DNA11	Dynein intermediate chain	SI, HTX, congenital HD	321, 322
	DNALI	Dynein light chain	SI	288, 323, 324
	CCDC114	Dynein docking	SI, HTX, congenital HD	325
	TTC25	Dynein docking	SI	326
	LRRC6	Dynein transcription	SI	320
	ZMYND10	Dynein transcription	SI	327
	CCDC11	Centriole	SI, HTX	328, 329
				330
Polycystin complex	PKD2		SI	331
	PKD1L1		HTX	332
Other	EVC	Hedgehog signaling	AVC	123
	EVC2	Hedgehog signaling	AVC	123
	MKS1	Hedgehog signaling	AVC	250
	DCHS1		Mitral prolapse	333

AVC indicates atrioventricular canal; HD, heart disease; HTX, heterotaxy; LRO, left-right organizer; RAI, right atrial isomerism; SI, situs inversus; TGA, transposition of the great arteries; and VSD, ventricular septal defect.

airway ciliary dysfunction could improve postoperative outcome by tailored modifications to their respiratory care. In addition, immotile cilia are found in the kidney and brain, and it is possible that cilial defects impact the kidney's response to injury,[337] neurodevelopment,[338,339] and metabolic function.[339]

NONSYNDROMIC CONGENITAL HD ATTRIBUTABLE TO SINGLE-GENE VARIATION

As discussed earlier, numerous genes have been implicated in the pathogenesis of congenital HD when it occurs in the setting of a genetic syndrome, but the identification of the genetic contributors of nonsyndromic congenital HD has proved to be more challenging. Although initial insights were based on studies of large, multigenerational kindreds in which multiple family members were affected with a cardiac malformation, these families are relatively uncommon, and the congenital HD is often less severe. With the elucidation of an increasing number of genes involved in the molecular regulation of cardiac morphogenesis by either a better understanding of cardiac developmental regulation through model organism studies or through identification of candidate cardiac genes within microdeletions/microduplications in individuals

with congenital HD, there has been a relative explosion of rare sequence variants in these heart development genes identified in children with congenital HD. Establishing disease causality, especially of a specific variant, remains a challenge. These genes mostly encode transcription factors, signaling molecules, or structural proteins important in cardiac development, structure, and function. The genes most strongly associated with congenital HD are briefly discussed in Transcription Factors, Cell Signaling and Adhesion Molecules, and Structural Proteins. Detailed information about the associated cardiac phenotypes and references to supporting studies are reviewed in Anderson et al[340] and Fahed et al[341] and are provided in Table 8. In addition to these "gold standard" congenital HD genes, there are several hundred genes with purported roles in cardiac development and congenital HD, and advances in genomic technology over the past 3 years have enabled us to unravel the genomic architecture of isolated or nonsyndromic congenital HD at a rapid pace. Many of the congenital HD genes identified to date can be assigned to one of the following functional categories.

Transcription Factors

Initial insights into the genetic pathogenesis of nonsyndromic congenital HD were based on the discovery of disease-causing sequence variants in critical cardiac transcription factors identified as important for normal heart development in multiple animal model systems.[401] Mutations in the transcription factor gene *NKX2-5* were reported in multiple familial and sporadic cases of congenital HD, with the first report in 1998 in 4 kindreds with autosomal dominant congenital HD.[358] Familial ASD with atrioventricular conduction abnormalities is the primary cardiac phenotype associated with *NKX2-5* mutations, but additional phenotypes include VSD, tetralogy of Fallot, subvalvar aortic stenosis, pulmonary atresia, and mitral valve abnormalities (reviewed in Stallmeyer et al[362] and Ellesøe et al[363]) These discoveries have been supported by the reports of similar cardiac phenotypes in mouse models harboring mutations in *Nkx2.5*.[402–404]

A similar approach identified heterozygous mutations in *GATA4*, a gene encoding another important cardiac transcription factor, in familial congenital HD.[113] The predominant and most penetrant phenotype is secundum ASD but can include VSD, PVS, AVSD, and tetralogy of Fallot (reviewed in Prendiville et al[405]). Evidence supporting these genetic associations has come from analysis of mice haploinsufficient for *Gata4* or harboring disease-causing *Gata4* mutations. These mouse models have replicated human disease phenotypes.[347,406,407] Additionally, another member of the GATA family of transcription factors, *GATA6*, has been implicated in sporadic and familial congenital

HD, with lesions including persistent truncus arteriosus, PVS, ASD, and PDA.[351] The link between *GATA6* mutations and human disease was expanded by the identification of de novo inactivating mutations in *GATA6* in ≈50% (15 of 27) of individuals with pancreatic agenesis, among whom 90% had congenital HD.[354]

Another important transcription factor family linked to congenital HD is the Tbox family. Besides the association with syndromic congenital HD (*TBX5* with HOS, *TBX1* with cardiac lesions in 22q11.2DS), mutations in *TBX5* and *TBX1* might be responsible for nonsyndromic congenital HD.[408,409] In addition, mutations in another family member, *TBX20*, were identified in 2 families with cardiac septation defects, mitral valve stenosis, and dilated cardiomyopathy.[366] Mutations in *TBX20* have subsequently been described in other cardiac malformations, including tetralogy of Fallot, truncus arteriosus, and double-outlet right ventricle. Other cardiac transcription factors implicated in congenital HD pathogenesis are listed in Table 8.[369]

Cell Signaling and Adhesion Molecules

Although mutations in the Notch signaling pathway are a cause of ALGS as well as AOS (both discussed previously),[141] this pathway is also implicated in nonsyndromic congenital HD. Garg et al reported a multigeneration family with autosomal dominant cardiovascular disease in which 9 members had aortic valve disease, primarily BAV, but also 1 member with tetralogy of Fallot.[185] Since then, mutations in *NOTCH1* have been implicated in familial nonsyndromic congenital HD, predominantly affecting the cardiac outflow tract and semilunar valves.[384] A recent report using clinical exome sequencing in a patient with HLHS identified a *NOTCH1* mutation although affected paternal family members had mostly right-sided heart lesions,[385] which suggests that the phenotype might not be limited to the left-sided semilunar valve.[383] Pathological sequence variants in other cardiac developmental signaling pathway genes have also been identified in patients with congenital HD (Table 8).

Structural Proteins

Mutations in sarcomeric genes, known to cause cardiomyopathy, have also been reported in congenital HD, for example, α-cardiac actin (*ACTC1*) and myosin heavy chain 6 (*MYH6*) mutations in familial ASD,[389,394] β-myosin heavy chain (*MYH7*) mutations in Ebstein's anomaly of the tricuspid valve and left ventricular noncompaction cardiomyopathy,[397,398] and myosin heavy chain 11 (*MYH11*) mutations in PDA, usually in association with thoracic aortic aneurysms.[399,400] As discussed, elastin (*ELN*) haploinsufficiency causes

Table 8. Disease Genes for Nonsyndromic Congenital Cardiovascular Disease

Gene	Cardiovascular Malformation	Nonsyndromic (NS) or Syndromic (S)	Gene MIM	References
Transcription factors				
CITED2	ASD, VSD	NS	602937	342
GATA4	ASD, VSD, AVSD, PVS, TOF	NS	600576	113, 343–350
GATA6	PTA, TOF	NS	601656	351–355
MED13L	TGA	NS	608771	356
NR2F2	AVSD, AS, CoA, VSD, HLHS, TOF	NS	107773	357
NKX2-5	ASD, atrioventricular conduction delay, TOF, HLHS	NS	600584	358–363
NKX2.6	PTA	NS	611770	364, 365
TBX20	ASD, VSD, MS, DCM	NS	606061	366–369
ZFPM2/FOG2	TOF, DORV	NS	603693	370–372
Cell signaling and adhesion proteins				
ACVR1/ALK2	AVSD	NS	102576	373
CRELD1	ASD, AVSD	NS	607170	374–376
GJA1	HLHS, VSD, PA	S (oculodentodigital dysplasia) and NS	121014	377–379
JAG1	TOF, PVS, PAS	S (Alagille syndrome) and NS	601920	380–382
NOTCH1	BAV, AS, HLHS, TOF, PVS	S (Adams-Oliver syndrome) and NS	190198	141, 185, 383–385
PDGFRA	TAPVR	NS	173490	386
SMAD6	BAV, CoA, AS	NS	602931	387
TAB2	BAV, AS, TOF	NS	605101	388
Structural proteins				
ACTC1	ASD, HCM, DCM, LVNC	NS	102540	389, 390
DCHS1	MVP	NS	603057	333
ELN	SVAS	S (Williams-Beuren syndrome) and NS	130160	80, 391–393
MYH6	ASD, HCM, DCM	NS	160710	394–396
MYH7	Ebstein's anomaly, LVNC, HCM, DCM	NS	160760	397, 398
MYH11	PDA, TAA	NS	160745	399, 400

AS indicates aortic valve stenosis; ASD, atrial septal defect; AVSD, atrioventricular septal defect; BAV, bicuspid aortic valve; CoA, coarctation of aorta; DCM, dilated cardiomyopathy; DORV, double-outlet right ventricle; HCM, hypertrophic cardiomyopathy; HLHS, hypoplastic left heart syndrome; LVNC, left ventricular noncompaction cardiomyopathy; MIM, Mendelian Inheritance in Man; MS, mitral valve stenosis; MVP, mitral valve prolapse; NS, nonsyndromic; PA, pulmonary atresia; PAS, pulmonary artery stenosis; PDA, patent ductus arteriosus; PS, pulmonic valve stenosis; PTA, persistent truncus arteriosus; PVS, pulmonary vein stenosis; S, syndromic; SVAS, supravalvar aortic stenosis; TAA, thoracic aortic aneurysm; TAPVR, total anomalous pulmonary venous return; TGA, transposition of great arteries; TOF, tetralogy of Fallot; and VSD, ventricular septal defect.

syndromic congenital HD in WS, whereas point mutations in *ELN* cause supravalvular aortic stenosis and other large artery stenoses without a syndromic phenotype.[392,393]

Recent Insights Into the Complex Genetic Architecture of Nonsyndromic Congenital HD

Given the large number of genes that contribute to congenital HD, NGS is being increasingly used in both research and clinical settings in congenital HD patients. In one of the earliest studies led by the Pediatric Cardiac Genomics Consortium that used WES in 362 severe congenital HD cases (parent-offspring trios), congenital HD cases showed a significant excess of protein-altering de novo sequence variants in genes expressed in the developing heart, with particular enrichment of histone-modifying genes that regulate expression of key developmental genes.[275] Of note, the involvement of histone modifier mutations in several forms of congenital HD suggests that epigenetic modifications more broadly (eg, ones that alter DNA methylation or noncoding RNAs) might prove relevant for congenital HD pathogenesis.[275,410] De novo point variants in several hundred genes together contributed to ≈10% of severe congenital HD. Of note, the vast majority of variants were "private" in that they were not identified in more

than 1 individual. The same group performed exome sequencing of 1213 congenital HD parent-offspring trios and identified an excess of protein-damaging de novo variants in genes highly expressed in the developing heart and brain. These potentially disease-causing variants accounted for 20% of patients with syndromic congenital HD, that is, congenital HD with neurodevelopmental disabilities and extracardiac congenital anomalies, but only 2% of patients with isolated congenital HD. These findings revealed shared genetic contributions to congenital HD, neurodevelopment, and extracardiac anomalies.[274]

A large international study using WES of 1891 probands found a significant enrichment of de novo protein-truncating variants but not inherited protein-truncating variants in known congenital HD genes in syndromic congenital HD. Conversely, in nonsyndromic congenital HD, there was a significant enrichment of protein-truncating variants inherited from unaffected parents in congenital HD genes. The study further identified 3 genome-wide significant syndromic congenital HD disorders caused by de novo variants in *CHD4*, *CDK13*, and *PRKD1*. This study underscored the distinct genetic architectures of syndromic versus nonsyndromic congenital HD.[411]

Although these studies involved heterogeneous cohorts of congenital HD, WES of a targeted cohort of nonsyndromic AVSD cases provided important insights into congenital HD genetic architecture, including the identification of *NR2F2*, a novel potentially causal gene in patients with nonsyndromic AVSD.[357] Because *NR2F2* mutations explained <5% of AVSDs, the investigators also performed a candidate gene search of 112 potential AVSD-associated genes and found a significant enrichment of rare, damaging variants in 6 genes, 3 of which were known syndrome-associated genes (*NIPBL*, *CHD7*, and *CEP152*).[412] This highlighted that syndrome-associated genes can contribute to nonsyndromic congenital HD. A follow-up study identified an even larger gene set enriched for potential disease-contributing variants compared with control subjects, with 32% of trios carrying at least 1 putatively disease-associated variant, either inherited or de novo, across a heterogeneous group of loci.[413] Together, these studies revealed the complex and oligogenic origins of AVSDs, as well as the ability of NGS to unravel some of this complexity.

Finally, a recent study using mouse forward genetics identified sequence variants in novel genes not previously associated with congenital HD, *Sap130* and *Pcdha9*, as being digenic causes of HLHS. *Sap130* mediated left ventricular hypoplasia, whereas *Pcdha9* increased penetrance of aortic valve abnormalities. The investigators also identified a subject with HLHS with both *SAP130* and *PCDHA13* sequence variants.[414] This study highlighted that complex congenital HD can be

caused by synergy between variants in multiple developmental genes rather than a single gene.

In summary, in recent years, NGS approaches in isolated congenital HD have revealed the following:

1. There are several hundred genes that either cause or contribute to congenital HD.[275]
2. Sequence variants in congenital HD genes can cause both sporadic and inherited congenital HD.
3. Sequence variants in congenital HD genes can cause both syndromic and nonsyndromic congenital HD, with strong association of de novo variants with syndromic CHD[274] and of inherited variants with nonsyndromic congenital HD.[411]
4. There is phenotypic heterogeneity, with sequence variants in the same genes often associated with different cardiac phenotypes, not only between families but also within families. This discordance in phenotype among family members was further highlighted in a Danish national study in which only 50% of siblings had the same type of congenital HD as the proband.[415]
5. Family studies often show incomplete segregation even in familial congenital HD, with could be attributable in part to incomplete penetrance but could also be related to oligogenic origins of congenital HD.[413] The occurrence of multiple variants in some patients might explain why some affected individuals have a more severe phenotype.[414]

Clinical Implications

The above findings have clinical implications. Although several laboratories offer congenital HD gene panels of various sizes for clinical testing, the relatively large numbers of genes involved and the role of novel and ultra-rare variants in causing rare disorders coupled with the oligogenic origins of some of the more complex congenital HDs suggest that a genome-wide search for congenital HD–associated variants might be cost-effective in the future as the accuracy of variant interpretation improves. Experience, challenges, and cost-effectiveness of clinical exome sequencing have been reported recently.[385,400] Also, given the overlap in genes associated with syndromic and nonsyndromic congenital HD, it is important to continue medical follow-up of patients with nonsyndromic congenital HD caused by a syndrome-associated gene, because noncardiac findings can sometimes manifest later than cardiac findings. Additional clinical implications of knowledge of genetic pathogenesis in screening, surveillance, and management of congenital HD have been discussed in detail in a recent American Heart Association scientific statement.[416]

FUNCTIONALITY OF CONGENITAL HD GENES

Widespread exome and genome sequencing of congenital HD patients is uncovering an ever-increasing number of candidate disease genes and disease-causing variants.[274,417] These sequence variants and candidate disease genes need to be studied in model systems to rapidly and accurately determine which variants are responsible for congenital HD causation, to characterize pathogenic mechanisms, and to identify new candidate genes for evaluation in clinical studies. Several in vitro and in vivo model systems are available, each with its own strengths and weaknesses. In vivo animal models, including mammalian (eg, mouse), "other" vertebrate (eg, zebrafish, frog, and chick), and invertebrate (eg, fruit fly) organisms, allow evaluation of the impact of genetic perturbations on cardiac development and function within the context of an intact organism. Recently, in vitro strategies have been developed in cell and tissue engineered models that complement the animal models and facilitate mechanistic studies.[418] In combination, these in vivo and in vitro model systems enable the elucidation of pathogenic mechanisms, the discovery of additional candidate congenital HD genes, and the development of novel, molecularly targeted therapeutic strategies.

Mouse Models

Because of the high degree of conservation between mouse and human cardiac development and the availability of well-established techniques for genetic manipulation, the mouse model has been used to study heart development for >25 years, resulting in an extensive knowledge base with bountiful reagents and resources. These key features have made the murine system the most widely studied animal model of cardiovascular development.

The mouse genome can be modified by many techniques. These can be grouped into methods that randomly insert DNA sequences into the genome (transgenesis) and those that modify an endogenous locus (targeted mutagenesis). Transgenesis, performed by introduction of foreign DNA (using a targeting vector) into a fertilized oocyte, is most commonly used to direct expression of a gene in an altered form or at an ectopic time, location, or level. The targeting vector used to modify the endogenous locus can be engineered to inactivate the gene (knockout), to introduce recombinase sites into the gene so that it can be conditionally inactivated by a second recombinase allele (eg, flank gene with loxP sites [floxed] that can be excised at a specific time in a specific tissue by Cre recombinase), or to modify the endogenous gene (knockin). More recently, nucleases that can be programmed to cut the genome

at a single specific site (eg, CRISPR/Cas9) have expedited targeted mutagenesis by dramatically increasing the efficiency of site-directed mutagenesis.[419]

These techniques for modifying the mouse genome have been used to study cardiovascular development and disease in a number of ways.[420] Transgenesis is often used for gain-of-function experiments, in which a promoter with specific spatiotemporal expression properties is used to drive a gene of interest. The opposite loss-of-function strategy is achieved by constitutive gene knockout or by excising an essential portion of a floxed gene of interest using Cre recombinase, expressed from a transgene or knocked into a second locus so that it is expressed in a known spatiotemporal domain. Gene knockout approaches have traditionally focused on coding regions. However, this strategy will also be useful to test the functional importance of conserved transcriptional regulatory elements that have been linked to congenital HD causation using high-throughput mapping technologies.[421–424]

Cre recombinase is also often used to dissect a cell's developmental history, a technique known as genetic lineage tracing.[425] Here, Cre recombination is used to activate expression of a reporter gene such as *LacZ* or *GFP* in a particular tissue beginning at a selected developmental stage. Because reporter activation involves modification of the genome, it is transmitted to all of the progeny of the Cre-expressing cells. Lineage tracing has been critical to deduce the developmental events that generate the heart, including the contributions of the second heart field, which adds cells onto the arterial and venous ends of the linear heart tube to form parts of the atria and most of the right ventricle and outflow tract,[426] as well as the contribution of the dorsal mesenchymal protrusion to form portions of the atrioventricular septae at the crux of the heart.[427,428] In spite of the similarities between mouse and human cardiac development, there also can be important differences. Because of practical considerations, congenital HD gene defects are often modeled in mice as homozygous gene knockouts, whereas most congenital HD mutations are heterozygous point or truncating mutations.[274,417] These point mutations can result in partial gain or loss of function or could have dominant-negative activity that is imperfectly modeled by gene knockout. Biologically, gene dosage and redundancy are important factors that influence the expression of mutations, and these parameters often vary between species. For instance, haploinsufficiency of *TBX1* in 22q11 deletion syndromes is an important contributor to congenital HD[429]; however, *Tbx1* haploinsufficiency is well tolerated in mouse models, and a more severe reduction of *Tbx1* dosage is required to produce cardiac defects.[430] Genetic modifiers modulate the expression of gene mutations in human congenital HD.[48] Exploration of genetic modifiers in mouse models can

be productive[405,431] but is rarely undertaken because these experiments are time and resource intensive. As a result of these technical and biological factors, mouse models often yield important principles and genetic pathways responsible for congenital HDs, but genotype-phenotype relationships can differ between the mouse and the human.

As noted above, cost and time are important considerations when developing mouse models of congenital HD. Creating a new mouse allele and characterizing it can take 6 to 12 months, and in models that require combining several different alleles, breeding mice to obtain the required genotype can be a critical practical bottleneck. Acquiring the correct mouse alleles for an experiment can also be time consuming and expensive. In some cases, these practical limitations can be circumvented through in vivo gene transfer and somatic mutagenesis.[432] Adeno-associated virus has proven to be an extremely efficient method for postnatal gene transfer to cardiomyocytes, and adeno-associated virus can be combined with CRISPR/Cas9 to efficiently introduce somatic mutations into cardiomyocytes. Although it is possible to deliver adeno-associated virus to late-stage mouse embryos,[433] unfortunately at the present time, adeno-associated virus transduction of mid-gestation mouse embryos and noncardiomyocytes (such as endothelial cells and valve cells) is inefficient and thus not applicable to many heart development studies.

Zebrafish and Other Vertebrate Models

The limitations of the mouse model have led to the use of alternative vertebrate models to study cardiovascular development. The zebrafish has become an attractive experimental model; the genes and signaling pathways involved in human cardiac development and responsible for human congenital cardiac defects are highly conserved in the zebrafish, and the zebrafish offers some important advantages for developmental studies. A key advantage of the zebrafish system over mice is that embryos are transparent and develop outside the body of the mother (ex utero), which permits the developing cardiovascular system to be imaged throughout the developmental process.[434,435] One can also rapidly and economically generate genetically modified zebrafish models. Rapid screening of candidate congenital HD disease genes for essential functions in heart development can be performed with antisense strategies to diminish expression of specific genes beginning very early in development. Unfortunately, this approach, which uses stable antisense RNA constructs called morpholinos,[436] can yield nonspecific morphological defects that can obscure gene function, requiring careful confirmation of observed phenotypes. Recent advances have enabled rapid and efficient stable gene targeting using CRISP-R/Cas9 approaches similar to those de-

scribed above for mice.[437] These approaches are more time consuming but have a lower level of nonspecific or off-target effects. One important consideration when performing targeted genetic modifications is that the zebrafish underwent a genome duplication event during evolution after divergence from its common ancestor with mammals.[438] As a result, for many mammalian genes, zebrafish often have 2 different genetic loci encoding for slightly different versions of the same gene, both of which might need to be targeted to have the same developmental effect as targeting the single gene in other vertebrates.

One popular approach to verifying that newly identified variants are indeed pathogenic is to determine whether a full-length expression construct of the wild-type and mutated versions of the gene is capable of rescuing the phenotype of zebrafish that lack a functional copy of the gene. This can be readily accomplished by directly injecting capped mRNA into 2- to 4-cell-stage embryos, leading to widespread expression of wild-type or mutant transcripts of genes known or suspected to be involved in cardiac development. Furthermore, expression of a mutant form of the gene in a wild-type embryo can help rapidly validate gain-of-function variants that have a dominant-negative effect on heart development.

The ex utero development of zebrafish embryos also permits interventions that are not possible in mouse. Addition of agents to the aquatic environment of the embryo can be used to examine the teratogenic effects of environmental toxins[439] or interrogate the developmental contributions of specific signaling pathways.[440] The embryo can also be directly accessed, and laser energy can be used to activate a specific gene in a specific cell,[441] photoconvert a green fluorescing cell to a red fluorescence (which allows examination of a particular cell with respect to the surrounding cells),[442] or lesion a specific tissue (to alter cardiovascular hemodynamics or ablate a specific group of cells).[443] Transfer of cells from one very early-stage embryo to another can enable the examination of genetically modified cells when surrounded by normal cells and tissues. Each of these interventions has helped to examine specific aspects of cardiac development in a manner that would not be possible in a higher vertebrate model in which the embryo develops in utero.

A major limitation of the zebrafish model is that the zebrafish has a 2-chambered heart (1 atrium and 1 ventricle), which makes it unsuitable for examination of the developmental process of septation. However, genetic mutations that lead to septal defects in humans cause detectable cardiac phenotypes in zebrafish embryos, which means the zebrafish is still a useful screening tool to examine the pathogenicity of mutations that are suspected of causing septation defects in humans.

Other Models

Another vertebrate model system that has the benefit of developing ex utero, which enables the observation and manipulation of developmental processes, is the chick embryo. It has the additional benefit of having a 4-chambered heart that is much more similar to the human heart and can be used to study septation and other, more complex processes in cardiac morphogenesis. As with the zebrafish model, delivery of gene expression or antisense RNA constructs can be used to manipulate gene expression, allowing examination of gene functions and the developmental pathways. A strength of the chick model system is the ability to examine the effects of embryo manipulation on cardiac development. For instance, surgical interventions such as left atrial or vitelline vein ligation alter intracardiac flow patterns and result in abnormalities of cardiac morphogenesis,[444,445] and ablation of specific developmental fields, such as the cardiac neural crest, allows determination of the contribution of those domains to the cardiac development. The combination of mechanical or pharmacological intervention with modification of gene expression can facilitate characterization of the effects of gene-environment interactions on heart development and is a particular strength of the chick embryo model system.

Other animal models that have been used to help characterize specific aspects of heart development include the frog (genus *Xenopus*), which has been very helpful in examining determination of "sidedness,"[253] and the fruit fly (genus *Drosophila*), which has been effectively used to examine cardiomyocyte specification.[446] Relatively high-throughput methods for cardiac gene knockdown and overexpression have been developed in *Drosophila*, which has enabled in vivo study of the functional significance of identified congenital HD mutations.[447]

In Vitro Model Systems

Although each animal model has its strengths in studying cardiac development and the pathological processes that cause human congenital cardiac defects, many lack the resolution to study the cellular interactions that are the foundation of organ development, and none can fully recapitulate the complex and unique genetic environment of a patient with congenital HD. Therefore, in vitro model systems, including those with the ability to directly examine the development of human patient–derived cells, have been developed to better understand cell-level interactions that guide heart development.

Although mammalian heart development cannot be fully recapitulated ex utero in culture systems, culture systems have been essential for mechanistic studies of cardiovascular development. Mammalian embryos remain viable and continue to develop for hours to days in culture environments, which permits key questions on lineage, mechanics, and molecular signaling to be studied. For example, whole embryo culture was used to dissect the interaction of vascular endothelial growth factor and calcineurin/NFAT (nuclear factor of activated T cells) signaling to regulate endocardial epithelial-mesenchymal transition.[448] Culture of microdissected pharyngeal arch arteries revealed a cardiac progenitor niche in the second pharyngeal arch artery that promotes renewal and expansion of cardiac progenitor cells, perturbation of which can contribute to congenital HD.[449] Atrial and ventricular explant culture has been used to demonstrate the origin of coronary endothelial cells from precursors on the atrial explant.[450]

Primary cell culture models have also been essential for studies of cardiovascular development. Primary fetal and neonatal cardiomyocytes can be maintained in culture for >1 week, and these systems have been essential for dissecting mechanisms that regulate cardiomyocyte survival, proliferation, and hypertrophy.[451,452] Culture of epicardial cells and explants has also been critical to dissect the function of these cells in regulating growth of myocardium and coronary vasculature.[453,454]

Stem cell differentiation into cardiomyocytes has become a powerful method to study cardiogenesis and early heart development. Cardiac progenitor cells are scarce in developing embryos, which makes studies that require thousands to millions of cells difficult. In contrast, millions of these cells can be efficiently generated in stem cell differentiation cultures. This has allowed key regulatory steps of cardiogenesis and early heart development to be carefully dissected.[455] Moreover, the availability of human pluripotent stem cells and protocols to efficiently direct their differentiation into cardiomyocytes has enabled the study of human cardiogenesis.[456]

The maturation of a number of transformative technologies has recently permitted the effect of gene mutations found in congenital HD patients to be studied using human "disease-in-a-dish" models. These technologies include (1) development of efficient methods for human stem cell differentiation; (2) reprogramming of somatic cells to induced pluripotent stem cells, which allows for the creation of patient-specific disease models; (3) facile genome editing, which permits rapid genetic manipulation of stem cells; and (4) development of bioengineered systems to build engineered heart tissues and assay them for relevant physiological parameters. This confluence of technical advances has allowed the impact of congenital HD mutations on cardiomyocyte gene expression, cardiac differentiation, and myocardial function to be evaluated in patient-specific genetic backgrounds, yielding new insights in disease pathogenesis.[457–460] Although these advances have opened exciting new approaches to studying congeni-

tal HD pathogenesis, a number of challenges need to be overcome before they can realize their full potential. Among these are developing in vitro, stem cell–based models of cardiac morphogenesis; enhancing directed differentiation of stem cells to the full gamut of cardiac cell types; and improving the maturation of in vitro differentiated cardiomyocytes.

Although beyond the scope of this scientific statement, it is important to mention the growing role of *in silico* modeling of genetic variants to determine the potential effects of the variant on protein structure and function. Early algorithms relied almost entirely on the effect of the amino acid alteration on protein structure to determine whether the mutation was likely to be pathogenic. More recently, algorithms have incorporated degree of evolutionary conservation, population frequency, and, in some cases, more advanced functional modeling to determine potential pathogenicity of a novel genetic variant. Increasingly, advances in the understanding of the functions of and pathophysiological mechanisms associated with specific disease-causing variants (gained from the above in vivo and in vitro models) will allow more refined disease gene–specific mathematical modeling to assess potential pathogenicity of specific genetic variants, better characterization of disease mechanisms, and identification of structural domains suitable for pharmacological targeting.

In summary, technical advances and our expanding knowledge base have fueled dramatic advances in the in vitro and in vivo modeling of cardiac development. Continued expansion of these modeling capabilities will allow the rapid screening and adjudication of potential congenital HD disease genes and pathogenic variants and the examination of potential therapeutic approaches. Advanced bioinformatics analyses of data generated in the in vitro and in vivo developmental models and genomic/genetic/epigenetic data from patients with congenital HD have the potential to better define developmental pathways involved in cardiac development, to greatly improve our understanding of congenital HD pathogenesis, and to identify novel approaches for prevention and treatment.

ETHICAL CONSIDERATIONS

As noted above, genetic testing for congenital HD has increased over the past 10 years[94,461] and is particularly helpful in diagnosing syndromes responsible for congenital HD and related noncardiac phenotypes that might require clinical management.[1,341,461] Benefits of genetic testing for congenital HD include establishing a genetic diagnosis, facilitating presymptomatic screening of at-risk family members, enabling anticipatory management of congenital HD, directing clinical screening and management of associated noncardiac conditions, and accelerating the development of novel therapeutic targets.[462] Because cardiac malformations

constitute a significant portion of birth defects,[1,341] knowledge of genetic predispositions to congenital HD could also be used by patients and their family members to support reproductive decisions and expectations, as well as to help guide prenatal and perinatal management. In addition, there is increasing evidence that certain types of genetic variations that cause congenital HD also affect clinical outcomes such as cognition, behavior, and motor skills (collectively termed *neurodevelopmental performance*).[341] Although this is still an emerging field, it is possible that in the near future, genetic testing could help identify patients at risk for abnormalities of neurodevelopment and help target intervention strategies.

Despite these potential benefits, uncertainty about the clinical significance of many genetic variants and the complexity of conveying this information present challenges. Sequencing has uncovered many more genetic associations with congenital HD,[94] but variant interpretation is imprecise, and the interplay between genetic and environmental factors that contribute to congenital HD continues to be elusive.[341,463,464]

With NGS, there is also the possibility that genetic variants associated with congenital HD might be discovered incidentally when testing for an unrelated phenotype, or that clinically significant incidental findings unrelated to congenital HD could be detected when testing for congenital HD. In 2013, the American College of Medical Genetics and Genomics (ACMG) recommended that certain clinically actionable secondary findings, including a number of cardiac-related variants, such as Ehlers-Danlos syndrome, familial thoracic aortic aneurysms, Marfan syndrome, and HCM, ought to be offered to all patients undergoing clinical WES or whole genome sequencing.[465,466] As a result, noncardiac specialists could be challenged with communicating complex genetic findings to potentially unsuspecting patients. In these situations, referral to a cardiovascular geneticist is advisable.

On the flipside, when performing a genomic test for congenital HD, patients ought to be informed about the potential to discover incidental findings unrelated to congenital HD and should be given an opportunity to opt out of those results.[466] This respects the patient's autonomy and preserves the "right not to know."[467] Pretest and posttest counseling is also important to facilitate informed decision making, and it is essential that it be offered to patients and their families.

Additional challenges are raised when performing genetic testing in children with congenital HD. For many years, there has been a general consensus that children should only receive genetic testing that offers the potential for direct clinical benefit during childhood.[1,468,469] The primary justifications for deferring testing for adult-onset conditions are respect for the child's future autonomy and right to an open future[470] and the potential psychosocial harm of knowing one's genetic risk of disease. Tar-

geted genetic testing is only plausible when there is a known family history of a mendelian condition that puts the child at risk for disease. In this situation, the affected family member already knows that he or she has, or is at risk for, the targeted genetic variant. In the context of genomic sequencing, however, a variant associated with an adult-onset condition that is discovered in a child could benefit parents or other family members, who would not otherwise know they are at risk.[465,471] Thus, increased attention is now being paid to the potential benefit of testing to families, and some professional organizations and scholars have recommended that the presence of clinically significant genetic variants discovered incidentally during the course of clinical WES or whole genome sequencing be offered to patients, regardless of age and irrespective of age of onset.[465,471,472] Comporting with standard ethical practice, children should be involved in the decision about whether to receive these incidental findings commensurate with their level of maturity and should provide assent whenever possible.[473]

Pretest genetic counseling for congenital HD should address the potential risks and benefits of testing, including the psychological and social impact of receiving a positive test result. Recent studies suggest that neither adults[474] nor children[475,476] experience significantly increased anxiety or distress after learning of their genetic status. However, the potential psychosocial impact of genetic testing can be greater when test results offer little therapeutic value and could include alterations of self-image and disruption in family relationships, including increased perceptions of child vulnerability that negatively impact development.[468] These risks must be weighed against the potential psychological benefits of testing. For example, patients and families might experience relief from the reduction of uncertainty when a genetic cause is discovered.[468] Additional research is needed to fully understand the impact of genetic testing for congenital HD on individuals, especially children, and their families, as well as to better appreciate how patients evaluate these trade-offs when deciding whether or not to undergo testing.

Even when the clinical and psychological benefits outweigh the risks, however, uptake of genetic testing for congenital HD can be limited if patients are concerned about the misuse of genetic information for discriminatory purposes. The 2008 Genetic Information Non-Discrimination Act (GINA) protects individuals in the United States from being discriminated against by health insurers and employers because of a genetic diagnosis.[477] However, GINA does not prevent life, disability, or long-term care insurers from using genetic information to make coverage decisions. Although there have been very few documented cases of genetic discrimination, even before the passage of GINA,[477] current regulatory uncertainty has the potential to negatively impact patients and families. A trustworthy system that provides robust protection against genetic discrimination is needed if we are to reap the benefits of advances in genetic testing for congenital HD that have been realized over the past 10 years.

GENETIC COUNSELING/RECURRENCE RISK/PRENATAL SCREENING

Genetic Counseling

The National Society of Genetic Counselors describes genetic counseling as the process of helping people understand and adapt to medical, psychological, and familial implications of genetic contributions to disease. This process integrates (1) interpretation of family and medical histories to assess the probability of disease occurrence or reoccurrence; (2) education about inheritance, testing, management, prevention, resources, and research; and (3) counseling to promote informed choices and adaptation to the risk or condition.[478] A genetic counselor is a graduate-level trained healthcare professional who receives training in medical genetics, genomics, and counseling. In the United States and Canada, this terminal degree leads to certification through the American Board of Genetic Counseling after the individual passes a national certification examination. As of May 2017, 20 states issue and require licensure for genetic counselors to practice, and 3 states have licensure laws in progress. As the need for cardiovascular genetic counseling is increasingly recognized, genetic counseling training programs are developing curricula and clinical rotations to meet this growing need.[415,479] Nevertheless, there are 3-fold more genetic counseling positions available than new graduates each year.

Genetic counselors skilled in cardiovascular genetics have become an invaluable clinical asset, helping not only to provide accurate recurrence risks but also to obtain family and medical histories, facilitate appropriate genetic testing, interpret test results, make necessary subspecialty referrals, and provide attendant psychosocial support for patients and their families. Physicians with subspecialty training in medical genetics are trained in dysmorphology, metabolism, monogenic conditions, genomics, and diagnostic testing and are able to generate a differential, determine a diagnostic evaluation approach, and provide specific management and treatment recommendations for patient care. In addition, geneticists can evaluate family members for other syndromic features and facilitate appropriate genetic testing or referrals. Studies have shown that genetics consultation increases the diagnostic rate of genetic syndromes in infants in the cardiac intensive care unit, as well as in older children with congenital HD seen for follow-up in a cardiac neurodevelopmental clinic.[480,481] Single-site studies have shown underutilization of cytogenetic testing in infants with congenital HD, but multisite studies to address genetic testing practices have not been performed.[482] Carey et al[47] found that pathogenic CNVs are identified

in >10% of single-ventricle forms of congenital HD and that patients with these cytogenetic abnormalities have more adverse outcomes. Dysmorphology evaluation is challenging in infants, and expanded testing identifies abnormalities missed even by trained dysmorphologists.[47] These findings support a more comprehensive, standardized approach to genetic testing in infants with congenital HD. Algorithms have been proposed based on expert recommendation; further evidence-based investigation is necessary.[13,483] Table 9 highlights indications for genetics involvement in patients with congenital HD.

Genetic counseling services are valued by families.[25,484] In the prenatal setting, cardiovascular genetic counseling is important for conveying information about the use and limitations of genetic testing and for providing psychosocial support to the patient and family.[485,486] Recent surveys of adult congenital HD populations have demonstrated that a majority of patients lack accurate understanding of their individual recurrence risk but that provision and recall of genetic information can be significantly improved by incorporating genetics providers into routine cardiovascular care.[484,487] In children, access to genetic services plays an important role in improving diagnostic yield, addressing ongoing health supervision needs of patients with genetic syndromes, and ensuring appropriate subspecialist referral.[481] Unfortunately, the shortage of both geneticists and genetic counselors limits more widespread integration of services within cardiology. Telemedicine services are emerging in response to this need. Many centers are developing triage algorithms and testing new counseling models. Genetic assistants are being piloted to augment genetic counselor functions and expand capacity. Continued integration of genetic evaluation and genetic counseling are important components for improving utilization of increasingly comprehensive and affordable genetic services. Furthermore, it is becoming increasingly important that practitioners in the care of patients with congenital HD develop a level of comfort and expertise in genetic concepts and terminology. With that in mind, the American Heart Association recently published a scientific statement on enhancing provider literacy in cardiovascular genetics.[415]

Recurrence Risk

On the basis of epidemiological studies such as the Baltimore-Washington Infant Study and the Danish national epidemiologic study, syndromic congenital HDs are thought to constitute at least 25% of all congenital HDs.[273,488,489] Identifying the underlying cause of congenital HD in these cases is important for medical management, surveillance, and communication of reproductive risks necessary for family planning. The distinction between syndromic and nonsyndromic, or isolated, congenital HD can be subtle. Technological

advancements in genetic testing have increased the diagnostic yield. Studies of patients with congenital HD do not always apply the same criteria to distinguish syndromic from nonsyndromic cases, and the age of patient evaluation influences assessment.

In general, recurrence estimates are more precise for syndromic than for isolated congenital HDs, because genes and associated inheritance patterns for many congenital HD–associated monogenic conditions are already known. Importantly, not all patients with a particular syndrome will present with structural heart defects, and the proportion who do can vary considerably depending on the specific diagnosis.[13] The presence or severity of a congenital HD in a parent is often not predictive of the risk for offspring. The likelihood of affected individuals reaching reproductive age or having children (reproductive fitness) is related to the new mutation rate that is a common cause of syndromic congenital HDs. As a result, some genetic syndromes that are highly penetrant for congenital HD contribute less to the congenital HD burden in the next generation than is the case for patients with isolated congenital HD or less severe lesions. Epidemiological studies can underestimate the number of familial cases because of the high rate of miscarriages of fetuses with congenital HDs and reproductive decisions to limit future pregnancies in families with a child with a congenital HD.[462]

As genetic testing technologies have evolved to offer higher resolution and higher diagnostic yields than those provided by conventional chromosomal analyses, CNVs have emerged as important causes of both syndromic and nonsyndromic congenital HDs. Moreover, an increasing recognition of contributing environmental[490] and epigenetic factors has revealed a previously unanticipated breadth to congenital HD pathogenesis.[13] Although all mendelian inheritance patterns have been identified in families with congenital HDs, the empiric sibling or offspring recurrence risk across all types of congenital HDs of 1% to 4% suggests that the majority of congenital HDs have a multifactorial pathogenesis.[491,492]

The study of groups of embryologically related cardiac malformations has identified subclasses of congenital HDs with strong familial clustering in first-degree relatives, ranging from 3-fold to 80-fold compared with the prevalence in the population.[273,491] Heritability rates of 70% to 90% support the strong genetic contribution for some types of congenital HDs.[493–495] Three classes of defects with the highest relative risk of recurrence of the same heart defect phenotype are heterotaxy, with a relative risk of 79.1 (95% confidence interval, 32.9–190); right ventricular outflow tract defects, with a relative risk of 48.6 (95% confidence interval, 27.5–85.6); and left ventricular outflow tract obstructive defects, with a relative risk of 12.9 (95% confidence interval, 7.48–22.2).[273] These findings are important because they alter information on recurrence

Table 9. Indications for Consultation With Genetics Professionals

Reason for Referral	Examples/Features	Care Provider
Genetic testing for known familial variant	Facilitation of genetic testing for infant born to a parent with long-QT syndrome	Genetic counselor Geneticist
Pretest or posttest genetic counseling	Provision of educational resources and anticipatory guidance after a positive genetic test result	Genetic counselor Geneticist
Preconception or prenatal genetic counseling regarding recurrence risk	Parent with congenital HD interested in discussing recurrence risk for offspring before pursuing pregnancy	Genetic counselor
Prenatal genetic counseling regarding fetal anomaly	Discussion of prenatal genetic testing options for fetus with congenital HD	Genetic counselor
Family history of congenital HD, other malformations, learning disability, or multiple miscarriages	Three generations with AVSD	Genetic counselor Geneticist
Isolated congenital HD highly associated with specific syndromes	Interrupted aortic arch, truncus arteriosus, etc	Genetic counselor Geneticist
Suspicion of an underlying genetic syndrome	Intellectual disability; learning disability; autism; other cognitive impairment Dysmorphic features Short stature Congenital anomalies Endocrine abnormalities Sensory deficits such as hearing loss or visual impairment Neurological deficits	Genetic counselor Geneticist

AVSD indicates atrioventricular septal defect; and HD, heart disease.

risk based on congenital HD subtype. In the case of left ventricular outflow tract obstructive defect, it also has implications for cardiac screening in family members. Not all families show evidence of similar types of congenital HDs, and familial clustering of discordant congenital HDs has also been documented, which suggests that common genetic pathways might underlie a spectrum of CHDs[496] (reviewed in Landis and Ware[462]). Because congenital HDs are so common, the majority of cases occur in individuals without a family history despite high heritability. Although the incidence of congenital HDs appears to be similar in most populations, there are some specific types of congenital HD that show important differences.[494,497,498] In addition, there is an increased rate of congenital HDs in populations with increased consanguinity, often attributed to autosomal recessive variants.[462] Studies have examined rates of recurrence among first-degree relatives of patients with isolated congenital HDs and collectively suggest an overall risk of 5% to 10% for any congenital HD when either 1 parent or >2 siblings are affected[499–501] or ≈3% with 1 affected child (Table 10; reviewed in Cowan and Ware[13]). Risk estimates for individual defects vary but are generally estimated in the range of 2% to 6%, with higher risk afforded to children of affected mothers (Table 10).[13] These figures are low relative to congenital HDs with demonstrable monogenic inheritance but can still have potentially important implications, particularly with respect to future reproductive decision making and prospective screening of presumably unaffected family members.[13] Family history of congenital HD remains one of the most consistently identified risk factors for identifying congenital HD prenatally.

Preimplantation Genetic Diagnosis

Preimplantation genetic diagnosis is an assisted reproductive technology that allows screening for a genetic condition after in vitro fertilization and before implantation. Preimplantation genetic diagnosis can be used in couples at risk for passing on a genetic condition, including carriers of X-linked disorders, single-gene disorders, and chromosomal disorders.[503,504] Prenatal genetic counselors provide information on risks and benefits of the procedure and work together with reproductive endocrinologists. Preimplantation genetic diagnosis has been used successfully in >100 genetic conditions, including inherited cardiac conditions such as Marfan syndrome, HCM, dilated cardiomyopathy, and muscular dystrophies.[505] Preimplantation genetic diagnosis requires a clear understanding of the cause of a genetic condition within a family.

Prenatal Screening

Until 2011, noninvasive prenatal screening consisted mainly of measurements of maternal serum analytes and ultrasonography. These tests have a false-positive rate of 5% and detection rates of 50% to 95%.[506] These techniques were used to provide families with information to optimize their pregnancy outcomes. In 2011, fcfDNA screening (also referred to as noninvasive prenatal testing, NIPT, or noninvasive prenatal diagnos-

Table 10. Recurrence Risks for Isolated (Nonsyndromic) Congenital HDs

Defect	Father Affected, %	Mother Affected, %	1 Sibling Affected, %	2 Siblings Affected, %
ASD	1.5–3.5	4–6	2.5–3	8
AVSD	1–4.5	11.5–14	3–4	10
VSD	2–3.5	6–10	3	10
AS	3–4	8–18	2	6
PVS	2–3.5	4–6.5	2	6
TOF	1.5	2–2.5	2.5–3	8
CoA	2–3	4–6.5	2	6
PDA	2–2.5	3.5–4	3	10
HLHS	21[495]		2–9*	6
TGA	2[502]		1.5	5
L-TGA	3–5		5–6	NR
EA	NR	6[502]	1	3
TrA	NR	NR	1	3
TA	NR	NR	1	3
PA	NR	NR	1	3

Data from references 499–501 except where otherwise noted. Merged cells indicate recurrence when 1 parent is affected, irrespective of sex, and are used in the absence of sex-stratified risks. AS indicates aortic stenosis; ASD, atrial septal defect; AVSD, atrioventricular septal defect; CoA, coarctation of the aorta; EA, Ebstein's anomaly; HD, heart disease; HLHS, hypoplastic left heart syndrome; L-TGA, congenitally corrected transposition of the great arteries; NR, not reported/insufficient data; PA, pulmonary atresia; PDA, patent ductus arteriosus; PVS, pulmonary valve stenosis; TA, tricuspid atresia; TGA, d-transposition of the great arteries; TOF, tetralogy of Fallot; TrA, truncus arteriosus; and VSD, ventricular septal defect.

*Eight percent recurrence risk for HLHS; up to 22% recurrence risk for any congenital HD.[495]

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tic screening) became clinically available.[507] This new testing uses bioinformatics algorithms and NGS of fetal DNA fragments present in maternal serum to estimate the probability of chromosome aneuploidy in the fetus. The ACMG has provided guidelines for the conditions for use of fcfDNA screening for aneuploidy.[506] These include providing up-to-date genetic counseling concerning the new technique so that families can select diagnostic or screening options according to their personal goals. The ACMG recommends informing all pregnant women that fcfDNA screening is the most sensitive noninvasive screening for aneuploidies such as trisomy 21, trisomy 18, and trisomy 13. One review analysis, published in 2016, found the pooled sensitivities for fcfDNA screening to be 99.3% for trisomy 21, 97.4% for trisomy 18, and 97.4% for trisomy 13.[508] If a positive screen test result is obtained, then the couple should be offered diagnostic testing. Screening for other autosomal aneuploidies besides trisomy 21, 18, and 13 is not yet recommended. The ACMG guidelines recommend offering fcfDNA screening to high-risk families (those with advanced maternal age or fetal anomalies on ultrasound), as well as lower-risk families.

Expanded fcfDNA screening for sex chromosome anomalies and CNVs is also currently offered by some laboratories. fcfDNA screening for sex chromosome abnormalities is less accurate than autosomal aneuploidies because of the potential for maternal X-chromosome biological variation. Clinically significant CNVs are rare in the population, and the positive predictive value is much lower than for whole chromosome aneuploidy. Therefore, at this time, the ACMG guidelines only recommend providing information on the availability of expanded use of fcfDNA screening for sex chromosome abnormalities and CNVs. If fcfDNA screening should identify a CNV or sex chromosome abnormality, then invasive diagnostic testing by chorionic villus sampling or amniocentesis is recommended by the ACMG guidelines to confirm the diagnosis.

If fetal ultrasounds or echocardiograms are abnormal, the American College of Obstetricians and Gynecologists and the Society of Maternal Fetal Medicine recommend prenatal CMA if invasive prenatal diagnosis is performed. Informed consent and comprehensive pretest and posttest genetic counseling are necessary.[509] Establishing a diagnosis before delivery can facilitate medical care plans.

Indications for Fetal Echocardiography

Fetal echocardiography is now widely used to detect, characterize, and help manage congenital cardiac malformations. Indications for performing a fetal echocardiogram have been formulated and were described in a recent American Heart Association scientific statement.[510] In addition to the further evaluation of concerns raised by screening ultrasounds performed as part of standard obstetrical care, specific risk factors for congenital HDs, including those related to maternal health (eg, diabetes mellitus and autoimmune conditions), maternal drug or toxin exposure, abnormalities of umbilical-placental development (eg, single umbilical artery and monochorionic twinning), and known, suspected, or potentially heritable genetic conditions, have achieved sufficient Classification of Recommendations (Class) and Level of Evidence (Level) to warrant performance of a fetal echocardiogram. As it pertains to known, suspected, or potentially heritable genetic conditions, performance of fetal echocardiograms in pregnancies in which the mother, father, or sibling has a congenital cardiac defect have been assigned a Class I/Level B indication, which means that it should be performed and that evidence from limited studies is supportive. As might be expected, the strength of Classification of Recommendation is reduced in more distantly related family members: with congenital HD in a second-degree family member, a fetal echocardiogram may be considered (Class IIb); with the nearest relative(s) with congenital HD being a third-degree or more distant family member(s), a fetal echocardiogram is not recommended (Class III). Similarly, fetal echocar-

diograms should be performed in pregnancies in which there is a heritable condition in a first-degree family member that is associated with a risk of heart defects (such as NS) even if the affected relative does not have a heart defect. However, for heritable cardiac conditions with a later onset of manifestation (such as HCM, Marfan syndrome, or Loeys-Dietz syndrome), fetal echocardiography may not be necessary if screening obstetrical ultrasound does not demonstrate any abnormalities.

Demonstration of noncardiac abnormalities suggestive of a potential genetic syndrome, teratogen, or malformation sequence is also an important indication to perform a fetal echocardiogram. The risk of a concomitant congenital HD in the fetus with another anomaly varies, but abnormalities of the central nervous system (microcephaly, hydrocephaly, agenesis of the corpus callosum, or other structural abnormalities), gastrointestinal system/abdomen (esophageal or duodenal atresia, diaphragmatic hernia, or omphalocele/gastroschisis), kidney (structural abnormalities), craniofacial structures, or limbs should prompt referral for evaluation with a fetal echocardiogram.[511] In addition, unexplained fetal growth delay[512] or features such as increased nuchal translucency[513] are associated with a significant risk of congenital cardiac abnormalities and are indications for performing a fetal echocardiogram even if fcfDNA screening does not detect a chromosomal abnormality. Recent data indicate that of fetuses with increased nuchal translucency and normal karyotype, ≈10% had positive testing for NS.[514] Furthermore, persistence of nuchal translucency into the second trimester as nuchal edema identified a high prevalence of NS (11 of 15 cases).[515]

SUMMARY

Our understanding of the role of genetics in the pathogenesis of congenital HD has advanced at a rapid pace over the past 10 to 15 years. The availability of new molecular techniques has facilitated gene discoveries that have changed the medical and cardiological care of many individuals with congenital HD. CNV detection and NGS gene panels are now in widespread use by geneticists, genetic counselors, and cardiologists for accurate diagnosis of congenital HD patients. Cardiovascular genetics clinics are now available in many major medical centers in the United States. Accurate diagnosis of congenital HD pathogenesis is allowing for determination of familial recurrence risks, providing reproductive options, identifying extracardiac manifestations of the genetic diagnosis that could affect clinical care, and improving long-term medical decisions in the care of congenital HD. Additionally, WES is now used in many centers for those congenital HD patients suspected of having a genetic disorder when no pathogenetic diagnosis was obtained by other molecular testing.

The future of understanding of other genetic factors important in the causation of congenital HD will be determined by (1) studies with larger numbers of individuals with congenital HD using existing technologies (eg, WES), as well as application of other "omic" approaches (eg, whole genome sequencing, DNA methylation analysis, RNA sequencing with discarded cardiac tissues, and increasingly advanced bioinformatics analyses), and (2) new research with animal and cell models to utilize innovative molecular technologies to study RNA expression, splicing alterations, signaling technology, transcription factor fate, and epigenetic processes.[13] Current induced pluripotent stem cell and gene-editing approaches have enabled the study of human cardiomyocytes relevant for congenital HD, whereas advances in generating tissues and organoids could allow the study of genetic variation relevant to congenital HD in contexts more relevant physiologically and developmentally in the near future.

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*Modest.

†Significant.

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*Significant.

APPENDIX

Appendix. Chromosomal Aneuploidies and Copy Number Variants Associated With Congenital HD[1,177]

Chromosome Change	Main Features	Percent With Congenital HD	Heart Anomaly	References
I. Aneuploidies (identifiable by routine karyotype)				
Trisomy 8 mosaicism	Widely spaced eyes, broad nasal bridge, small jaw, high arched palate, cryptorchidism, renal anomalies, skeletal/vertebral anomalies	25	VSD, PDA, CoA, PVS, TAPVR, TrA	516
Trisomy 9/mosaicism	Prenatal and postnatal growth retardation, microcephaly, deep-set eyes, low-set ears, severe intellectual disability	65	PDA, LSVC, VSD, TOF/PA, DORV	517
Trisomy 13 (Patau syndrome)	Cleft lip and palate, scalp defects, hypotelorism, microphthalmia or anophthalmia, colobomata of irides, holoprosencephaly, microcephaly, deafness, severe intellectual disability, rib abnormalities, polydactyly, omphalocele, renal abnormalities, hypospadias, cryptorchidism, uterine abnormalities	57–80	ASD, VSD, PDA, HLHS, laterality defects, atrial isomerism	518, 519
Trisomy 18 (Edwards syndrome)	IUGR, polyhydramnios, micrognathia, short sternum, hypertonia, rocker-bottom feet, overlapping fingers and toes, TEF, CDH, omphalocele, renal anomalies, biliary atresia, severe intellectual disability	80–90	ASD, VSD, PDA, TOF, DORV, TGA, CoA, BAV, BPV, polyvalvular nodular dysplasia	518
Trisomy 21 (Down syndrome)	Hypotonia, hyperextensibility, epicanthal folds, up-slanting palpebral fissures, single palmar transverse crease, clinodactyly of fifth finger, brachydactyly, variable intellectual disability, premature aging	40–50	AVSD, VSD, ASD, (TOF, TGA less common)	33, 37
Monosomy X (Turner syndrome, 45,X)	Lymphedema of hands and feet, widely spaced hypoplastic nipples, webbed neck, primary amenorrhea, short stature, normal intelligence or mild learning disability	23–35	CoA, BAV, AS, HLHS, aortic dissection	41, 520
II. Chromosome abnormalities (identifiable on karyotype and more recently using chromosomal microarray)				
3p25 deletion	Prenatal and postnatal growth deficiency, polydactyly, microcephaly, intellectual disability, renal anomalies	33	VSD, AVSD, tricuspid atresia	521
Deletion 4p16.3 (Wolf-Hirschhorn syndrome)	Microcephaly, widely spaced eyes, broad nasal bridge (Greek helmet appearance), downturned mouth, micrognathia, preauricular skin tags, severe intellectual disability, seizures, growth retardation	50–65	ASD, VSD, PDA, LSVC, aortic atresia, dextrocardia, TOF, tricuspid atresia	522
Deletion 4q	Growth retardation, intellectual disability, cleft palate, broad nasal bridge, micrognathia, abnormal ears, genitourinary defects	50	VSD, PDA, AS, ASD, TOF, CoA	523
Deletion 5p (cri-du-chat)	Catlike cry, prenatal and postnatal growth retardation, round face, widely spaced eyes, epicanthal folds, single palmar transverse crease, severe intellectual disability	30–60	VSD, ASD, PDA	524, 525
Deletion 9p syndrome	Craniosynostosis, trigonocephaly, up-slanting palpebral fissures, abnormal ear pinnae, scoliosis, micropenis, cryptorchidism, intellectual disability	35–50	VSD, PDA, PVS	526
Deletion 10p	Frontal bossing, short down-slanting palpebral fissures, small low-set ears, micrognathia, cleft palate, short neck, urinary/genital and upper-limb anomalies	42	BAV, ASD, VSD, PDA, PVS, CoA	527
Duplication 10q24-qter	Prenatal growth retardation, intellectual disability, camptodactyly, renal anomalies, cryptorchidism	50	AVSD, VSD	528
III. Copy number variants (identifiable by chromosomal microarray)				
1p36 deletion	Growth deficiency, intellectual disability, microcephaly, deep-set eyes, low-set ears, hearing loss, hypotonia, seizures, CNS defects, genital anomalies	70	PDA, VSD, ASD, BAV, Ebstein anomaly, noncompaction cardiomyopathy	99
1q21.1 deletion	Short stature, microcephaly, colobomas, microphthalmia, hearing loss, seizures, mild intellectual disability, autism spectrum disorder, skeletal malformations	N/A	PDA, VSD, ASD, TrA, TOF	102, 105
1q21.1 duplication	Large head size, hemivertebrae, variable intellectual disability, variable autistic features, hypospadias, clubfoot	N/A	TOF, TGA, PVS	105

(Continued)

Appendix. Continued

Chromosome Change	Main Features	Percent With Congenital HD	Heart Anomaly	References
III. Copy number variants (identifiable by chromosomal microarray) (Continued)				
1q41q42 microdeletion	Growth retardation, intellectual disability, microcephaly, diaphragmatic hernia, seizures, short limbs	40	BAV, ASD, VSD, TGA	529
1q43q44 microdeletion	Prenatal and postnatal growth retardation, intellectual disability, limited speech, microcephaly, deep-set eyes, microcephaly, large low-set ears, cleft palate, agenesis of corpus callosum	N/A	VSD, CoA, HLHS	530
2q31.1 microdeletion	Prenatal and postnatal growth retardation, large ventricles, microcephaly, narrow forehead down-slanting palpebral fissures, cleft palate/cleft lip, limb defects, hypoplastic genitalia	25	VSD, ASD, PDA	531
2q37 microdeletion	Short stature, obesity, intellectual disability, sparse hair, arched eyebrows, epicanthal folds, thin upper lip, small hands and feet, clinodactyly	30	VSD, ASD, CoA, hypoplastic aortic arch	532, 533
Deletion 7q11.23(Williams-Beuren syndrome)	Infantile hypercalcemia, skeletal and renal anomalies, cognitive deficits, "social" personality, elfin facies	53–85	Supravalvar AS and PS, PPS	41, 74
8p23.1 deletion	Microcephaly, growth retardation, deep-set eyes, malformed ears, small chin, genital anomalies in males, intellectual disability	50–75	AVSD, PVS, VSD, TOF	112
9q34.3 Subtelomeric deletion (Kleefstra syndrome)	Short stature, obesity, intellectual disability, microcephaly, behavior abnormalities, brain anomalies, hypertelorism, arched eyebrows, midface hypoplasia	31–44	ASD, VSD, TOF, pulmonary arterial stenosis	534
Deletion 11q (Jacobsen syndrome)	Growth retardation, developmental delay, thrombocytopenia, platelet dysfunction, widely spaced eyes, strabismus, broad nasal bridge, thin upper lip, prominent forehead, intellectual disability	56	HLHS, AS, VSD, CoA, Shone's complex	91, 92
15q24 microdeletion	Prenatal and postnatal growth retardation, intellectual disability, abnormal corpus callosum, microcephaly, high forehead, down-slanting palpebral fissures, tapered eyebrows, abnormal ear pinnae, hearing loss, hypospadias, scoliosis, coloboma, strabismus	40	PDA, pulmonary arterial stenosis, PVS	535
16p11.2p12.2 microdeletion	Hypotonia, intellectual disability, long narrow face, deep-set eyes, low-set malformed ears	33	TOF, BAV, pulmonary atresia	536
17q21 microdeletion	Abnormal hair pigmentation, up-slanting palpebral fissures, epicanthal folds, bulbous nasal tip, strabismus, ptosis, long slender fingers, hip dislocation, renal anomalies, spine deformities, cryptorchidism, global developmental delay	27	PVS, ASD, VSD, BAV	537
Deletion 20p12 (Alagille syndrome)	Bile duct paucity, cholestasis, skeletal or ocular anomalies, broad forehead, widely spaced eyes, underdeveloped mandible	85–94	Peripheral PA hypoplasia, TOF, PVS (left-sided heart lesions and septal defects less common)	538
22q11.2DS (DiGeorge, velocardiofacial, and conotruncal anomaly face syndrome)	Hypertelorism, micrognathia, low-set posteriorly rotated ears, thymic and parathyroid hypoplasia, hypocalcemia, feeding/speech/learning/behavioral disorders, immunodeficiency, palate/skeletal/renal anomalies, learning disability	75	IAA-B, TrA, isolated aortic arch anomalies, TOF, conoventricular VSD	60, 70
22q11.2 duplication	Very variable phenotype, some with velopharyngeal insufficiency, cleft palate, hearing loss, minor facial anomalies, mild learning disability to normal learning ability, hypotonia, scoliosis, frequent infections	15	TOF, HLHS, VSD, PVS, TrA	66
22q13 microdeletion (Phelan-McDermid syndrome)	Normal growth, intellectual disability, dolichocephaly, dysplastic ears, pointed chin, large fleshy hands, hypotonia	>25	PDA, VSD, ASD, TAPVR	539

22q11.2DS indicates 22q11.2 deletion syndrome; AS, aortic stenosis; ASD, atrial septal defect; AVSD, atrioventricular septal defect; BAV, bicuspid aortic valve; BPV, bicuspid pulmonary valve; CDH, congenital diaphragmatic hernia; CoA, coarctation of the aorta; DORV, double-outlet right ventricle; HD, heart disease; HLHS, hypoplastic left heart syndrome; IAA-B, interrupted aortic arch type B; IUGR, intrauterine growth retardation; LSVC, persistent left superior vena cava; N/A, not available; PA, pulmonary artery; PDA, patent ductus arteriosus; PPS, peripheral pulmonary stenosis; PS, pulmonary stenosis; PVS, pulmonic valve stenosis; TAPVR, total anomalous pulmonary venous return; TEF, tracheoesophageal fistula; TGA, d-transposition of the great arteries; TOF, tetralogy of Fallot; TOF/PA, tetralogy of Fallot with pulmonary atresia; TrA, truncus arteriosus; and VSD, ventricular septal defect.

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