Genetic Basis for Congenital Heart Defects: Current Knowledge: A Scientific Statement From the American Heart Association Congenital Cardiac Defects Committee, Council on Cardiovascular Disease in the Young: Endorsed by the American Academy of Pediatrics

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AHA Scientific Statement

Genetic Basis for Congenital Heart Defects: Current Knowledge

A Scientific Statement From the American Heart Association Congenital Cardiac Defects Committee, Council on Cardiovascular Disease in the Young

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Mary Ella Pierpont, MD, PhD, Chair; Craig T. Basson, MD, PhD, FAHA; D. Woodrow Benson, Jr, MD, PhD, FAHA; Bruce D. Gelb, MD; Therese M. Giglia, MD; Elizabeth Goldmuntz, MD; Glenn McGee, PhD; Craig A. Sable, MD; Deepak Srivastava, MD; Catherine L. Webb, MD, MS, FAHA

***Abstract*—**The intent of this review is to provide the clinician with a summary of what is currently known about the contribution of genetics to the origin of congenital heart disease. Techniques are discussed to evaluate children with heart disease for genetic alterations. Many of these techniques are now available on a clinical basis. Information on the genetic and clinical evaluation of children with cardiac disease is presented, and several tables have been constructed to aid the clinician in the assessment of children with different types of heart disease. Genetic algorithms for cardiac defects have been constructed and are available in an appendix. It is anticipated that this summary will update a wide range of medical personnel, including pediatric cardiologists and pediatricians, adult cardiologists, internists, obstetricians, nurses, and thoracic surgeons, about the genetic aspects of congenital heart disease and will encourage an interdisciplinary approach to the child and adult with congenital heart disease. **(*Circulation*. 2007;115:3015-3038.)**

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The goal of this review is to provide more information for clinicians on the expanding knowledge of the involvement of genetic contributions to the origin of congenital heart disease (CHD). There has been a long-standing clinical view that most CHD occurs as isolated cases. On the basis of studies of recurrence and transmission risks, a hypothesis of multifactorial etiology was proposed.[1] In this type of inheritance, the genetic predisposition of the individual interacts with the environment to cause the congenital heart defect. In recent years, separate environmental[2] and genetic causes have been identified. Classic mendelian transmission of congenital heart defects in some families has been described in the literature. In the past decade, molecular genetic studies have exploited these observations of families with multiple affected individuals and have provided insights into the genetic basis of several forms of CHD, such as atrial septal defect or patent ductus arteriosus.[3,4] These initial discoveries demonstrate that the genetic contribution to CHD has been significantly underestimated in the past. This review includes descriptions of the currently available diagnostic tools and their applications. Some syndromes, including DiGeorge syndrome, Williams-Beuren syndrome, Alagille syndrome, Noonan syndrome (NS), and Holt-Oram syndrome, have been highlighted in the text for the purpose of illustrating some of these new technologies. For further clinical details, interested readers are referred to a genetics textbook such as *Smith's Recognizable Patterns of Human Malformation*.[5]

In reading this review, it is important to remember that human cardiovascular genetics is in the early phase of gene discovery; consequently, the field is changing rapidly. Ge-

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netic testing of embryos, fetuses, children, and adults, in both research and clinical settings, is expanding more quickly than are regulatory and surveillance programs. As part of these changes, clinically available genetic tests for various forms of CHD move from the research laboratory to the bedside or clinic at variable speeds. The pace of discovery is such that today's state of the art quickly becomes outdated. As a means of keeping abreast of the latest genes and availability of testing, the reader is referred to online resources such as Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/omim/) and GeneTests (http://www.genetests.org/), which are updated regularly.

Prevalence of CHD

Cardiac malformations present at birth are an important component of pediatric cardiovascular disease and constitute a major percentage of clinically significant birth defects, with an estimated prevalence of 4 to 50 per 1000 live births. For example, it is estimated that 4 to 10 liveborn infants per 1000 have a cardiac malformation, 40% of which are diagnosed in the first year of life.[6,7] The true prevalence, however, may be much higher. For example, bicuspid aortic valve, the most common cardiac malformation, is usually excluded from this estimate. Bicuspid aortic valve is associated with considerable morbidity and mortality later in life and by itself occurs in 10 to 20 per 1000 in the general population.[8] Recent studies are finding a high degree of heritability of bicuspid aortic valve, alone and with other cardiovascular anomalies, especially left ventricular outflow tract obstructive disorders.[9–11] When isolated aneurysm of the atrial septum and persistent left superior vena cava, each of which occurs in 5 to 10 per 1000 live births, are taken into account, the incidence of cardiac malformations approaches 50 per 1000 live births.[12] The incidence of ventricular septal defect (VSD) has also been demonstrated to be as high as 5% in 2 independent cohorts of 5000 serial newborns and 5000 serial premature infants in Israel.[13,14] In light of the above considerations, an incidence of CHD of 50 per 1000 live births is a conservative estimate.[15,16]

In the year 2000, the prevalence of CHD in the pediatric population was estimated at approximately 623 000 (320 000 with simple lesions, 165 000 with moderately complex disease, and 138 000 with highly complex CHD).[16] Tremendous advances in medical and surgical care of children with CHD over the past decade have made survival into adulthood a reality. At the time of the Bethesda Conference in 2000, an estimated total of 787 000 adults were living with CHD (368 800 with simple disease, 302 500 with moderately complex disease, and 117 000 with highly complex disease).[17,18] This assessment of prevalence in the adult population is likely low, because many adult patients, particularly minorities, have been lost to follow-up. It has been estimated that the population of adults with CHD is growing by ≈5% per year, which predicts that the total adult CHD population likely reached 1 million by 2005.[19] This means that the number of adults living with CHD has for the first time surpassed the number of children with CHD. Clearly, it is imperative that many disciplines within the medical community, including adult cardiologists and thoracic surgeons, internists, obstetricians, family practitioners, and ancillary healthcare personnel, acquire an understanding of CHD and its inheritance so that proper lifetime care can be provided for this burgeoning patient population, which to date has been largely unfamiliar to all but pediatricians and pediatric cardiologists.

Importance of Identifying the Genetic Basis of CHD

Extraordinary diagnostic precision and definitive therapies with relatively low morbidity and mortality characterize the state of the art in the management of most CHD (eg, the arterial switch operation for transposition of the great arteries or device closure of intracardiac shunts). These types of therapies indicate that more and more individuals with CHD are going to live to adulthood and may have the opportunity to reproduce. Although there have been tremendous advances in diagnosis and treatment of CHD, our knowledge of the causes of CHD has been limited but has advanced in recent years. Despite the many advanced therapies currently available for a number of heart defects, significant morbidity and mortality are still associated with some types of CHD, for example, hypoplastic left heart syndrome. Improved understanding of possible causes will permit insight into the pathobiological basis of the congenital heart problem and allow definition of disease risk, 2 critical elements for disease prevention. For the clinician caring for a child with CHD, it is very important to determine whether there is an underlying genetic pattern (eg, deletions, duplications, or mutations), for the following reasons: (1) there may be other important organ system involvement; (2) there may be prognostic information for clinical outcomes; (3) there may be important genetic reproductive risks the family should know about; and (4) there may be other family members for whom genetic testing is appropriate. The following sections describe currently available techniques for evaluating infants and children with CHD.

Current Genetic Techniques for Evaluation of Congenital Heart Defects

Congenital heart defects often occur in the setting of multiple congenital anomalies, including abnormal facial features, or in association with limb anomalies, other organ malformations, developmental abnormalities, or growth abnormalities. We now have a number of genetic tests that can assist the clinician in diagnosing genetic alterations in the child with CHD. These include cytogenetic techniques, fluorescence in situ hybridization (FISH), and DNA mutation analysis. After discussion of these techniques, some syndromes that illustrate the use of these genetic techniques will be highlighted, and finally, a suggested approach for comprehensive assessment of these children is provided with an algorithm.

Chromosome Analysis

Before the availability of advanced cytogenetic techniques such as FISH, standard chromosome analysis revealed chromosomal aberration in 8% to 13% of neonates with CHD.[20] With improved resolution in cytogenetic analysis and the availability of molecular techniques, the prevalence of chromosomal abnormalities in selected congenital heart defects is now estimated to be much higher.[21] In contrast, of all children with chromosomal abnormalities, at least 30% have a con-

genital heart defect, with the incidence varying from that of the general population to nearly 100%, as in trisomy 18.[22] Therefore, chromosomal analyses in children with various types of CHD, especially if they have other organ system anomalies, is currently an important part of their medical evaluation (Appendix 1).

The standard metaphase karyotype (450 to 550 bands) is diagnostic for many chromosomal disorders, especially those of chromosome number such as trisomy (trisomy 21) or monosomy (45,X or Turner syndrome). A more sensitive test, high-resolution banding, evaluates chromosomes in prometaphase, which allows for the visualization of a greater number of bands (550 to 850 bands) than the standard karyotype. This technique better defines chromosomal structural abnormalities such as duplications, translocations between chromosomes, and interstitial or terminal deletions.[23] In most centers, 7 to 14 days is required for standard karyotyping and up to 3 weeks for high-resolution banding. More advanced cytogenetic techniques, such as FISH, are required to diagnose more subtle structural abnormalities, such as microdeletions, tiny duplications, and/or subtle translocations. FISH probes (see below) for chromosomes 13, 18, and 21 are currently available for use on interphase (nondividing) cells to diagnose chromosomal trisomies in a more timely fashion, ie, 1 to 2 days, as would be helpful if one of these trisomies were suspected in a neonate.[24]

Chromosomes can be analyzed from a number of sources, including peripheral blood lymphocytes, cord blood, skin fibroblasts, amniotic fluid, chorionic villi, and bone marrow, with peripheral blood most commonly used. Prior blood product transfusions are not likely to interfere with chromosome testing considering the small volume of the transfusion in relation to the total blood volume of the patient, and especially if leukoreduced and/or irradiated blood products have been used.[25]

Amniotic fluid cells are the primary means of prenatal chromosomal diagnosis. Amniocentesis is routinely performed at 15 to 16 weeks' gestation. Amniotic fluid cells, however, take 1 to 2 weeks to grow and harvest before karyotyping can be done. Chorionic villus sampling involves the biopsy of tissue from the villous area of the chorion transcervically or transabdominally, between 10 and 12 weeks' gestation. These results are usually available in 10 to 14 days. The major advantage of chorionic villus sampling compared with midtrimester amniocentesis is that chorionic villus sampling allows the results to be available at an earlier stage of the pregnancy, which reduces the period of uncertainty.

In the current era of in vitro fertilization, preimplantation genetic diagnosis for chromosomal abnormalities/aneuploidies and single-gene defects has recently become possible.[26] Preimplantation genetic diagnosis provides chromosomal and mutational analysis of blastocysts that result from in vitro fertilization before implantation. Preimplantation genetic diagnosis is primarily used by patients choosing assisted reproductive services who have concerns regarding risks of specific genetic disorders. The techniques used for prenatal or preimplantation diagnosis have inherent risks and benefits, which should be discussed on an individual basis with the treating physician. For more detail, the reader is referred to recent reviews of prenatal or preimplantation diagnosis.[27,28]

FISH Technology

FISH is a method by which biotinylated test and control DNA probes are hybridized with metaphase chromosomes to determine whether 1 (deletion), 2 (normal), or 3 (duplication) copies of the test region are present.[29] Specific DNA probes can be located by fluorescence microscopy and will identify well-known deletion syndromes such as del 5p (cri-du-chat). Other fluorescent DNA probes are useful in determining microdeletion syndromes that cannot be detected visually. Several disorders, including Williams-Beuren, Alagille, and the 22q11 deletion syndromes, have been associated with a consistent microdeletion that frequently can be detected only by FISH technology. This technology is widely available in almost every cytogenetics laboratory for the syndromes noted.

Telomere Analysis by Subtelomere FISH

Tiny deletions, duplications, or subtle translocations involving the most distal ends of each chromosome (telomeres) may be quite difficult to detect by standard or high-resolution karyotype techniques. Newly developed fluorescent DNA probes for many interstitial chromosomal regions now provide the ability to detect abnormalities that involve the subtelomere-telomere regions (subtelomere FISH). The distal segments of the chromosomal telomeres are composed of telomere-associated repeat sequences, and these extend 100 to 300 kb from the terminal repeat sequences.[30] Chromosome-specific unique sequences are present in these terminal regions, and fluorescent DNA probes can be specifically targeted to these areas. The subtelomere regions are thought to contain a very high concentration of genes; thus, rearrangements in these regions may have a significant impact on the phenotype of the individual.[31] Subtelomere FISH probes with fluorescent DNA have been commercially developed for each end of the chromosome arms except for the short arms of the acrocentric (centromere near 1 end) chromosomes.[32] If the karyotype is normal in a patient with dysmorphic facial features, congenital anomalies, developmental delay, and mental retardation, then the clinician should consider ordering subtelomere FISH studies for further genetic evaluation.

Cardiac malformations reported to date in children with subtelomere chromosomal rearrangements include aortic arch anomalies, VSD, atrial septal defect, mitral valve insufficiency, and concomitant pulmonary stenosis with VSD.[33,34] Most of the published studies of subtelomere abnormalities indicate that a 4% to 9% prevalence of subtle chromosome rearrangements can be detected in children or adults with microcephaly, hydrocephaly, tracheoesophageal fistula, skeletal anomalies, multiple congenital anomalies, polycystic kidney, duodenal atresia, syndactyly, epilepsy, mental retardation, developmental delay, and/or dysmorphic facial features.[30,35]

The use of subtelomeric FISH analysis has significant utility in individuals with normal karyotypes, especially if there are multiple congenital anomalies that include mental

retardation or CHD.[36] By finding a tiny deletion, duplication, or unbalanced translocation, further investigation of other family members can uncover the exact genetic risks faced by the family and the affected individual. As many as 50% of families can have other individual members with subtelomeric abnormalities.[37] Because some polymorphic variants and cross-hybridizations of subtelomeric FISH probes are known,[30] families in whom a subtelomeric abnormality is identified should be seen by a medical genetics specialist to provide appropriate evaluation and counseling.

Methods of Gene Discovery

Initial strategies of gene discovery were directed toward isolating a protein of interest, sequencing a portion of it, and then cloning the gene that produces that protein. This approach works well for disorders for which the function of the target protein is obvious and facilitates its identification, eg, Pompe disease (acid α-glucosidase deficiency). Currently, disease gene discovery can be accomplished by positional cloning, a candidate gene approach, or a combination of these 2 methods.[38] Positional cloning has been referred to as reverse genetics. In this paradigm, investigators study families with affected individuals to identify a position on a chromosome that must contain the disease gene of interest, utilizing linkage analysis. That disease gene is then identified from among the set of all genes residing in that chromosomal region through cloning techniques. An example of the successful use of this strategy was the identification of the *NKX2.5* gene, for which the locus was defined from linkage analysis of large families.[3] Some investigators have used this approach to identify a CHD gene in a syndromic disorder that is a single-gene trait. This approach is far less robust for finding disease genes when the disorder arises in a more complex genetic fashion or is heterogeneous, for example, patent ductus arteriosus.[4] This may be the case for many forms of CHD. Using the candidate gene approach, investigators look for mutations in genes that encode proteins with relevance to the process in question. For CHD, this means that genes that control the formation and development of the heart (also known as cardiogenic genes) are candidates. A combination of these 2 methods, or the positional candidate approach, uses linkage analysis or identification of karyotypic abnormalities to find a region of a chromosome likely to contain the gene of interest. Candidate genes (cardiogenic) in that particular chromosomal region are then evaluated for mutations.

DNA Mutation Analysis

The cytogenetic methods described above identify large changes in chromosome number or structure. However, in certain disorders, changes occur at the level of a single gene and must be detected by alternative techniques. Genes are complex structures that include not only regions coding for the protein itself but also other sequences involved in regulation of gene activity. Currently, the coding region for the protein is evaluated for sequence changes for which the biological significance of an altered coding sequence can generally be interpreted. In contrast, the regulatory domains are not usually studied for sequence changes, because the regulatory domains for the gene may not be known, and the biological significance of the altered sequence is difficult to interpret.

Mutation analysis identifies changes in the coding sequence of the gene, including small deletions, insertions, or substitutions of nucleotides that alter the encoded amino acid and consequently protein structure. Most methods employ polymerase chain reaction–based assays. Indirect screening methods, such as denaturing high-performance liquid chromatography[39] or single-strand conformation polymorphism,[40] have been used extensively. More expensive exon-by-exon sequencing of genomic DNA has recently emerged. Additionally, newer, more cost-effective direct sequence analysis methods have become available.[41] Such testing is usually done on DNA obtained from peripheral blood lymphocytes, but other tissues, such as skin, liver, muscle, buccal cells, or saliva, can be used, depending on their availability. DNA testing technology does have some limitations. For example, several types of mutations, including large deletions, other chromosomal structural abnormalities, and some changes that cause splicing errors, are difficult to detect by these approaches.

Once a sequence variation is identified, it is important to consider whether this variation is disease related. The basic criteria used to establish the disease-causing potential of the nucleotide sequence change are that it (1) is predicted to alter the gene coding sense, gene splice site, or regulatory region of the encoded protein; (2) segregates with disease in a kindred; and (3) is not found in unrelated, unaffected control chromosomes. The occurrence of a change in an evolutionarily conserved sequence domain provides additional support that the sequence change is disease causing. Although each of these criteria should be met by any disease-causing mutation, supporting evidence will come from the demonstration that affected individuals from other unrelated families have mutations in the same gene.

Another major problem is the interpretation of the biological importance of mutations. In many instances, little is known of the role of the normal gene product in cardiac development or function, and in some instances, genes were not known to have any role in the heart before mutation identification (eg, in Alagille syndrome). To date, a variety of mutations that cause pediatric cardiovascular disease, including missense and frameshift mutations, have been identified. The extent and heterogeneity of the genes and the mutations identified thus far suggest that they are associated with a variety of pathogenetic mechanisms, including loss of expression, inactivation, or loss of function or gain of function of the mutated allelic products. The challenge of the future is to define the pathogenesis of disease-causing mutations, which in turn will provide opportunities to develop diagnostic and therapeutic strategies as alternatives to those now used.

Loci and Genes Associated With Congenital Heart Defects Identified to Date

Deletion Syndromes Identified by FISH Technology

DiGeorge Syndrome

DiGeorge syndrome was originally considered to be a rare developmental field defect encompassing derivatives of the branchial arch/pharyngeal pouch system.[42,43] The syndrome is characterized by aplasia or hypoplasia of the thymus, aplasia or hypoplasia of the parathyroid glands, cardiac malformations, and specific facial features. Infants present with CHD, hypocalcemia, immunodeficiency, and facial dysmorphia. Ten to twenty percent of patients with DiGeorge syndrome have visible alterations that result in the loss of the proximal long arm of 1 copy of chromosome 22.[44] On FISH, ≈90% of patients with the DiGeorge phenotype have a microdeletion of part of 1 copy of chromosome 22.[45] The prevalence of the 22q11 deletion has been estimated at 1 per 5950 live births.[46]

Subsequently, it has been shown that patients with the clinical diagnosis of DiGeorge, velocardiofacial (Shprintzen), or conotruncal anomaly face syndromes most often share a common genetic origin, namely, a 22q11 deletion.[47] Not all patients with the clinical features of these syndromes have a 22q11 deletion, consistent with heterogeneous causes for the clinical features. For instance, some patients with similar clinical features may have a small deletion of the short arm of chromosome 10, or some of these features may also result from maternal diabetes mellitus or maternal alcohol use.

The clinical features of the 22q11 deletion syndrome are highly variable between affected individuals, even when they are related.[48] The most common features include cardiovascular anomalies, palate anomalies, feeding disorders, speech and learning disabilities, renal anomalies, and behavioral disorders. Other abnormalities may include hypocalcemia, immunodeficiency, skeletal abnormalities, and growth hormone deficiency. Typical facial features may also include tubular nose, hypoplastic alae nasi, bulbous tip nose, low-set and/or dysplastic ears, and myopathic facies. A 22q11 deletion is inherited in an autosomal dominant fashion from a parent in approximately 6% to 28% of cases.[48] In many familial cases, one of the parents is found to have a 22q11 deletion only after their child with CHD has been diagnosed as affected. All parents affected with 22q11 deletions are then found on further analysis to have subtle syndromic features that were not recognized previously.[48,49] Also, given that approximately 6% to 28%[48] of parents are found to carry the deletion, this has significant implications for future pregnancies, because there is a 50% chance that the deletion-bearing chromosome from an affected parent will be transmitted to the offspring. This is very important information for genetic family counseling.

The most common cardiovascular defects associated with a 22q11 deletion include tetralogy of Fallot, interrupted aortic arch type B, truncus arteriosus, conoventricular VSDs, and aortic arch anomalies.[50–52] Pulmonary stenosis, atrial septal defects, heterotaxy syndrome, and hypoplastic left heart syndrome have also been reported.

TABLE 1. Estimated 22q11 Deletion Frequency in Congenital Heart Disease

Cardiac Defect	Estimated Deletion Frequency, %	Reference(s)
Interrupted aortic arch	50–89	56, 57
VSDs	10	58
With normal aortic arch*	3	
With aortic arch anomaly†	45	
Truncus arteriosus	34–41	51, 56, 59–61
Tetralogy of Fallot	8–35	51, 56, 59, 61, 62
Isolated aortic arch anomalies	24	55
Double-outlet right ventricle	<5	51, 56, 59
Transposition of the great arteries	<1	51, 59

*Left-sided aortic arch with normal branching pattern.

†Includes right aortic arch and/or abnormal branching pattern, cervical location, and/or discontinuous branch pulmonary arteries.

Several studies have demonstrated that a 22q11 deletion is commonly found in a subset of patients with specific types of CHD (Table 1). Individuals with both a cardiac defect and an aortic arch anomaly (right aortic arch, cervical location, or abnormal branching pattern) are more likely to have a 22q11 deletion, as are a subset of patients with tetralogy of Fallot associated with absent pulmonary valve syndrome or aortopulmonary collaterals.[53–55] Children with double-outlet right ventricle or transposition of the great arteries are rarely found to have a 22q11 deletion (Table 1).[51,55–62]

It is important to identify the cardiac patient with a 22q11 deletion by FISH testing to evaluate for associated noncardiac features of the syndrome in a timely fashion and to offer accurate genetic counseling. Additionally, a higher operative mortality in some individuals with a 22q11 deletion has been documented,[63,64] and the clinician and surgeon should be aware of this when planning surgery and postoperative care, particularly as related to calcium metabolism or immunologic issues.

Discussions have centered around which cardiac patients should be routinely tested for a 22q11 deletion and at what age. It appears reasonable to test all infants with interrupted aortic arch type B or truncus arteriosus for a 22q11 deletion given the high frequency of a 22q11 deletion in those patients (Table 1). Using the same logic, data also support the testing of all infants with tetralogy of Fallot and one of the following associated features: absent pulmonary valve syndrome, aortic arch anomalies (including right aortic arch), pulmonary artery anomalies, or aortopulmonary collaterals (Table 1).[53–55] A high frequency of 22q11 deletion also supports testing of patients with both perimembranous VSD and associated aortic arch abnormalities[58] or those with isolated aortic arch abnormalities[55] (Table 1).

Much debate on testing strategies has focused on infants with tetralogy of Fallot who have a normal aortic arch and branching pattern. This subset comprises a large patient population, of which 6% are estimated to have a 22q11 deletion.[51] To clinically detect the deletion-bearing patient, the infant should be evaluated for hypocalcemia, thymic size, typical facial features, palate anatomy, or nasal regurgitation

TABLE 2. Age-Related Features of the 22q11 Deletion Syndrome

Newborn/infant age group
Specific types of congenital heart disease (interrupted aortic arch, truncus arteriosus, tetralogy of Fallot, VSD, aortic arch anomaly)
Aortic arch anomaly or discontinuous branch pulmonary arteries
Overt or submucous cleft palate, high arched palate, bifid uvula
Absent, hypoplastic, or abnormally located thymus
Hypocalcemia
Nasal regurgitation of feeds
Feeding disorders/failure to thrive/gastroesophageal reflux
Facial dysmorphia (especially abnormal ear or nose)
Toddler/school-aged child
Findings detailed above
Feeding disorders
Delayed emergence in speech
Hypernasal speech
Learning disabilities
Behavioral disorders, including attention deficit hyperactivity disorder (ADHD)
Adolescent/adult
Findings detailed above
Psychiatric disorders, including bipolar disorders and/or schizophrenia

TABLE 3. Suggested Testing Strategy for a 22q11 Deletion in the Congenital Heart Disease Population

All newborns/infants with:
IAA
TA
TOF
VSD* with AAA
Isolated AAA
Discontinuous branch pulmonary arteries
Any newborn/infant/child with CHD and another feature of the 22q11 deletion syndrome
Any child/adolescent/adult with TOF, TA, IAA, VSD, or AAA not previously tested who has 1 other feature of the 22q11 deletion syndrome (see Table 2)
All fetuses with IAA, TAA, TOF, VSD, or AAA (if amniocentesis performed for diagnostic purposes)
Consider all newborns/infants with VSD with normal aortic arch

IAA indicates interrupted aortic arch; TA, truncus arteriosus; TOF, tetralogy of Fallot; and AAA, aortic arch anomaly.

*Perimembranous, conoseptal hypoplasia or malalignment VSD.

with feeding on a routine examination (Table 2). The older child with a suspected 22q11 deletion could be evaluated for speech and learning disabilities, endocrine abnormalities, immune dysfunction, or other recognized syndromic abnormalities (Table 2). However, clinical assessment for syndrome features alone of the at-risk individual may not consistently identify the infant carrying a 22q11 deletion. Therefore, more routine FISH testing of at-risk infants is likely warranted.

In particular, facial features may be the only associated syndromic finding in the newborn and can be difficult to detect in that age group.[62] Such patients may be uncommon and would presumably be identified at an older age when other syndromic features and symptoms became more apparent. But these data also argue for a more comprehensive testing strategy to identify all infants with tetralogy of Fallot and a 22q11 deletion. Ultimately, early diagnosis of the patient with a 22q11 deletion allows for appropriate treatment of associated noncardiac anomalies, including appropriate handling of blood products at the time of surgery (leukocyte-depleted and cytomegalovirus-negative blood for the immunocompromised patient). In addition, accurate and timely genetic counseling can be provided to the family, including information on recurrence issues. Other family members can then be tested appropriately. Therefore, early FISH testing in patients with specific types of CHD is currently suggested as outlined in Table 3.

Finally, prenatal testing for a 22q11 deletion should be strongly considered in the fetus with either interrupted aortic arch, truncus arteriosus, tetralogy of Fallot, VSD (perimembranous, conoseptal hypoplasia, or malalignment types only), or aortic arch anomaly.[51,55,58] In the fetus, it is much more difficult to diagnose the 22q11 deletion syndrome by clinical appearance alone, because other features, such as facial dysmorphia, will not be sufficiently apparent to exclude the diagnosis. Appropriate genetic and family counseling is of critical importance in this situation.

Williams-Beuren Syndrome

Williams-Beuren syndrome (Williams syndrome) is an autosomal dominant disorder characterized by specific cardiovascular defects, infantile hypercalcemia, skeletal and renal anomalies, cognitive deficits, "social personality," and elfin facies. Most cases arise de novo due to a chromosomal microdeletion. As with other deletion syndromes, Williams syndrome has a broad range of clinical presentations. Typical cardiovascular anomalies include supravalvular aortic stenosis, often in conjunction with supravalvular pulmonary stenosis and peripheral pulmonary stenosis. These arterial abnormalities constitute an elastin arteriopathy or vasculopathy caused by deletion of the elastin gene.[65] The degree of cardiovascular involvement and the involvement of the pulmonic or aortic vessels varies widely. The supravalvular aortic stenosis has been shown to progress in many cases, whereas the supravalvular pulmonary stenosis or peripheral pulmonary artery stenosis usually regresses with time.[66,67]

Approximately 90% of individuals with a clinical diagnosis of Williams syndrome have been found by FISH to have a microdeletion at chromosome 7q11.23.[65,68] Molecular analyses comparing clinical phenotype to genotype have demonstrated that this syndrome is a contiguous gene-deletion syndrome, ie, the deletion or alteration of specific genes in the deleted region corresponds with specific clinical features. Deletion of 1 copy of the elastin gene corresponds with the development of vascular manifestations of this disorder. Deletion of different genes in the region accounts for different manifestations of the disorder. Larger deletions, particularly deletions visible cytogenetically, can be associated with more severe clinical phenotypes, including seizures, which

TABLE 4. Clinical Features of Williams-Beuren Syndrome

Cardiovascular
Supravalvular aortic stenosis
Pulmonary arterial stenosis
Multiple arterial stenoses
Aortic/mitral valve defects
Adult systemic hypertension
Distinctive facies
Periorbital fullness
Stellate iris pattern
Full lips/wide mouth
Elfin appearance
Ophthalmologic
Strabismus
Hyperopia
Neurological
Mental retardation/cognitive disability
Unique personality
Hyperacusis
Feeding difficulties
Infantile failure to thrive
Adult height <third percentile
Endocrine
Hypercalcemia
Hypercalciuria
Hypothyroidism
Adult diabetes mellitus
Renal/bladder disorders
Chronic urinary tract infections
Structural anomalies
Nephrocalcinosis

are not typically seen in Williams syndrome. Given the clinical variability of Williams syndrome and the fact that many aspects of Williams syndrome are not particularly evident in a young infant or child, especially characteristic facial features, it is appropriate to consider testing all patients with supravalvular aortic or pulmonic stenosis for this specific microdeletion by FISH at the time of diagnosis of the cardiac disease. In addition, if peripheral pulmonary stenosis persists beyond infancy, it is also appropriate to assess these patients with FISH analysis for the Williams syndrome critical region.

Early diagnosis of Williams syndrome is important to initiate treatment for other potential medical problems (Table 4). In particular, hypercalcemia, which often occurs in the first year of life along with hypercalciuria, can be treated with appropriate diet or medication. Because hypercalcemia can be a risk factor for the development of nephrocalcinosis, making this diagnosis is important for prevention of extensive kidney damage, which can lead to renal failure. Screening for thyroid and renal anomalies will uncover anomalies that are unsuspected clinically.[69] Routine follow-up of blood pressure measurements is needed because at least half of adults with Williams syndrome have systemic hypertension, and this can often be detected in childhood or adolescent years.[70] Early identification of Williams syndrome is also essential for planning educational strategies that can enhance learning and development in children with Williams syndrome. The detection of a deletion also adds diagnostic certainty for the family and the responsible clinician. Appropriate testing of other family members and genetic counseling can then occur.

Single-Gene Disorders

In the past 15 years, considerable progress has been made toward identifying molecular genetic causes of selected congenital heart defects. As illustrated in the first part of Table 5, a number of selected congenital heart defects have been found to be associated with mutations in a variety of single genes.[3,4,71–104] Some cardiac defects are related to mutations in >1 gene. It is highly likely that additional single-gene abnormalities (mutations) will be defined in the future. DNA testing for most of the genes for isolated congenital heart defects is unavailable except on a research basis at this time; however, testing of some of these genes is transitioning from the research laboratory to clinical availability. The clinician is advised to consult the Gene Tests Web site (http://www.genetests.org), a publicly funded medical genetics information resource, for updates on what testing is currently available.

The identification of causative gene mutations for genetic syndromes is also occurring at a rapid pace. A select group of syndromes in which the underlying single gene has been discovered is also listed in Table 5. For illustration purposes, Alagille syndrome, NS, and Holt-Oram syndrome will be discussed in greater detail. These single-gene disorders reflect the recent identification of genes responsible for congenital heart defects and for multiple other clinical features.

Alagille Syndrome

Alagille syndrome, an autosomal dominant disorder, was originally defined as the presence of bile duct paucity on liver biopsy in conjunction with 3 of the 5 following characteristics: cholestasis; cardiovascular, skeletal, or ocular anomalies; or typical facial features. Cardiovascular anomalies occur in >90% of individuals with Alagille syndrome.[79] The most common cardiovascular features include peripheral pulmonary hypoplasia, tetralogy of Fallot, and pulmonary valve stenosis, although left-sided lesions and septal defects are also seen. Liver disease is highly variable from patient to patient and also within affected members of the same family.[105] It is characterized by a paucity of intrahepatic bile ducts and can include chronic cholestasis, minimal liver enzyme elevation, hypercholesterolemia, or liver failure. Additional clinical features of Alagille syndrome are listed in Table 6.

A subset of Alagille patients (3% to 7%) have deletions of chromosome 20p12 detectable by karyotype or FISH analysis.[106] The gene *JAG1*, which encodes a Notch ligand protein product, has been mapped into the commonly deleted region of 20p12. Mutations of *JAG1* have been identified in patients with a broad spectrum of clinical phenotypes of Alagille syndrome, including patients with a predominant cardiac phenotype.[89]

TABLE 5. Genes Associated With Congenital Heart Defects in the Young

Condition	Gene(s)	Chromosome Location	Reference(s)
Congenital heart defects			
Familial congenital heart disease (ASD, atrioventricular block)	*NKX2.5(CSX)*	5q34-q35	3, 71–74
D-TGA, DORV	*CFC1*	2q21	75, 76
D-TGA	*PROSIT240*	12q24	77
Tetralogy of Fallot	*ZFPM2/FOG2*	8q23	78
	NKX2.5	5q34-q35	72
	JAG1	20p12	79
Atrioventricular septal defect	*CRELD1*	3p21	80
ASD/VSD	*GATA4*	8p23	81
Heterotaxy	*ZIC3*	Xq26	82
	CFC1	2q21	75, 76
	ACVR2B	3p21.3-p22	83
	LEFTYA	1q42.1	84
Supravalvar aortic stenosis	*ELN*	7q11	85, 86
Syndromes			
Holt-Oram syndrome	*TBX5*	12q24	87, 88
Alagille syndrome (PPS)	*JAG1*	20p12	89
Char syndrome (PDA)	*TFAP2B*	6p12	4
Noonan syndrome	*PTPN11*	12q24	90, 91
	KRAS	12p1.21	92
	SOS1	2p21	115, 116
CHARGE association	*CHD7*	8q12	93, 94
Ellis-van Creveld	*EVC, EVC2*	4p16	95, 96
Marfan syndrome	*FBN1*	15q21.1	97
Marfan-like syndrome	*TGFBR2*	3p22	98, 99
Cardiofaciocutaneous syndrome	*KRAS*	12p12.1	100
	BRAF	7q34	100
	MEK1	15q21	101
	MEK2	7q32	101
Costello syndrome	*HRAS*	11p15.5	102–104

ASD indicates atrial septal defect; D-TGA, D-transposition of great arteries; DORV, double-outlet right ventricle; PPS, peripheral pulmonary stenosis; PDA, patent ductus arteriosus; and CHARGE, coloboma, heart anomaly, choanal atresia, retardation, and genital and ear anomalies.

Patients suspected of having Alagille syndrome should undergo a karyotype and FISH analysis to check for a 20p12 rearrangement or deletion. Karyotype and FISH analysis are readily available in most cytogenetics laboratories, and the finding of a deletion or chromosomal rearrangement can be diagnostic for Alagille syndrome. If this diagnosis is confirmed by the cytogenetic testing, the child can be evaluated for other important features of Alagille syndrome, such as liver disease or additional vascular involvement.[107] In addition, the cytogenetic results will most likely have a significant impact on the reproductive decisions some families will make in the future.

More than 90% of individuals with the classic phenotype of Alagille syndrome have a *JAG1* mutation when the most sensitive and rigorous methods for mutation detection are used.[108] *JAG1* mutation analysis is now clinically available for those patients whose karyotype and FISH analyses are normal. Growing evidence suggests that patients with a strong family history of right-sided defects, such as peripheral pulmonary stenosis, valvar pulmonary stenosis, or tetralogy of Fallot, who do not otherwise fulfill the criteria for Alagille syndrome may also be appropriate for testing in this specific region.[109,110] The finding of peripheral pulmonary stenosis or hypoplasia of the branch pulmonary arteries in a child, alone or in combination with tetralogy of Fallot, should prompt consideration of testing for Alagille syndrome. All patients with documented *JAG1* mutations or suspected Alagille syndrome should have cardiac, hepatic, ophthalmologic (anterior chamber defects, pigmentary retinal anomalies, posterior embryotoxon), orthopedic (butterfly vertebrae), hematologic (bleeding tendency), and renal (structural, cysts, tubular acidosis) evaluations.[111] The finding of a *JAG1* mutation in an individual establishes the diagnosis and allows for further testing of appropriate family members in whom the diagnosis

TABLE 6. Clinical Features of Alagille Syndrome

Cardiovascular
Pulmonary artery stenosis or hypoplasia
Tetralogy of Fallot
Valvar pulmonary stenosis
Atrial septal defect
Labile systolic hypertension
Liver
Persistent cholestasis/jaundice
Hepatic ductular hypoplasia
Hepatocellular carcinoma
Hypercholesterolemia
Abnormal liver function tests
Distinctive facies
Triangular face
Prominent forehead and chin
Hypertelorism
Ophthalmologic
Posterior embryotoxon
Axenfeld anomaly
Ectopic pupils
Pigmentary retinopathy
Neurological
Normal intelligence to moderate mental retardation
Hoarse voice
Endocrine
Delayed puberty
Growth retardation
Hypothyroidism
Renal
Horseshoe kidney
Renal compromise
Other
Butterfly vertebra
Conductive hearing loss

has not yet been suspected. This is helpful to make appropriate arrangements for comprehensive evaluation of clinical issues and to provide appropriate genetic counseling to the family regarding recurrence risk.

Noonan Syndrome

NS is a genetic multiple malformation disorder that includes short stature, typical facial dysmorphism, webbed neck, chest deformity, and cardiovascular abnormalities.[112] The cardiac involvement is observed in 80% to 90% of affected individuals, with valvar pulmonic stenosis and hypertrophic cardiomyopathy being the most common.[112,113] Other congenital heart defects observed in NS are secundum atrial septal defect, atrioventricular septal defect, mitral valve abnormalities, aortic coarctation, and tetralogy of Fallot. Other noncardiac features of NS include cryptorchidism, bleeding diathesis, and developmental delay. Additional features are listed in Table 7. Population prevalence has been estimated at

TABLE 7. Clinical Features of Noonan Syndrome

Cardiovascular
Congenital heart defects
Pulmonic stenosis
Atrioventricular septal defects
Aortic coarctation
Secundum atrial septal defects
Mitral valve defects
Tetralogy of Fallot
VSDs
Patent ductus arteriosus
Hypertrophic cardiomyopathy
Dysmorphic features
Epicanthal folds
Ptosis
Down-slanting palpebral fissures
Triangular facies
Low-set, thickened pinnae
Light-colored irides
Curly, coarse hair
Webbed neck with low posterior hairline
Skeletal
Short stature
Pectus excavatum and/or carinatum
Cubitus valgus
Scoliosis
Vertebral anomalies
Genitourinary
Cryptorchidism
Developmental
Developmental delay
Attention deficit/hyperactivity disorder
Feeding difficulties
Hematologic
Bleeding diathesis
Von Willebrand disease
Factor XI, XII, XIII deficiency
Thrombocytopenia, amegakaryocytic
Leukemia
Juvenile myelomonocytic
Acute lymphoblastic
Ophthalmologic
Strabismus
Myopia
Other
Hearing loss, sensorineural
Dental malocclusion
High-arched palate
Lymphatic
Lymphedema
Lymphangiectasia

1 per 1000 to 1 per 2500 live births. The trait is inherited in an autosomal dominant fashion, although a substantial fraction of cases are sporadic.

NS is genetically heterogeneous, which means that there are at least 3 NS disease genes, *PTPN11*, *SOS1*, and *KRAS*.[92,114–116] With genetic linkage analysis and then positional candidacy, an NS disease gene on chromosome 12 was identified.[90] It is *PTPN11*, which encodes a protein tyrosine phosphatase called SHP-2. SHP-2 plays an important role in signal transduction for a wide variety of biological processes, including the formation of the semilunar valves.[117,118] Mutations in the *PTPN11* gene are observed in 40% to 50% of NS patients and are more prevalent among familial cases and among NS patients with pulmonary valve stenosis.[91] NS patients with hypertrophic cardiomyopathy are unlikely to harbor a *PTPN11* mutation. Otherwise, there does not appear to be a strong correlation between the presence or absence of a *PTPN11* mutation and most other aspects of the NS phenotype (eg, mental retardation). Disease penetrance is nearly complete among those with *PTPN11* mutations, although phenotypic variability within families can be substantial.

Clinical mutation testing for *PTPN11*, *SOS1*, and *KRAS* is now available in the United States and elsewhere. These DNA tests can confirm the diagnosis of NS but cannot exclude it due to the genetic heterogeneity (ie, the individual could harbor a mutation in another NS gene that has not been identified as yet). Molecular confirmation is useful in borderline cases, especially in neonates and adults in whom the facial features of NS may not be obvious. Prenatal testing can be done when the fetus is at risk for inheriting a defined *PTPN11* mutation from an affected parent. Similar testing of suspicious prenatal, sporadic cases (eg, a fetus with cystic hygroma and pulmonic stenosis) suffers from the uncertainty that arises from the genetic heterogeneity.

There are 3 NS-related conditions for which *PTPN11* mutations can be found: LEOPARD syndrome, Noonan-like with multiple giant cell lesions, and certain hematopoietic disorders. LEOPARD syndrome is also a multiple malformation disorder; the name is an acronym that designates the cardinal features: multiple lentigines, electrocardiographic conduction abnormalities, ocular hypertelorism, pulmonic stenosis, abnormal genitalia, retardation of growth, and sensorineural deafness. A high percentage of affected individuals have *PTPN11* mutations with certain missense defects that appear to be specific for LEOPARD syndrome rather than NS.[119,120] Noonan-like with multiple giant cell lesions includes all of the features of NS plus the giant cell lesions of bone. The proclivity for involving the maxilla with expansile lesions leads to this disorder being a form of cherubism. Cardiac involvement appears to be highly similar to NS. Unlike LEOPARD syndrome, the *PTPN11* mutations have no specificity in predicting this disorder versus NS.[91,120]

Holt-Oram Syndrome

Holt-Oram syndrome is an autosomal dominant "heart-hand" syndrome that is characterized by congenital heart defects in patients with upper-limb deformities.[122] This syndrome occurs in approximately 1 per 100 000 individuals, and although it can be inherited in a mendelian fashion, a significant portion of cases are sporadic.[123] All patients have preaxial radial ray malformation (eg, triphalangeal, hypoplastic, or absent thumb and/or radial dysplasia), and three fourths of patients have septation (atrial and/or ventricular) defects and/or progressive atrioventricular conduction disease.[124–126] Human genetic linkage analyses and positional cloning studies of affected families revealed that Holt-Oram syndrome is caused by mutations in the *TBX5* transcription factor gene (chromosome 12q24.1).[87,124,126,127] The *TBX5* transcription factor has proven to be a key regulator, particularly in combination with other transcription factors such as *NKX2.5* and *GATA-4*, of gene expression during embryogenesis, and loss of its activity markedly impairs development of the heart and limb.[81,128,129]

Although there is significant genetic heterogeneity to the broader class of heart-hand syndromes,[130] there is little if any genetic heterogeneity among Holt-Oram patients. Mutational analyses of the *TBX5* gene-coding regions will detect mutations in approximately three fourths of such patients, and the remainder are likely to have mutations in regulatory regions or to have deletions/insertions not detectable by current mutational analysis.[131] Some studies find that fewer than half of Holt-Oram patients have *TBX5* mutations, which suggests genetic heterogeneity.[126,127,132] However, these studies have been confounded by aggregation of patients who have other heart-hand syndromes with those who have Holt-Oram.[133] Thus, careful and detailed clinical evaluations of the cardiovascular and other organ systems are essential to distinguish other such clinical syndromes (eg, Rothmund-Thomson syndrome, Okihiro syndrome, thrombocytopenia absent radius syndrome, and VACTERL association [vertebral anomalies, anal atresia, cardiac defect, tracheoesophageal fistula, renal abnormalities, and limb abnormalities]) that share features with Holt-Oram syndrome but are nonetheless clinically and genetically distinct.[134–136]

Key to the accurate diagnosis of Holt-Oram syndrome is the uniform presence of upper-limb radial ray defects, which may be symmetrical or asymmetrical (even unilateral) regardless of the presence or absence of cardiovascular disease. Such limb deformity, for example, altered structure of a single carpal bone, may be quite subtle and only detectable radiographically, but individuals without such radial ray defects do not have Holt-Oram syndrome.[124,137] Other limb malformations (eg, syndactyly of digits other than the thumb, polydactyly, or lower-limb defects), craniofacial abnormalities, and/or evidence of noncardiac visceral organ abnormalities (including heterotaxy) make Holt-Oram syndrome unlikely.[124,125,131,138] Most Holt-Oram structural cardiac defects are either ostium secundum atrial septal defects or muscular VSDs. Complex congenital heart defects have been seen in Holt-Oram syndrome patients with *TBX5* mutations, but they are rare events.[87,88,139] Therefore, the demonstration of ostium primum atrial septal defects, membranous VSDs, or congenital valvular disease should at least prompt further detailed clinical evaluations of other organ systems and consideration of other diagnoses.

Among those individuals with Holt-Oram syndrome, most will have *TBX5* mutations that are nonsense or frameshift

mutations that are predicted to produce a 50% reduction in *TBX5* gene dosage, that is, haploinsufficiency. Interestingly, there have been several reports[140–142] of individuals with duplications of chromosome 12q segments encompassing *TBX5* (and therefore potentially *TBX5* overexpression), and such patients have clinical phenotypes that overlap with Holt-Oram syndrome.[87] A minority of Holt-Oram syndrome is due to missense *TBX5* mutations that do not alter the gene's dosage. Although large family-based studies have suggested that many such missense *TBX5* mutations have their greatest impact on either heart or limb development, compared with haploinsufficient *TBX5* mutations that markedly deform both organ systems, these genotype-phenotype associations are not necessarily evident in the individual patient with Holt-Oram syndrome and are not clinically useful for predicting the individual patient's phenotype.[88,132]

Thus, in the setting of careful clinical evaluations of patients with suspected Holt-Oram syndrome, there is a rather limited role for *TBX5* mutational analyses. When diagnostic clarity is not achieved clinically, *TBX5* mutational analyses can provide adjunctive information. However, due to technical limitations of genetic assays used, the absence of a detected *TBX5* mutation in an individual with a typical clinical presentation does not preclude a diagnosis of Holt-Oram syndrome. Thus, the most valuable setting for *TBX5* genetic testing may be in establishing diagnoses for family members of a patient with previously established Holt-Oram syndrome and a known *TBX5* mutation. For instance, McDermott et al[131] used genetic testing to rule out Holt-Oram syndrome in an individual with tetralogy of Fallot whose cousin had well-established Holt-Oram syndrome. *TBX5* genetic testing has also been a useful addition to our assisted reproductive armamentarium.[26] When in vitro fertilization is used as a reproductive strategy for an individual affected by Holt-Oram syndrome, blastocysts can be subjected to preimplantation genetic testing in vitro before their transfer back to the mother. If the affected parent's *TBX5* mutation is established before the in vitro fertilization cycle is begun, mutational analyses can occur in a sufficiently rapid and sensitive fashion that they can be the basis for embryo selection to achieve offspring who will not carry the *TBX5* mutation and will therefore be unaffected by Holt-Oram syndrome.

Nonsyndromic Single-Gene Disorders

Studies have recently shown that nonsyndromic CHD can result from single-gene defects. Schott et al[3] identified mutations in *NKX2.5* in 4 kindreds with atrial septal defects and atrioventricular conduction delay without other apparent syndromic features. The mutations were found only in affected individuals, were not present in control samples, and were demonstrated to change protein structure or function. Given that some members of these kindreds had either isolated atrioventricular conduction delay or other types of CHD, investigators subsequently studied additional kindred and sporadic cases with isolated atrioventricular conduction delay or CHD for *NKX2.5* mutations. These studies identified likely disease-related mutations in a subset of cases with atrioventricular conduction delay and additional sequence alterations in patients with selected types of CHD.[71,72,143–145] The gene changes in patients with sporadic CHD were not identified in control subjects, and it was difficult to demonstrate their functional significance; thus, their relationship to the disease may not be proved. These studies demonstrate the complexity of the biological interpretation of some alterations and the likely complexity of the genetic contribution to CHD.

Investigators have also identified mutations of *GATA4* in 2 kindreds with septal defects and no apparent syndromic features.[81] Once again, the mutations identified were found in affected individuals but not in control samples and were shown to confer changes in protein function. Mutations in additional kindreds and subjects with septal defects have been reported subsequently.[146–148] It remains to be seen whether mutations of *GATA4* will be identified widely in patients with septal defects or in other sporadic cases of CHD; however, these studies highlight the utility of studying large kindreds to identify novel disease genes for CHD, and they demonstrate that single-gene disorders may be found in a subset of CHD. In addition, these studies identify critical molecular pathways involved in cardiovascular development and disease, given that the proteins encoded by *NKX2.5*, *GATA4*, and *TBX5* are known to interact with one another in experimental systems.

Many cases of nonsyndromic CHD are unlikely to result from simple single-gene disorders. Instead, many cases of CHD are likely the result of multiple genetic alterations that increase susceptibility to CHD and interact with environmental factors. Already there is evidence of decreased penetrance and marked variability in expressivity of identical genetic alterations. For example, only 40% to 50% of children with trisomy 21 have CHD, and patients with a 22q11 deletion or even a single-gene defect (eg, *JAG1*) can present with markedly variable features. Such variable expressivity and penetrance is presumably explained by other genetic and environmental factors. These observations and the marked genetic heterogeneity already evident demonstrate the complexity of deciphering the genetic basis of CHD.

Evaluation for Genetic Basis in Children With CHD

Chromosome analysis and FISH testing for specific deletions are now accepted tools for the clinician. If the clinician finds a specific chromosome abnormality, it will provide the family with a clear explanation of the cause, allow the clinician to provide appropriate counseling about recurrence or lack of recurrence, and prompt the physician to investigate other potential medical problems known to be associated with the particular chromosomal anomaly.

Despite the rapidly advancing fund of knowledge, a genetic defect can only be identified through available testing in a minority of patients with CHD.[149] Many of these children have abnormalities of other organ systems that indicate the presence of a known phenotype. In some cases, there may be a single-gene defect for which no testing is clinically available. In other instances, polygenic inheritance with or without an additive environmental component may be implicated. A complete understanding of the interactions between abnormal cardiac physiology and derangements in other organs is important for appropriate management and counseling in such patients. Therefore, it is useful for the physician caring

for these patients to have an algorithm based on the initial presentation to assess for the presence of noncardiac abnormalities (Appendix 2).

The approach to the newly diagnosed patient with CHD should include routine examination of all relatives for a potential genetic contribution. Identification of some genetic causes of CHD has highlighted the importance of obtaining an accurate medical history of other family members and documenting an extended pedigree. In some forms of cardiovascular disease, for example, hypertrophic cardiomyopathy and Marfan syndrome, the familial nature (autosomal dominant inheritance) is well recognized; however, for other problems, for example, bicuspid aortic valve, family clustering has not been widely appreciated in the past. Recent studies have shown that a familial bicuspid aortic valve is likely to be inherited as an autosomal dominant condition with reduced penetrance.[9,150] There is a 24% prevalence of bicuspid aortic valve in first-degree relatives of patients with left ventricular outflow tract obstruction.[150] Increasingly, medical practice is evolving toward a recommendation that other family members undergo clinical evaluation, which may include an electrocardiogram and echocardiogram.

Specific assessment for physical features is warranted. The physical examination should focus on dysmorphic facies, eye and ear abnormalities, limb reduction defects, polydactyly, other skeletal defects, gastrointestinal and urologic defects, and neurological status. This assessment may be more difficult in the newborn who is intubated and/or sedated, and it may be more fruitful before rather than after cardiac surgery. In these situations, it is helpful and important to have a geneticist perform a complete examination to help uncover more subtle abnormalities. Other consultants, for example, from neurology, ophthalmology, orthopedic surgery, and otolaryngology, may be needed based on the suspected diagnoses.

Chest radiographs are performed in all newborn inpatients and many older patients who are diagnosed with CHD. Particular attention should be paid to skeletal defects and cardiac aortic arch, pulmonary, liver, and stomach situs. Additional radiographic tests that may also be indicated include abdominal/renal ultrasound, upper gastrointestinal series, liver-spleen scan, head ultrasound, and brain computed tomography or magnetic resonance imaging.

Cytogenetic testing should be considered in the following situations:

1. Any infant or child with the phenotype of a recognizable chromosomal syndrome (eg, trisomy 21 or 18)
2. Because not all chromosomal abnormalities result in a clinically recognizable syndrome, any infant or child with a congenital heart defect combined with (a) dysmorphic features, (b) growth retardation that cannot be explained by the heart defect, (c) developmental delay or mental retardation, or (d) multiple congenital anomalies
3. Infants or children with a family history of multiple miscarriages and/or siblings with birth defects
4. If major cardiac and/or other visceral organ malformations are documented by prenatal ultrasound and/or fetal echocardiogram

Genetic consultation is recommended in the presence of mental retardation, multiple congenital anomalies, or facial dysmorphia or if the standard karyotype is normal despite the clinical suspicion of a genetic abnormality (ie, normal karyotype in the presence of dysmorphism, mental retardation, and/or multiple congenital anomalies that include cardiac defects). In this situation, high-resolution banding or more advanced cytogenetic techniques may be indicated (FISH for specific defects or telomeric and subtelomeric probes). In addition, chromosome analysis is warranted as described above. Consultation with a clinical geneticist is recommended when a chromosomal abnormality is discovered so that appropriate counseling and evaluation of family members may be undertaken.

It is anticipated that the emphasis in the evaluation of patients with CHD will increasingly focus on the family in addition to the patient. Given the regularity with which the phenomenon of variable expression (ie, phenotype variation in individuals carrying the same gene mutation) is being recognized, the evaluations may need to be very comprehensive. For example, the evaluation may extend to noncardiac organs (eg, upper-extremity [Holt-Oram syndrome, *TBX5* mutations] and liver, skeleton, or eyes [Alagille syndrome, *JAG1* mutations]).

Impact on Patients and Families

For individuals with CHD and their families, identification of a genetic cause is very beneficial. This allows confidence in the diagnosis and allows the physician to explain the exact genetic mechanisms to the family. It also alerts the clinician to investigate other organ systems that may be involved in the syndrome and broadens the context of evaluation from the individual to other family members. In instances where a genetic cause such as Alagille syndrome has been identified in a family, genotyping may be very useful for stratifying "asymptomatic" family members into groups who should have cardiac evaluations and those for whom it is not necessary. Genotype-negative individuals have a low risk of developing pediatric cardiovascular disease, and clinical evaluation of such patients is not warranted. On the other hand, serial evaluation of genotype-positive individuals is essential to monitor development of the phenotype.

Ethical Considerations

Predictive genetic testing of children and adolescents has been the subject of numerous recommendations.[151–153] Although there is no universal agreement about acceptable practices in pediatric genetic testing, consensus exists that pediatric genetic testing should not take place unless there are clinical benefits to be reaped as a direct result of testing before the patient reaches the age of majority. In addition, the struggle to obtain the pediatric analogue of informed consent is particularly important in genetic testing, in part because the long-term social and legal risks of genetic testing for pediatric patients are difficult to predict, and the risks are more difficult for a child to judge. On the other hand, genetic testing may determine a genetic mechanism of disease that provides an important opportunity for genetic counseling that benefits the entire family.

Summary

Ongoing research is now demonstrating that variations or alterations in genes contribute to the origin of CHD to a greater degree than previously suspected. This review has summarized the current knowledge of the genetics of CHD and has provided guidelines and algorithms to aid the clinician in making diagnoses and planning care. Many types of genetic testing are currently clinically available; other testing is still in the research phase. Awareness of this rapidly advancing field is important for all clinicians, and a multidisciplinary team approach to the child with CHD is necessary for comprehensive, state-of-the-art care. In addition to physicians and surgeons with expertise in CHD, a geneticist is a highly important member of this team.

Patients with CHD require multidisciplinary care. Their families deserve up-to-date genetic information as it relates to their child's prognosis and to the kindred's risk for future inheritance of genetic abnormalities associated with cardiac defects. Obstetricians will have involvement in these issues if prenatal echocardiography demonstrates CHD or if preimplantation genetic diagnosis and in vitro fertilization are requested. Pediatricians require knowledge about these issues in caring for multiple organ systems in children with genetic syndromes that include CHD. Families of these children will need information about recurrence risk. Pediatric cardiologists and pediatric cardiac surgeons are currently well equipped to care for patients with CHD, but they need to constantly update their understanding of the contribution of genetic abnormalities to these birth defects. As children grow into adulthood, internists, obstetricians, cardiologists, and thoracic surgeons will step in to care for CHD as it is superimposed on adult medical issues.

Research discoveries regarding the genetics and inheritance of CHD are rapidly occurring. As in all genetic research, ethical considerations for children with heart disease demand thorough and thoughtful reflection. It is hoped that dissemination of the information in the present report will result in improved diagnoses and care for children and adults with congenital cardiac disease. Through multidisciplinary care and research, the goal to prevent and improve clinical outcomes in CHD will guide future investigations.

Appendix 1

Representative Chromosomal Disorders Associated With Congenital Heart Defects

Chromosomal Disorder	Main Features	Percent With CHD	Heart Anomaly	Reference(s)
Deletion 4p (Wolf-Hirschhorn syndrome)	Pronounced microcephaly, widely spaced eyes, broad nasal bridge (Greek helmet appearance), downturned mouth, micrognathia, preauricular skin tags, elongated trunk and fingers, severe mental retardation and seizures; 1/3 die in infancy	50–65	ASD, VSD, PDA, LSVC, aortic atresia, dextrocardia, TOF, tricuspid atresia	22, 154
Deletion 5p (cri-du-chat)	Catlike cry, prenatal and postnatal growth retardation, round face, widely spaced eyes, epicanthal fold, simian crease, severe mental retardation, long survival	30–60	VSD, ASD, PDA	22, 155, 156
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	67, 157, 158
Trisomy 8 mosaicism	Skeletal/vertebral anomalies, widely spaced eyes, broad nasal bridge, small jaw, high arched palate, cryptorchidism, renal anomalies (50%), long survival	25	VSD, PDA, CoA, PS, TAPVR, truncus arteriosus	22, 159–162
Deletion 8p syndrome	Microcephaly, growth retardation, mental retardation, deep-set eyes, malformed ears, small chin, genital anomalies in males, long survival	50–75	AVSD, PS, VSD, TOF	163–165
Trisomy 9	Severe prenatal and postnatal growth retardation, marked microcephaly, deep-set eyes, low-set ears, severe mental retardation; 2/3 die in infancy	65–80	PDA, LSVC, VSD, TOF/PA, DORV	22, 166
Deletion 10p	Frontal bossing, short down-slanting palpebral fissures, small low-set ears, micrognathia, cleft palate, short neck, urinary/genital, upper-limb anomalies	50	BAV, ASD, VSD, PDA, PS, CoA, truncus arteriosus	22, 167, 168
Deletion 11q (Jacobsen syndrome)	Growth retardation, developmental delay, mental retardation, thrombocytopenia, platelet dysfunction, widely spaced eyes, strabismus, broad nasal bridge, thin upper lip, prominent forehead	56	HLHS, valvar AS, VSD, CoA, Shone's complex	169
Trisomy 13 (Patau syndrome)	Polydactyly, cleft lip and palate, scalp defects, hypotelorism, microphthalmia or anophthalmia, colobomata of irides, holoprosencephaly, microcephaly, deafness, profound mental retardation, rib abnormalities, omphalocele, renal abnormalities, hypospadias, cryptorchidism, uterine abnormalities; 80% die in first year	80	ASD, VSD, PDA, HLHS, laterality defects, atrial isomerism	170, 171
Trisomy 18 (Edwards syndrome)	IUGR, polyhydramnios, micrognathia, short sternum, hypertonia, rocker-bottom feet, overlapping fingers and toes, TEF, CDH, omphalocele, renal anomalies, biliary atresia, profound mental retardation; 90% die in first year	90–100	ASD, VSD, PDA, TOF, DORV, D-TGA, CoA, BAV, BPV, polyvalvular nodular dysplasia	22, 172, 173
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, PS, (left-sided heart lesions and septal defects less common)	79, 174
Trisomy 21 (Down syndrome)	Hypotonia, hyperextensibility, epicanthal fold, simian crease, clinodactyly of fifth finger, brachydactyly, variable mental retardation, premature aging	40–50	AVSD, VSD, ASD, (TOF, D-TGA less common)	22, 175–180
Deletion 22q11 (DiGeorge, velocardiofacial, and conotruncal anomaly face syndrome)	Hypertelorism, micrognathia, low-set posteriorly rotated ears, "fish mouth," thymic and parathyroid hypoplasia, hypocalcemia, feeding/speech/learning/behavioral disorders, immunodeficiency, palate/skeletal/renal anomalies	75	IAA-B, truncus arteriosus, isolated aortic arch anomalies, TOF, conoventricular VSD	181, 182
Monosomy X (Turner syndrome, 45,X)	Lymphedema of hands and feet, widely spaced hypoplastic nipples, webbed neck, primary amenorrhea, short stature, normal intelligence	25–35	CoA, BAV, valvar AS, HLHS, aortic dissection	22, 183–187
Klinefelter syndrome (47,XXY)	Usually normal appearing, tall stature, small testes, delayed puberty, emotional and behavioral problems common, variable mental retardation	50	MVP, venous thromboembolic disease, PDA, ASD	22, 188

CHD indicates congenital heart defects; ASD, atrial septal defect; VSD, ventricular septal defect; PDA, patent ductus arteriosus; LSVC, persistent left superior vena cava; TOF, tetralogy of Fallot; AS, aortic stenosis; PS, pulmonic stenosis; PPS, peripheral pulmonary stenosis; CoA, coarctation of the aorta; TAPVR, total anomalous pulmonary venous return; AVSD, atrioventricular septal defect; TOF/PA, tetralogy of Fallot with pulmonary atresia; DORV, double-outlet right ventricle; BAV, bicuspid aortic valve; HLHS, hypoplastic left heart syndrome; IUGR, intrauterine growth retardation; TEF, tracheoesophageal fistula; CDH, congenital diaphragmatic hernia; D-TGA, D-transposition of the great arteries; BPV, bicuspid pulmonary valve; PA, pulmonary artery; IAA-B, interrupted aortic arch type B; and MVP, mitral valve prolapse.

Appendix 2

Genetic Algorithms for Cardiac Defects

I. Pulmonary outflow obstruction
- A. Pulmonary valve stenosis
  - 1. Noonan syndrome
    - a) Autosomal dominant
    - b) 25% to 70% of cases result from de novo mutation
    - c) More likely if pulmonary valve is dysplastic
    - d) Also associated with hypertrophic cardiomyopathy (right and/or left ventricle)
    - e) Noncardiac phenotype features
      - (1) Male or female
      - (2) Short stature
      - (3) Broad or webbed neck
      - (4) Unusual chest shape
      - (5) Characteristic facies
      - (6) Developmental delay
      - (7) Cryptorchidism
    - f) Genetic testing clinically available
      - (1) *PTPN11* gene mutation analysis
      - (2) *KRAS* gene mutation analysis
      - (3) *SOS1* gene mutation analysis
  - 2. Alagille syndrome (see below)
  - 3. Costello syndrome
    - a) Sporadic occurrence
    - b) Also associated with hypertrophic cardiomyopathy
    - c) Noncardiac phenotype features
      - (1) Failure to thrive
      - (2) Feeding difficulties
      - (3) Mental retardation
      - (4) Increased risk of malignancy
      - (5) Coarse facial features with thick lips
      - (6) Loose skin
      - (7) *HRAS* mutations
  - 4. LEOPARD syndrome
    - a) Autosomal dominant
    - b) Noncardiac phenotype features
      - (1) Hearing loss
      - (2) Lentigines
      - (3) Short stature
      - (4) Similarities with Noonan syndrome
    - c) Genetic testing clinically available
      - (1) *PTPN11* gene mutation analysis
  - 5. Other chromosomal anomalies
    - a) Deletions of chromosome 1p, 8p, 10p, 22q
    - b) Duplications of chromosome 6q, 15q, 19q
    - c) Trisomy 8
- B. Pulmonary artery branch stenosis
  - 1. Alagille syndrome
    - a) Autosomal dominant
    - b) 50% to 60% of cases result from de novo mutation
    - c) Noncardiac phenotype features
      - (1) Bile duct paucity
      - (2) Cholestasis
      - (3) Eye findings (posterior embryotoxon)
      - (4) Vertebral anomalies
      - (5) Characteristic facies
      - (6) Growth retardation
    - d) Genetic testing clinically available:
      - (1) Microdeletion in chromosome locus 20p12 detectable by FISH
      - (2) *JAG1* gene mutation analysis
  - 2. Williams-Beuren syndrome (see below)
  - 3. Other
    - a) Congenital rubella
    - b) Ehlers-Danlos syndrome
    - c) Noonan syndrome (see above)
    - d) LEOPARD syndrome (see above)
- C. Pulmonary valve atresia (intact ventricular septum)
  - 1. Ring 9 chromosome abnormality

II. Aortic outflow obstruction
- A. Aortic valve stenosis
  - 1. Chromosome abnormalities
    - a) Deletion of chromosome 11q (Jacobsen syndrome)
    - b) Autosomal trisomies (13, 18)
    - c) Deletion of 10q
    - d) Duplications of 1q, 2p, 2q, 6q, 11q
  - 2. Noonan syndrome (see above)
  - 3. Turner syndrome (see below)
- B. Supravalvular aortic stenosis
  - 1. Williams-Beuren syndrome
    - a) Autosomal dominant
    - b) Most cases result from de novo mutation
    - c) Noncardiac phenotype features
      - (1) Characteristic elfin facies
      - (2) Loquacious personality
      - (3) Hypercalcemia
      - (4) Developmental delay/cognitive defects
      - (5) Connective tissue abnormalities
      - (6) Renal anomalies
      - (7) Thyroid disorder
    - d) Genetic testing clinically available:
      - (1) Microdeletion in chromosome 7q11 (elastin gene) detectable by FISH (>95% of cases)
    - e) Rare translocations involving 7q11 locus
  - 2. Isolated supravalvular aortic stenosis, Eisenberg type
    - a) Distinct entity from Williams syndrome
    - b) Abnormal facies and mental retardation absent
    - c) Elastin gene mutations
- C. Coarctation of the aorta
  - 1. Turner syndrome
    - a) Noncardiac phenotype features
      - (1) Female
      - (2) Unusual chest shape
      - (3) Widely spaced nipples
      - (4) Webbed neck
      - (5) Lymphedema
      - (6) Short stature
      - (7) Streak ovaries

Appendix 2. Continued

- b) Karyotype is diagnostic: 45,X or mosaics (45,X/46,XX)
- 2. Other chromosomal abnormalities
  - a) Deletion of 18p
  - b) Duplications of 4p, 4q, 6q, 10p
  - c) Autosomal trisomies 8, 9
- 3. Familial aggregation of left-sided obstructive heart defects
  - a) Frequent occurrence in first-degree relatives (9.4%)

D. Aortic atresia/hypoplastic left heart syndrome

- 1. Chromosomal anomalies
  - a) Deletion of 11q (Jacobsen syndrome)
  - b) Turner syndrome
  - c) Trisomy 13, 18
  - d) Deletion of 4p (Wolf-Hirschhorn)
- 2. Familial aggregation of left-sided obstructive heart defects
  - a) Frequent association with bicuspid aortic valve in a parent (5%)
  - b) Sibling recurrence risk (2% to 9%)
  - c) Proposed inheritance patterns
    - (1) Multifactorial
    - (2) Autosomal dominant with reduced penetrance
    - (3) Autosomal recessive

E. Bicuspid aortic valve

- 1. Very common cardiac anomaly (incidence 0.9% to 1.36% in population)
- 2. Association with familial aggregation of left-sided obstructive heart defects
  - a) Frequent finding of bicuspid aortic valve in parents of children with other left-sided obstructive anomalies
  - b) Frequent association of cardiac anomalies in first-degree relatives (19.3%)
- 3. Familial bicuspid aortic valve
  - a) Autosomal dominant with reduced penetrance
  - b) Prevalence 24% in first-degree relatives
- 4. Turner syndrome (see above)
- 5. Chromosomal anomalies
  - a) Autosomal trisomies 13, 18
  - b) Deletion 10p
  - c) Duplication 6q

III. Laterality defects (heterotaxy, asplenia/polysplenia)

A. Phenotype

- 1. Asplenia syndrome (also known as right atrial isomerism)
  - a) Cardiac defects
    - (1) Right atrial isomerism
    - (2) Complex conotruncal defects
    - (3) AVSD
    - (4) Anomalous location of inferior vena cava (on same side as abdominal aorta)
  - b) Pattern of visceral organs
    - (1) Asplenia: 99% of patients, more severe than polysplenia
    - (2) Bilateral "right-sidedness"
    - (3) Symmetrical liver
    - (4) Gastrointestinal malrotation
    - (5) Right-sided stomach
    - (6) Genitourinary, bronchopulmonary, axial skeletal, and central nervous system abnormalities
- 2. Polysplenia syndrome (also known as left atrial isomerism)
  - a) Cardiac defects
    - (1) Left atrial isomerism
    - (2) Septal defects
    - (3) Interrupted inferior vena cava
    - (4) Bilateral superior vena cavae
    - (5) Partial anomalous pulmonary venous return
  - b) Pattern of visceral organs
    - (1) Polysplenia: 90% of patients
    - (2) Bilateral "left-sidedness"
    - (3) Symmetrical or inverted (larger lobe on left) liver
    - (4) Gastrointestinal malrotation
    - (5) Two or more spleens, can be functionally asplenic
    - (6) Extrahepatic biliary atresia
    - (7) Genitourinary, bronchopulmonary, axial skeletal, and central nervous system abnormalities

B. Genotype

- 1. No well-described genetic syndromes with clinical testing available
- 2. Reported chromosomal abnormalities
  - a) Autosomal
    - (1) Chromosome 2 (*CFC1* gene encoding CRYPTIC protein)
    - (2) Chromosome locus 6q (*HTX3* gene)
  - b) X-linked: locus Xq26.2 (*ZIC3* gene)

IV. Atrial septal abnormalities

A. Secundum ASD

- 1. Holt-Oram syndrome
  - a) Autosomal dominant
  - b) Variable expression
  - c) Also associated with VSD, variable other defects
  - d) Noncardiac phenotype features
    - (1) No sex predilection
    - (2) Variable preaxial limb defects
    - (3) Absent, hypoplastic, or triphalangeal thumbs
  - e) Mutations of *TBX5* gene on 12q24.1
- 2. Familial ASD and progressive atrioventricular block
  - a) Autosomal dominant
  - b) No demographics known
  - c) Variable onset of conduction abnormality
  - d) Other cardiac anomalies can include VSD, tetralogy of Fallot, and others
  - e) No noncardiac features reported
  - f) Mutations or haploinsufficiency of *NKX2.5* gene on chromosome 5
- 3. Familial ASD without progressive atrioventricular block
  - a) Other cardiac anomalies can include VSD or pulmonary stenosis
    - (1) *GATA 4* mutations
- 4. Ellis-van Creveld syndrome
  - a) Autosomal recessive
  - b) Often single atrium
  - c) Noncardiac features
    - (1) Male or female
    - (2) Polydactyly
    - (3) Deformity of upper lip
    - (4) Dwarfism with narrow thorax

Appendix 2. Continued

(5) Mutations have been described in Ellis-van Creveld gene at 4p16.1
5. Noonan syndrome (see above)
6. Other chromosomal abnormalities
a) Deletions of 1, 4, 4p, 5p, 6, 10p, 11, 13, 17, 18, and 22
b) Trisomy 18, 21
c) Klinefelter syndrome
7. Other syndromes
a) Rubinstein-Taybi syndrome
b) Kabuki syndrome
c) Williams syndrome
d) Goldenhar syndrome
e) Thrombocytopenia–absent radius syndrome
f) Marfan syndrome (rare)

B. Single atrium (see Ellis-van Creveld syndrome)
C. Ostium primum ASD (see atrioventricular septal abnormalities)

V. Ventricular septal abnormalities
A. VSD
1. Holt-Oram syndrome (see under ASD)
2. Familial ASD and progressive atrioventricular block (see ASD)
3. Familial ASD without progressive atrioventricular block
a) Other cardiac anomalies can include VSD or pulmonary stenosis
b) *GATA 4* mutation
4. Chromosome abnormalities
a) Deletions of many chromosomes
b) Duplications of many chromosomes
c) Autosomal trisomies 13, 18, and 21
5. Other syndromes
a) Rubinstein-Taybi syndrome
b) Goldenhar syndrome
c) VACTERL association
d) Costello syndrome
e) Williams syndrome (see above)
f) Kabuki syndrome
g) Cornelia de Lange syndrome
h) Apert syndrome
i) Carpenter syndrome

VI. Atrioventricular septal abnormalities
A. AVSD, partial and complete
1. Autosomal trisomies
a) Down syndrome
(1) 60% of infants with AVSD have Down syndrome
b) Occurs also in trisomy 13 and 18
2. Other chromosome abnormalities
a) Deletions of 3p25, 8p2, 22q
b) Duplications of 10q, 11q, 22q
3. Isolated AVSD
a) Autosomal dominant AVSD
(1) Partial and complete
(2) Gene locus mapped to 1p21p31
4. Other syndromes
a) Holt-Oram syndrome (see above)
b) Noonan syndrome (see above)
c) Chondrodysplasias
d) Smith-Lemli-Opitz syndrome
e) Ellis-van Creveld syndrome (see above)
f) Hydrolethalus

VII. Patent ductus arteriosus
A. Familial patent ductus arteriosus
1. Char syndrome
a) Autosomal dominant
b) Noncardiac phenotypic features
(1) Characteristic facies
(2) Aplasia/hypoplasia of middle phalanges of fifth fingers
c) Variable expression
d) Mutations of *TFAP2B*

VIII. Conotruncal defects
A. Tetralogy of Fallot (51 entries in OMIM)
1. 22q11 deletion syndrome
a) Clinical features of DiGeorge/velocardiofacial/conotruncal anomaly face syndromes
b) Associated with a chromosome 22q11 deletion
c) Familial inheritance approximately 6% to 28%, autosomal dominant
d) Most are de novo deletions of 22q11
e) Highly variable clinical presentation
f) Most common noncardiac defects include
(1) Hypocalcemia
(2) Hypoplastic/aplastic thymus
(3) Immune deficiency
(4) Palate anomalies, including velopharyngeal insufficiency
(5) Feeding disorders
(6) Speech disabilities
(7) Learning disabilities
(8) Behavioral/psychiatric disorders
(9) Facial dysmorphia
g) Genetic testing available
(1) FISH for deletion 22q11
(2) Chromosome analysis for translocation or other 22q rearrangement
2. Alagille syndrome (see pulmonary artery branch stenosis)
3. Cat-eye syndrome
a) Associated with duplication of chromosomal region 22pter22q11
b) Most arise de novo
c) Highly variable clinical presentation
d) Most common noncardiac anomalies include
(1) Anal atresia
(2) Coloboma
(3) Microphthalmia
(4) Cleft palate
(5) Renal anomalies
(6) Facial dysmorphia, particularly misshapen ears
e) Genetic testing available
(1) FISH for extra marker 22 chromosome
4. Nearly 50 other syndromes in which tetralogy of Fallot is diagnosed (for details, search OMIM for tetralogy of Fallot)
a) Chromosomal abnormalities

Appendix 2. Continued

- (1) Deletions of many chromosomes
- (2) Duplications of many chromosomes

B. Truncus arteriosus/interruption of the aortic arch
- 1. 22q11 deletion syndrome (see above)
- 2. Trisomy 8
- 3. Deletion 10p

C. Transposition of the great arteries (D-TGA, L-TGA)
- 1. Chromosome abnormalities
  - a) Trisomy 18, 21
  - b) 22q11 deletion syndrome (very rarely)
  - c) Many other partial deletions of different chromosomes

D. Double-outlet right ventricle
- 1. Chromosome abnormalities
  - a) Autosomal trisomies 9, 13, 18
  - b) Duplication 2p, 12p
  - c) 22q11 deletion syndrome (very rarely)

IX. Tricuspid atresia

A. Most cases are sporadic

B. Familial occurrences reported but rare
- 1. In siblings
- 2. In association with a conotruncal malformation or annular hypoplasia in family members

C. Chromosome abnormalities reported but rare
- 1. Deletions: 22q11, 4p (Wolf-Hirsch3horn syndrome)
- 2. Duplications: partial duplication 22 (Cat-eye syndrome)

D. Targeted mutation of gene encoding Fog-2 in mice resulted in tricuspid atresia, thereby suggesting a genetic basis for the disease

X. Ebstein anomaly

A. Most cases are sporadic

B. Familial occurrences reported but rare
- 1. In siblings and other family members
- 2. In association with other mitral valve abnormalities in family members
- 3. In association with familial atrial standstill

C. Chromosome abnormalities reported but rare
- 1. Trisomy 21
- 2. Rearrangements of chromosome 11q in association with renal malformation and Pierre Robin sequence

D. Animal studies implicate several possible candidate genes on chromosome 17q

XI. Total anomalous pulmonary venous return

A. Most cases are sporadic

B. Familial occurrences reported
- 1. In siblings, twins, parents/children, first cousins
- 2. Chromosome 4p13-q12, autosomal dominant, variable expressivity, reduced penetrance in large Utah-Idaho family
- 3. Familial scimitar syndrome

C. Trisomy 8

LEOPARD syndrome indicates syndrome consisting of cardinal features of multiple lentigines, electrocardiographic conduction abnormalities, ocular hypertelorism, pulmonic stenosis, abnormal genitalia, retardation of growth, and sensorineural deafness; AVSD, atrioventricular septal defect; ASD, atrial septal defect; OMIM, Online Mendelian Inheritance in Man; and TGA, transposition of the great atries.

Clinical testing is not yet available for many of the syndromes listed in this appendix.

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Therese M. Giglia	Schneider Children's Hospital, Albert Einstein College of Medicine	None	None	None	None	Secretary, Pediatric Cardiac Intensive Care Society	None
Elizabeth Goldmuntz	Children's Hospital of Philadelphia	None	None	None	None	None	None
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Craig A. Sable	Children's National Medical Center	None	None	None	None	None	None
Deepak Srivastava	Gladstone Institute of Cardiovascular Disease, University of California at San Francisco	None	None	None	None	None	None
Catherine L. Webb	Children's Memorial Hospital, Northwestern University Feinberg School of Medicine	None	None	None	None	None	None

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Calum A. MacRae	Massachusetts General Hospital/Harvard Medical School	None	None	None	None	None	None
Luisa Mestroni	University of Colorado Health Sciences Center	None	None	None	None	None	None
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