

# New Genetic Insights into Congenital Heart Disease

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## Abstract

There has been remarkable progress in understanding the genetic basis of cardiovascular malformations. Chromosome microarray analysis has provided a new tool to understand the genetic basis of syndromic cardiovascular malformations resulting from microdeletion or microduplication of genetic material, allowing the delineation of new syndromes. Improvements in sequencing technology have led to increasingly comprehensive testing for aortopathy, cardiomyopathy, single gene syndromic disorders, and Mendelian-inherited congenital heart disease. Understanding the genetic etiology for these disorders has improved their clinical recognition and management and led to new guidelines for treatment and family-based diagnosis and surveillance. These new discoveries have also expanded our understanding of the contribution of genetic variation, susceptibility alleles, and epigenetics to isolated congenital heart disease. This review summarizes the current understanding of the genetic basis of syndromic and non-syndromic congenital heart disease and highlights new diagnostic and management recommendations.

**Keywords:** Gene; Mutation; Cardiovascular genetics; Genetic syndrome; Chromosome

Recent advances in genetic technology have had a significant impact on the practice of clinical genetics and the diagnosis of genetic syndromes associated with cardiac malformations as well as sporadic congenital heart disease. The development of chromosome microarray technology has largely replaced routine chromosome analysis and has led to the identification of a number of new genomic disorders resulting from microdeletions or microduplications of genetic material. This technology has also led to expansion of the spectrum of well described genetic syndromes as individuals with non-classic features are increasingly being given a genetic diagnosis. Similarly, the rapid progress in the development of novel, high throughput, cost effective sequencing technology – so called next generation sequencing – has made it possible to sequence the entire genome or all genes (whole exome sequencing). This technology is moving rapidly from the research realm to clinical practice, with the launch of clinical whole exome sequencing first occurring in the United States in November, 2011. Taken together, these new technologies will substantially increase the diagnostic yield in patients with cardiovascular malformations. As cohorts of patients with rare syndromes increase and the molecular precision continues to evolve, there will be an increasing emphasis on the recognition of genotype-phenotype correlations and the implementation of personalized medicine. In keeping with the goals of this special issue, this review will highlight new insights and recent developments in the understanding of genetic syndromes and isolated cardiovascular malformations.

## Noonan Syndrome and RASopathies

The RAS/mitogen activated protein kinase (MAPK) pathway is important for control of cell proliferation and differentiation. Dysregulation of this pathway results in a spectrum of disorders known as “RASopathies” including Noonan, LEOPARD, Costello, and Cardiofaciocutaneous syndrome, along with Legius syndrome, and Neurofibromatosis type 1 [1,2]. The former syndromes are associated with a high rate of cardiac involvement whereas cardiac abnormalities are infrequent in Legius and NF1.

*PTPN11* was the first gene identified as causing Noonan syndrome in 2001 [3]. Subsequently a number of additional causative genes have been identified in the RAS-MAPKinase signaling pathway. Currently,

clinical testing is available for 10 genes causing Noonan syndrome and related disorders including LEOPARD syndrome, Costello syndrome, Cardiofaciocutaneous syndrome, and Noonan-like syndrome with loose anagen hair. The genes are: *PTPN11*, *RAF1*, *SOS1*, *KRAS*, *NRAS*, *BRAF*, *MAP2K1 (MEK1)*, *MAP2K2 (MEK2)*, *HRAS*, and *SHOC2*. Importantly, in the vast majority of cases Noonan and related syndromes result from point mutations and therefore require specific gene testing and will not be identified by broader methods such as chromosome microarray analysis. Table 1 provides the genetic testing yield by gene for RASopathies with cardiac features.

Noonan syndrome is a well-recognized genetic syndrome with

	Noonan	LEOPARD	Costello	Cardiofaciocutaneous
<i>PTPN11</i>	50%	90%	N/A	N/A
<i>RAF1</i>	3-17%	<5%	N/A	N/A
<i>SOS1</i>	10-20%	N/A	N/A	N/A
<i>KRAS</i>	<5%	N/A	N/A	rare
<i>NRAS</i>	<1%	N/A	N/A	N/A
<i>BRAF</i>	<2%	<5%	N/A	75%
<i>MAP2K1</i> or <i>MAP2K2</i>	<2%	N/A	N/A	25%
<i>HRAS</i>	N/A	N/A	80-90%	N/A
<i>SHOC2</i>	<1% <sup>a</sup>	N/A	N/A	N/A

<sup>a</sup>5% in cohort negative for other genes known to cause Noonan syndrome  
Compiled from Pagon RA, Bird TD, Dolan CR, Stephens K, editors. GeneReviews [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2007 [accessed Nov 30, 2011]

**Table 1:** RASopathies with cardiac features: molecular etiologies.

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a prevalence of approximately 1 in 3500 [4,5]. It is inherited in an autosomal dominant pattern although new cases are common because the de novo mutation rate is high. The classic manifestations of Noonan syndrome include short stature, pulmonic stenosis and/or hypertrophic cardiomyopathy, dysmorphic features including hypertelorism, downsloping palpebral fissures, low set posteriorly rotated ears, and webbing of the neck. Pectus excavatum or mixed pectus carinatum superiorly with pectus excavatum inferiorly are common. A variety of lymphatic abnormalities have been associated with Noonan syndrome, especially in the prenatal period where cystic hygroma, polyhydramnios and hydrops fetalis have all been described [6,7]. Genitourinary abnormalities are also common, especially cryptorchidism in males, and ophthalmologic problems and sensorineural hearing loss (10%) can occur throughout the lifetime of the affected individual. Developmental delay of variable severity occurs in approximately 25% [4,8]. Patients may also have a coagulopathy and this is important to evaluate and manage appropriately prior to any surgical or invasive procedure [9]. Finally, there is a threefold increased risk of malignancy in Noonan syndrome. Juvenile myelomonocytic leukemia is most classically associated with Noonan syndrome, but acute lymphoblastic leukemia, acute myeloid leukemia, rhabdomyosarcoma, and neuroblastoma are all seen at higher rates than in the general population as are myeloproliferative disorders [10-14].

The frequency of cardiac disease is estimated at 50-80% in patients with Noonan syndrome [4]. Pulmonary valve stenosis, often with dysplasia, is the most common cardiovascular malformation, occurring in 25-50% of patients. Other common structural defects include branch pulmonary artery stenosis, septal defects, especially secundum atrial septal defects and partial atrioventricular canal defects, and tetralogy of Fallot. Although coarctation of the aorta was originally thought to be a differentiating feature between Noonan and Turner syndromes, more recent data indicate that aortic coarctation is not rare in Noonan syndrome. Hypertrophic cardiomyopathy is found in approximately 20% of patients and may occur at any age. Up to 50% of patients with Noonan syndrome have an abnormal electrocardiographic pattern characterized by left axis deviation, an abnormal R/S ratio over the left precordial leads, and an abnormal Q wave. These findings are independent of underlying cardiovascular malformations [15]. Patients with Noonan syndrome need lifetime cardiac follow-up because left sided obstructive lesions may develop in adulthood and hypertrophic cardiomyopathy can develop at any age.

One of the interesting new developments in the genetics of Noonan syndrome is the emergence of genotype-phenotype correlations. *PTPN11* mutations are more common in individuals with pulmonary stenosis and characteristic facial features and stature [3,16,17]. Specific exons of *PTPN11* are more highly associated with risk for hematologic malignancy. Mutations in *RAF1* are associated with hypertrophic cardiomyopathy in up to 95% of individuals, as compared to the overall prevalence of hypertrophic cardiomyopathy in this population of approximately 20%. This correlation is protein domain specific, with mutations occurring in the N-terminal 14-3-3 consensus site or the C-terminus. Overall, the growth and development of patients with *SOS1* mutations is better but ectodermal abnormalities are common [18-20]. Patients with *SHOC2* mutations have a specific phenotype associated with loose anagen hair, a distinctive hyperactive behavior, and growth hormone deficiency. There is an over-representation of mitral valve dysplasia and septal defects, especially atrial septal defects, in this population [21].

LEOPARD syndrome (lentiginos, ECG abnormalities, ocular hypertelorism, pulmonary stenosis, abnormalities of genitalia, retardation of growth, deafness) is an autosomal dominant condition. Early diagnosis can be difficult due to phenotypic overlap with Noonan syndrome. Overlap with the phenotype of NF1 also occurs because both disorders have skin findings of café-au-lait macules. Age related development of lentiginos with or without hearing loss was necessary for diagnosis prior to the availability of molecular testing. Approximately 85% of patients have cardiac involvement, with HCM being most common [22]. Pulmonary valve stenosis and other structural defects have also been reported. ECG abnormalities are poorly characterized and longitudinal follow-up of a larger cohort is necessary to determine the natural history of cardiac involvement and appropriate surveillance.

Costello syndrome shares features with Noonan syndrome but is generally regarded as more severe. Unlike Noonan and Cardiofaciocutaneous syndromes which are genetically heterogeneous, Costello syndrome is only caused by *HRAS* mutations that result in constitutive or prolonged activation of the protein [23]. These mutations typically originate from the paternal germline [24]. Facial features in Costello syndrome are coarse. Ectodermal features are common and include hyperpigmentation, papillomas, and curly hair. In infancy, excessive wrinkling of the skin, especially of the hands and feet, is notable. Individuals with Costello syndrome have an approximately 15% lifetime risk for malignancy. Cardiac involvement includes structural anomalies, HCM, and conduction system abnormalities. Approximately 65-75% of Costello patients with *HRAS* mutations have cardiac involvement [2,25,26]. Pulmonic stenosis occurs in approximately 25%, arrhythmia in 42%, and HCM in 47%. The arrhythmia most commonly described is supraventricular tachycardia, especially chaotic atrial rhythm/multifocal atrial tachycardia, or ectopic atrial tachycardia [26].

Cardiofaciocutaneous syndrome (CFC) has substantial overlap with Noonan syndrome but can also be confused with Costello syndrome because of its common ectodermal involvement and more severe intellectual impairment. Skin abnormalities can be extensive and include hyperkeratosis, eczema, palmoplantar hyperkeratosis, and keratosis pilaris. The hair is typically sparse and curly. CFC syndrome has similar cardiac and lymphatic findings to Noonan syndrome [20]. Approximately 75% of patients have cardiac involvement. HCM is identified in 40% of patients and is most frequently diagnosed in infancy but can occur at any age. Pulmonary valve stenosis is identified in 25% of patients. Atrial septal defects, ventricular septal defects, mitral or tricuspid valve dysplasia and BAV are all identified with lesser frequency.

In summary, the RASopathies with cardiac involvement exhibit genetic heterogeneity and phenotypic overlap. While each syndrome has characteristic features and classic cases do exist, the overlap can make definitive diagnosis difficult without molecular testing. Genotype-phenotype correlations are emerging and will improve risk assessment, surveillance, and management. Finally, other genetic syndromic conditions characterized by short stature, congenital heart disease, and dysmorphic features such as Williams-Beuren syndrome, Turner syndrome (in females), Aarskog syndrome, and Kabuki syndrome need to be considered in the differential.

## Turner Syndrome

Turner syndrome is a relatively common aneuploidy characterized by short stature, gonadal dysgenesis, and cardiovascular malformations, most typically coarctation of the aorta. Some features

of Turner syndrome overlap with Noonan syndrome including short stature, webbed neck, and a broad shield chest. Turner syndrome is estimated to affect 1 in 2000 live births although only about 1% of 45, X fetuses survive to term. Guidelines for health supervision of patients with Turner syndrome have been developed to address their long term developmental, endocrine, cardiac and other medical management needs [27].

From a cardiac standpoint, approximately 30% of patients have a congenital heart defect with left ventricular outflow tract obstructive defects predominating. Bicuspid aortic valve is most common (30-50%) followed by coarctation of the aorta (15-30%). Elongation of the transverse aortic arch (30%), atrial and ventricular septal defects, and PAPVR (16%) are other common structural abnormalities. MRI is beneficial to more precisely define the anatomy. Recommendations exist for reimaging children using MRI when they can do so without sedation [28]. In addition, conduction or repolarization defects are common and an electrocardiogram should be obtained.

Mortality is significantly increased in women with Turner syndrome. Aortic dissection is estimated to occur in 1-2% of the Turner syndrome population and is most often preceded by dilation of the aortic root and/or ascending aorta (Figure 1). In one study, the median age for dissection was 35 years [29]. A national study in Great Britain followed a cohort of 3439 women diagnosed with Turner syndrome between 1959-2002 identified a 3-fold higher mortality than in the general population [30]. Circulatory disease accounted for 41% of excess mortality with the greatest risk being derived from aortic aneurysm (standardized mortality ratio = 23.6; 95% CI = 13.8-37.8) and aortic valve disease. The overall risk of aortic dissections is 100-fold increased in Turner syndrome [31]. One of the major contributing risk factors for cardiovascular events is hypertension, which affects up to 25% of adolescents and 40-60% of adults with Turner syndrome [32], highlighting the need for careful blood pressure monitoring at each clinic visit and institution of appropriate therapy. The significant association between elongation of the transverse aortic arch and CoA, BAV, and aortic sinus dilatation may also play a role in the risk of aortic dissection. A recent study identified BAV as a risk factor for acceleration of aortic dilation. Although longitudinal follow-up was limited, this study documented increases in the aortic sinus, sinotubular junction and mid-ascending aorta at rates 0.4 mm/year [33]. Currently there are no guidelines for medical management in this patient population. Surveillance imaging is necessary, but further studies are required to determine the most appropriate interval. Current guidelines suggest repeat imaging every 5 to 10 years if initial imaging is normal without evidence of BAV, CoA, or dilation of the ascending aorta [34].

### Connective Tissue Disorders

Hereditary disorders of connective tissue include syndromic conditions such as Marfan syndrome, Loeys-Dietz syndrome, and Ehlers-Danlos syndromes as well as non-syndromic conditions such as isolated thoracic aortic aneurysm and dissection (TAAD) with or without structural heart defects such as patent ductus arteriosus, bicuspid aortic valve (BAV), and coarctation of the aorta (CoA). These conditions are genetically heterogeneous and encompass a spectrum of clinical presentations. They share in common genetic defects in structural connective tissue proteins or pathways that affect these proteins. This abnormal connective tissue represents a risk for aneurysm formation and dissection of the aorta.

### Marfan syndrome

Marfan syndrome is an autosomal dominant connective tissue disorder with a high degree of clinical variability and a prevalence of 1 in 5000-10,000. The cardinal features of Marfan syndrome involve the ocular, skeletal, and cardiovascular systems. Up to 90% of individuals with a clinical diagnosis of Marfan syndrome have mutations in *FBNI*, a gene that codes for fibrillin-1, a structural component of microfibrils which provides mechanical stability and elastic properties to connective tissues [35-37]. Five to 21% of individuals with a known or suspected diagnosis of Marfan syndrome who did not have mutations in *FBNI* had mutations in *TGFBR2*, a gene more commonly associated with the related connective tissue disorder Loeys-Dietz syndrome [38,39].

In 2010, the clinical diagnostic criteria for Marfan syndrome, also known as the Ghent criteria, were revised [40]. Non-cardiac and -ophthalmologic features are now grouped into a category of systemic features (Table 2), and the previous system of major and minor criteria has been removed. The new criteria also integrate molecular genetic testing into the diagnostic features. Aortic root enlargement is required for the diagnosis of Marfan syndrome. In the absence of a family



Figure 1: Cardiac magnetic resonance imaging of adult with Turner syndrome revealing mild dilation of the aortic root and ascending aorta.

Phenotype	Score
Wrist or thumb sign	1
Wrist and thumb sign	3
Pectus carinatum deformity	2
Pectus excavatum or chest asymmetry	1
Hindfoot deformity	2
Plain flat foot (pes planus)	1
Pneumothorax	2
Dural ectasia	2
Protrusio acetabulae	2
Reduced upper segment / lower segment AND increased arm span/ height ratios	1
Scoliosis or thoracolumbar kyphosis	1
Reduced elbow extension	1
3 of 5 facial features	1
Skin striae	1
Pneumothorax	2
Dural ectasia	2
Protrusio acetabulae	2
Myopia	1
Mitral valve prolapse	1

<http://www.marfan.org>

Table 2: Systemic features in new diagnostic criteria for Marfan syndrome.

history, a second finding of either ectopia lentis, a pathogenic *FBNI* mutation, or a systemic score greater than or equal to 7 is required. Alternatively, if the patient has an *FBNI* mutation previously associated with aortic enlargement and also has ectopia lentis, diagnostic criteria are satisfied. In cases where there is a positive family history of Marfan syndrome, isolated findings of ectopia lentis, aortic root enlargement, or a systemic score of 7 or greater suffices.

Cardiovascular manifestations in Marfan syndrome include dilatation of the aorta at the level of the sinuses of Valsalva, a propensity for aortic tear and rupture, mitral valve prolapse with or without regurgitation, tricuspid valve prolapse, and enlargement of the proximal pulmonary artery (Figure 2). Marfan syndrome accounts for approximately 5% of all thoracic aortic aneurysms (TAAs). Recent guidelines for management of genetic syndromes associated with TAA suggest an echocardiogram at diagnosis and 6 months thereafter to determine the rate of enlargement of the aorta. Subsequently, annual imaging is recommended for patients with stable aortic diameter less than 4.5 cm [34]. These guidelines may be modified in the pediatric population, especially around the time of puberty [41]. Evaluation for surgical repair usually occurs at a threshold external diameter of 5.0 cm. Mitigating factors affecting the timing of surgical repair include rapid growth (greater than 0.5 cm/yr), family history of aortic dissection at less than 5.0 cm, or the presence of significant aortic regurgitation [34].

Genotype-phenotype correlations have been investigated for Marfan syndrome. Overall, the inter- and intra-familial variability is high. Exons 24-32 have been associated with a severe phenotype, including neonatal Marfan [42]. In addition, missense mutations in which cysteine is replaced or added are more highly associated with ectopia lentis. Mutations in exon 59 are associated with isolated ectopia lentis and mutations resulting in isolated skeletal features have also been described. Currently, the molecular basis of these genotype-phenotype correlations remains elusive [43].

Over the past decade there has been significant progress in understanding the pathogenesis of Marfan syndrome. The primary pathology is related to alterations in TGF $\beta$  signaling due to sequestration of TGF $\beta$  in the extracellular matrix triggered by loss of fibrillin-1 [44-46]. The increase in active TGF $\beta$  signaling has been shown to cause aortic root dilation, lung bullae, and impaired muscle regeneration. Angiotensin-II receptor blockers antagonize TGF $\beta$  signaling. Early trials with losartan, an angiotensin-II receptor blocker, showed marked success in ameliorating the effects of elevated TGF $\beta$  signaling in a mouse model and in a small trial in patients with Marfan syndrome [47,48]. A multi-site clinical study is ongoing to compare losartan vs. atenolol in the treatment of aortic root dilation in patients with Marfan syndrome.

### Loeys-Dietz syndrome (LDS)

This is an autosomal dominant condition that includes many features of Marfan syndrome including aortopathy (arterial tortuosity, aneurysms, and dissections), skeletal involvement (pectus deformity, scoliosis, arachnodactyly, joint laxity), dural ectasia, and aortic root aneurysm with dissection. Some features of Marfan syndrome are either less prominent (dolichostenomelia) or absent (ectopia lentis). Unique features can include hypertelorism, broad or bifid uvula, cleft palate, learning disability (rare), craniosynostosis, cervical spine instability, talipes equinovarus, soft and velvety skin, translucent skin, easy bruising, generalized arterial tortuosity and aneurysms, and dissection throughout the arterial tree. Congenital heart disease may be present including atrial septal defects and patent ductus arteriosus [49].

Aortic aneurysms are typically more aggressive than those in Marfan syndrome, with frequent dissection and rupture at small dimensions and in early childhood being demonstrated in early reports, as well as diffuse arterial involvement. Both the thoracic and abdominal aorta may be involved in LDS (Figure 3). As the cohort of LDS patients increases in size, it is important to determine whether the initial reports represent the most severe cases due to ascertainment bias since subsequent reports document more similar clinical outcomes [38]. LDS is caused by mutations in either *TGFBR1* or *TGFBR2* [49,50]. Genetic testing has an increasingly important role in both the diagnosis and management of connective tissue disorders since identification of a mutation in *TGFBR1* or *TGFBR2* may alter the timing of surgical management. Repair is currently recommended at diameters less than 5 cm. Current guidelines recommend that LDS patients should have yearly MRI surveillance from the cerebrovascular circulation to the pelvis due to the propensity for aneurysm development in other vessels [34]. The differential diagnosis includes Marfan syndrome and other connective tissue disorders with vascular involvement such as Ehlers-Danlos syndrome, vascular type, caused by mutations in *COL3A1*. Vascular EDS can be clinically differentiated from LDS on the basis of characteristic facial features, thin skin, prominent vasculature, and propensity for rupture of medium sized arteries, bowel, and uterus.



Figure 2: Cardiac magnetic resonance imaging of an adolescent with Marfan syndrome depicting moderate dilation of the aortic root.



Figure 3: Cardiac magnetic resonance imaging of adolescent with Loeys-Dietz syndrome revealing tortuosity of the infrarenal abdominal aorta.

Tissue fragility significantly complicates surgical procedures in vascular EDS.

### Familial thoracic aortic aneurysms and aortic dissection (TAAD)

This is an autosomal dominant disorder without other systemic or syndromic manifestations. Structural heart defects such as bicuspid aortic valve, aortic coarctation, or patent ductus arteriosus may also be identified. The aortic disease observed is similar to that observed in Marfan syndrome and includes dilatation of the aorta and dissections either at the level of the sinuses of Valsalva or the ascending thoracic aorta. Recent guidelines for the management of TAA have been established [34]. Mutations in *MYH11*, *ACTA2*, *TGFBR1*, *TGFBR2*, and *MYLK* have been described in individuals with TAAD [51,52]. Taken together, genetic testing identifies a cause in less than 20% of current TAAD cases, indicating substantial locus heterogeneity in this condition. Positive genetic testing results in the index case are highly informative for at risk first degree family members who can then be tested to determine whether ongoing cardiac surveillance is necessary. In the absence of an identifiable disease causing mutation in the index case, aortic imaging is recommended for all first degree relatives to identify those with asymptomatic disease.

### 22q11.2 Deletion Syndrome

Disorders resulting from the gain or loss of chromosomal material are termed genomic disorders. 22q11.2 deletion syndrome, also known as DiGeorge syndrome or Velocardiofacial syndrome is a prototypical genomic disorder and is a multiple gene deletion syndrome with an estimated prevalence of 1 in 4000 live births. The submicroscopic chromosomal deletions are detected by FISH, multiplex ligation-dependent probe amplification (MLPA), or by chromosome microarray analysis. A common deletion size of approximately 3 Mb leads to deletion of about 45 genes and results from recombination at rearrangement hotspots [53]. However, smaller and large deletions have been identified.

Clinically, 22q11 deletion is characterized by dysmorphic features, aplasia or hypoplasia of the thymus and parathyroids, learning disability, and frequent cardiovascular malformations. Palatal abnormalities, especially velopharyngeal incompetence, submucous cleft palate, and cleft palate, are common and result in hypernasal speech. Immune deficiency, hypocalcemia, feeding problems, and renal anomalies are all frequently identified. The syndrome is characterized by marked variability and a high degree of suspicion can be required to make the diagnosis in adolescents and adults [54]. New guidelines for managing patients with 22q11.2 deletion syndrome were developed in 2011 [55].

Congenital heart defects occur in approximately 75% of individuals with 22q11.2 deletion and it is the second most common cause of developmental delay and congenital heart disease after Down syndrome. A subset of conotruncal defects are common in 22q11.2 deletion syndrome, leading to the development of AHA consensus guidelines recommending FISH for 22q11.2 deletion in all infants with interrupted aortic arch type B or truncus arteriosus; tetralogy of Fallot associated with absent pulmonary valve, aortic arch anomalies (including right aortic arch), pulmonary artery anomalies, or aortopulmonary collaterals; perimembranous ventricular septal defect and associated aortic arch abnormalities; or infants with isolated aortic arch abnormalities [56]. Pulmonary stenosis, atrial septal defects, heterotaxy syndrome, and hypoplastic left heart syndrome have also been reported.

Understanding the developmental mechanisms that cause congenital heart disease in patients with 22q11.2 deletion is an active area of research. Analysis of genes within the deleted interval led to identification of the transcription factor *TBX1* as responsible for cardiovascular malformations [57]. Genome wide association studies are in progress to better understand the genetic contributors to the phenotypic variability in 22q11.2 deletion syndrome. Common variants in *TBX1* itself do not explain the phenotypic variability, suggesting the existence of additional genetic modifiers that influence phenotype [58].

### Williams-Beuren Syndrome

Williams-Beuren syndrome, like 22q11.2 deletion, is a genomic disorder – in this case caused by a 1.5-1.8 Mb microdeletion of chromosome 7q11.23. It is estimated to occur in 1 in 10,000 individuals. The diagnosis can be made by chromosome microarray analysis or by FISH for 7q11.23. The deletion leads to haploinsufficiency of approximately 28 genes, including elastin (*ELN*) [59,60]. The clinical features of Williams syndrome include short stature, characteristic dysmorphic features, intellectual disability with a characteristic outgoing personality, endocrine abnormalities including hypercalcemia, hypothyroidism, and abnormal glucose metabolism, and cardiovascular malformations.

The classic congenital heart defect associated with Williams syndrome is supravalvular aortic stenosis, often in conjunction with supravalvular pulmonary stenosis and peripheral pulmonary stenosis. Dosage sensitivity is a central concept to the understanding of genomic disorders. While many genes can exist in a single copy or in triplicate without consequence, the function of some genes requires exact dosage. In Williams syndrome, haploinsufficiency of the *ELN* has been shown to be primarily responsible for the associated cardiac features and generalized arteriopathy [59,60]. The degree of cardiovascular involvement varies widely. There is potential involvement of any medium to large-sized artery with narrowing due to medial hypertrophy. 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 [61]. Stenosis involving the aorta does not usually respond to balloon dilation. Idiopathic hypertension is seen in approximately 50% of patients, diffuse aortic hypoplasia in 10-20%, renovascular stenosis in 5-10% and structural cardiac defects such as atrial and ventricular septal defects in 10% [54]. Whereas Williams syndrome is a contiguous gene deletion syndrome, point mutations in *ELN* result in familial autosomal dominant supravalvular aortic stenosis without other syndromic features associated with Williams syndrome [62]. Recently, a new syndrome caused by duplication rather than deletion of the Williams syndrome critical region has been identified [63,64]. It is characterized by severe expressive speech delay and autism, intellectual disability in some patients, hypotonia, and cardiovascular malformations, especially patent ductus arteriosus.

### Rare Genetic Syndromes

A search of the Online Mendelian Inheritance in Man (OMIM) database reveals that the number of genetic syndromes with cardiac involvement is greater than 1300. Advancing genetic technology has led to the recognition of the molecular basis of these syndromes and further delineation of the phenotypic features. Here we briefly highlight three genomic disorders and three single gene syndromic disorders that have relevant new findings.

## Microdeletion and microduplication syndromes

Three genomic disorders that have significant new developments with regard to cardiac or genetic findings within the last 5 years include 1p36 syndrome, Jacobsen syndrome, and 17p11.2 duplication syndrome (also known as Potocki-Lupski syndrome, PTLs).

**1p36 deletion syndrome:** 1p36 deletion syndrome, also called monosomy 1p36, is the most common terminal chromosome deletion and is estimated to occur in at least 1 in 5000 livebirths [65-68]. The majority of cases are de novo deletions. The diagnosis is most frequently made by chromosome microarray analysis since the deletion can be very difficult to visualize by routine chromosome analysis. Characteristic features include severe intellectual disability, seizures, hearing loss, dysmorphic facial features, microbrachycephaly, large anterior fontanelles and cardiac defects. The first systematic clinical characterization of a cohort of 1p36 deletion patients identified 23% with a history of dilated cardiomyopathy in infancy and 43% with structural defects, the most common of which was patent ductus arteriosus followed by ventricular septal defects, dilated aortic root, atrial septal defects, and BAV [66]. A second cohort study identified an even larger degree of cardiac involvement, with 71% of patients having a structural abnormality [69]. More recently, left ventricular noncompaction has been recognized as a common feature of 1p36 syndrome and is estimated to occur in greater than 20% of patients [69-71]. Guidelines for longitudinal surveillance have not been established and given that the identification of 1p36 deletions is relatively new, the natural history of cardiomyopathy in this patient population is not yet known.

**Jacobsen syndrome:** Jacobsen syndrome is a well-recognized genetic syndrome caused by partial deletion of the long arm of chromosome 11. The size of the deletion is variable, ranging from 7 Mb to 20 Mb in size. These deletions are detectable by routine chromosome analysis. Chromosome microarray analysis is useful for more specific delineation of deletion size. While Jacobsen syndrome is quite rare, cardiac features are common and often severe, occurring in more than 50% of patients [72]. Left ventricular outflow tract obstructive defects are the most common anomalies and Jacobsen is one known syndromic cause of hypoplastic left heart syndrome [73]. The syndrome is also characterized by growth retardation, dysmorphic features, intellectual disability, and abnormal platelet number and/or function (Paris-Trousseau) or pancytopenia [74]. Noonan syndrome and Turner syndrome are in the differential given shared features of small size and facial dysmorphism. Some recent breakthroughs in the investigation of Jacobsen syndrome have stemmed from the establishment of mouse models and identification of *ETS-1* as a gene within the deletion region important for congenital heart defects [75].

**17p11.2 duplication syndrome:** 17p11.2 duplication syndrome, also known as Potocki-Lupski syndrome, is a recently identified syndrome resulting from the reciprocal duplication of the genomic region that causes Smith-Magenis syndrome when deleted [76]. This genomic disorder is most frequently caused by duplication of a 3.7 Mb region containing the *RAI1* gene on 17p, although larger and smaller duplication sizes have been identified [77]. The diagnosis is made by chromosome microarray analysis. Characteristic features include failure to thrive, intellectual disability, autistic features, apraxia, sleep apnea, and cardiovascular malformations. Although relatively small numbers of patients have been evaluated in this newly described disorder, there has been significant effort to characterize the cardiac anomalies [78,79]. Approximately 50% of patients have cardiac involvement including structural heart disease, aortopathy, and

electrocardiographic abnormalities. Dilated aortic root was the most common abnormality, occurring in 20% of patients.

## Single gene syndromic disorders

The molecular basis of single gene disorders underlying Mendelian disease is being identified at a rapid pace. Alagille syndrome, CHARGE syndrome, and Kabuki syndrome are single gene syndromic disorder in which cardiovascular malformations have been regularly identified.

**Alagille syndrome:** Alagille syndrome is a multisystem disorder with heart, skeletal, liver, eye, and facial features. It is classically characterized by paucity of bile ducts on liver biopsy, cholestasis and/or conjugated hyperbilirubinemia. Other findings include skeletal abnormalities such as butterfly vertebrae, eye anomalies such as posterior embryotoxon, and right sided heart defects. The diagnosis is based on clinical features and molecular testing. Mutations in *JAG1* account for the majority of cases, occurring in approximately 89% of patients that fulfill clinical criteria [80,81]. *JAG1* encodes a ligand in the Notch signaling pathway. Microdeletions containing *JAG1* on chromosome 20p12 account for up to 7% of cases and can be detected by FISH. A second gene, *NOTCH2*, has been shown to cause less than 1% of cases. The clinical features are highly variable, even within families. While detection rate is greater than 80% in patients with involvement of all five systems, mutations are identified in up to 20% of patients with involvement of only one system, illustrating that many atypical or mild cases exist that would go unrecognized without molecular testing [82,83]. Sequence variants in *JAG1* have also been identified in a small number of apparently isolated cases of tetralogy of Fallot or pulmonic stenosis [84].

The prevalence of cardiac findings is unclear due to ascertainment bias, but early descriptions of Alagille documented structural defects in greater than 90% [85]. Right sided defects predominate, occurring in 75%. Peripheral and branch pulmonic stenosis are the most common findings. Tetralogy of Fallot is seen in approximately 15% of patients. Less frequent cardiac malformations include ventricular septal defect, atrial septal defect, aortic stenosis and coarctation [85,86]. *NOTCH* and *JAG1* are known to be important for vascular development and at least 10% of patients with Alagille have documented extra-cardiac vascular anomalies including internal carotid artery anomalies, basilar artery aneurysms, middle cerebral artery aneurysm, and Moyamoya disease. Aortic aneurysms have also been documented as have intracranial vascular events without documented vessel abnormalities, with the latter accounting for 34% mortality in one cohort [87].

**CHARGE syndrome:** CHARGE is an acronym for ocular coloboma, congenital heart defects, choanal atresia, retardation of growth and development, genital hypoplasia, and ear anomalies associated with deafness. The phenotype is highly variable and the spectrum of anomalies has been further expanded since the identification of *CHD7* as the causative gene in 2004 [88]. *CHD7* is a chromatin remodeling ATPase that is important for epigenetic regulation of gene expression [89]. Clinical testing for *CHD7* mutations identified mutations in approximately 60-70% of patients with CHARGE syndrome. The remaining patients are diagnosed based on clinical findings including temporal bone imaging. Clinical criteria for diagnosis have been established [90,91]. Unilateral or bilateral coloboma occurs in 80-90%. Abnormal outer ears with or without Mondini defect of the cochlea and absent or hypoplastic semicircular canals are nearly pathognomonic, occurring in greater than 90%. Cranial nerve dysfunction is also very characteristic of classic CHARGE, but occurs in only 40% of patients.

Cardiovascular malformations are found in 75-85%. The most characteristic defects include conotruncal anomalies. AV canal defects, vascular ring, and aberrant subclavian artery are also described frequently. A subset of CHARGE patients has significant phenotypic overlap with 22q11.2 deletion or DiGeorge sequence [92] including conotruncal defects and cell-mediated and humoral immunodeficiencies along with multiple congenital anomalies; however, abnormalities of the semicircular canals, if present, are a differentiating feature. The differential also includes Kabuki syndrome, Kallman syndrome, VACTERL association, and Cat-Eye syndrome, among others.

**Kabuki syndrome:** Recently, *MLL2* was identified as the gene causing a majority of cases of Kabuki syndrome. Mutations are detected in 56-76% of individuals with a clinical diagnosis of Kabuki and it seems likely that the availability of molecular testing will assist in further delineation of associated features [93-97]. Kabuki syndrome (KS) is characterized by typical facial features (elongated palpebral fissures with eversion of the lateral third of the lower eyelid; arched and broad eyebrows; large, prominent, or cupped ears), minor skeletal anomalies, persistence of fetal fingertip pads, mild to moderate intellectual disability, and postnatal growth deficiency. Approximately 40-50% of patients with Kabuki syndrome have cardiovascular involvement. Left sided obstructive defects, especially coarctation of the aorta, are most common [98]. Atrial and ventricular septal defects are also common findings. Other structural abnormalities that may occur include genitourinary anomalies, cleft lip and/or palate, and gastrointestinal anomalies including anal atresia, ptosis and strabismus.

## The Genetic Basis of Non-Syndromic Cardiovascular Malformations

Congenital heart disease is the most common birth abnormality and the etiology is unknown in the overwhelming majority of cases. Epidemiologic and population based studies estimate that syndromic cardiovascular malformations comprise approximately 25% of cases, with the remainder being isolated malformations [99-101]. A study of normal and abnormal cardiac development in a variety of animal models has provided information on the genes important for cardiac morphogenesis. Mutations in genes known to be essential for cardiac development such as *NKX2.5*, *TBX20*, *GATA4*, *GATA6*, and *MYH6* have been identified in small families with isolated, non-syndromic cardiovascular malformations with autosomal dominant inheritance [102-106]. While families exhibiting autosomal dominant inheritance of isolated cardiovascular malformations have been genetically informative, in the majority of isolated cases, it is likely that the cardiovascular malformation results from a complex mixture of genetic and environmental factors. In many cases, congenital heart defects may be inherited as a complex trait resulting from inheritance of rare or common variants as susceptibility alleles. Higher throughput analysis afforded by next generation sequencing technology will allow comprehensive and simultaneous detection of multiple deleterious variants in genes/developmental pathways important for cardiac morphogenesis, thereby providing insight into these more complex inheritance models in which the cumulative effect of multiple genetic risk factors leads to disease.

Multiple epidemiologic studies show that isolated cardiovascular malformations show familial clustering and have high heritability. A recent Danish population-based study investigating absolute and relative recurrence risk of congenital heart disease strongly suggests that gene mutations are the underlying cause [99]. Two of the classes

of defects with the highest relative risk of recurrence of the same heart defect phenotype were heterotaxy, with a relative risk of 79.1 (95% CI 32.9-190) and left ventricular outflow tract defects, with a relative risk of 12.9 (95% CI 7.48-22.2). Familial clustering of dissimilar types of heart defects also had an elevated relative risk of 3.02 [107], suggesting that common pathways that involve shared susceptibility genes may underlie a continuum of heart defects. Recent studies provide further insight into the genetics of specific classes of cardiovascular malformations such as septal defects, LVOTO defects and heterotaxy, as discussed below.

## Septal defects

Atrial septal defects, ventricular septal defects, and atrioventricular septal defects are genetically and phenotypically heterogeneous cardiovascular malformations that together account for a large proportion of all congenital heart disease. In some cases, these defects are highly associated with syndromic congenital heart disease, such as the common finding of atrioventricular septal defects in patients with Trisomy 21. While the underlying molecular basis of most cases of nonsyndromic septal defects is unknown, autosomal dominant pedigrees have been identified and have proven useful for identification of genes or chromosomal loci (Table 3). Many of these genes have been shown to be essential for cardiac development in animal models and biological networks are emerging. For example, *GATA4*, *NKX2.5*, and *TBX5* (the gene causing Holt-Oram syndrome) may function in a complex to regulate a subset of genes required for cardiac septal formation [102]. Interestingly, mutations in *MYH6* or *ACTC1* can cause either congenital heart disease or cardiomyopathy. Genetic testing is clinically available for *GATA4*, *NKX2.5*, and *ACTC1*. The yield of testing in individuals with cardiovascular malformations without a significant positive family history of autosomal dominantly inherited disease is not known with certainty although some studies indicate that it is relatively low [108].

Phenotype	Chromosome locus	Gene
Atrial septal defect 1	5p	unknown
Atrial septal defect 2	8p23.1	<i>GATA4</i>
Atrial septal defect 3	14q11.2	<i>MYH6</i>
Atrial septal defect 4	7p14.2	<i>TBX20</i>
Atrial septal defect 5	15q14	<i>ACTC1</i>
Atrial septal defect 6	4q32.3	<i>TLL1</i>
Atrial septal defect 7	5q35.1	<i>NKX2.5</i>
Atrial septal defect 8	6q24.1	<i>CITED2</i>
Ventricular septal defect 1	8p23.1	<i>GATA4</i>
Ventricular septal defect 2	6q24.1	<i>CITED2</i>
Ventricular septal defect 3	5q35.1	<i>NKX2.5</i>
Atrioventricular septal defect 1	1p31-21	unknown
Atrioventricular septal defect 2	3p25.1	<i>CRELD1</i>
Atrioventricular septal defect 3	6q22.31	<i>GJA1</i>
Atrioventricular septal defect 4	8p23.1	<i>GATA4</i>

**Table 3:** Chromosomal loci and genes implicated in non-syndromic septal defects.

## LVOTO defects

Left ventricular outflow tract obstructive (LVOTO) defects include bicuspid aortic valve (BAV), aortic valve stenosis (AVS), coarctation of the aorta (CoA), and hypoplastic left heart syndrome (HLHS). A recent national epidemiologic study from Denmark indicated that these defects exhibited a high relative recurrence risk [99]. LVOTO malformations are a leading cause of infant mortality and the majority of cases are non-syndromic. These malformations are thought to share developmental pathogenic mechanisms. Mutations in *NOTCH1* have been identified as a cause of aortic valve malformations, including BAV and early aortic valve calcification, via linkage analysis in an affected family [109]. Linkage to multiple loci, including chromosomes 2p, 6q, 10q, and 16p have been identified for AVS, CoA, and HLHS indicating that they are genetically heterogeneous [110,111]. Additional loci for BAV have been found on chromosomes 5q, 13q, and 18q [112]. The presence of an LVOTO lesion increases the risk of identifying BAV in a parent or children and the overall relative risk of BAV in relatives is 5.05 (95% confidence interval: 2.2-11.7) [113]. The high heritability of these malformations has been established with recurrence risks ranging from 5% risk of BAV in first degree family members of individuals with AVS, CoA, or HLHS to 22% recurrence risk of cardiovascular malformations in siblings of patients with HLHS [113-115]. As a result these findings, first degree relatives of an individual with AVS, CoA, or HLHS should be screened by echocardiography.

## Heterotaxy

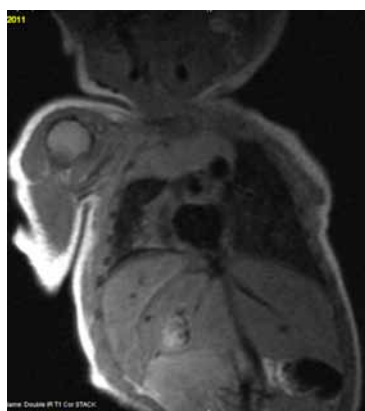
Heterotaxy spectrum defects have the highest relative risk of recurrence of all classes of cardiovascular malformations [99]. Despite this, heterotaxy is sporadic in the vast majority of cases, and occurs without an identifiable family history, a fact that has made the identification of causative genes challenging. These lesions have high morbidity and mortality and because of the substantially decreased reproductive fitness it is possible that some fraction of heterotaxy could be caused by highly penetrant *de novo* mutations. Mechanistically, they have their basis in abnormal patterning of the left and right sides of the body during early embryogenesis prior to organ formation, with an end result of abnormal organ positioning (Figure 4) [116]. While heterotaxy can be associated with genetic syndromes, including aneuploidies, most cases are non-syndromic. Inheritance can be X-linked, autosomal dominant, autosomal recessive, or sporadic [117-119]. The X-linked form is caused by mutations in the zinc finger transcription factor *ZIC3* [120]. Mouse models and expression analyses of patient mutations

demonstrate that heterotaxy results from loss of function of *ZIC3* [121]. Mutations account for approximately 75% of familial cases and 5% of sporadic cases. Affected females have been identified [121-123]. Testing for mutations in *ZIC3* should be performed for any male with heterotaxy since the results significantly affect familial recurrence risk: 50% recurrence risk if positive vs. 5-10% recurrence risk if negative. Mouse models have been useful for elucidating other genes important for the development of LR patterning. Mutations in genes within the TGF $\beta$  pathway required for left-right patterning such as *NODAL*, *CFC1*, *ACVR2B*, *LEFTYA*, and *FOXH1* have been identified in patients with heterotaxy but also with isolated congenital heart defects [124-127]. Likewise, mutations in *ZIC3* have been identified in patients with isolated heart defects as well as patients with more complex phenotypes including VACTERL association [121,128]. Clinical testing is available for heterotaxy genes. In addition, there is a newly identified overlap between heterotaxy and primary ciliary dyskinesia (PCD). Published data indicate that at least 6.4% of PCD patients have heterotaxy and unpublished data indicate that true numbers may be 10-20% [116]. This has important management implications with regard to pulmonary toilet and institution of chest physiotherapy and surveillance in order to prevent bronchiectasis. The increasing sophistication and availability of genomic technology is leading to the identification of additional genetic causes, including a new candidate pathway involving *ROCK2* and *SHROOM3* [129,130]. Rare variants in genes in developmental pathways important for left-right patterning may confer susceptibility to heterotaxy with the phenotype resulting from a combination of inherited susceptibility risk factors and environmental risks such as maternal diabetes and vascular insufficiency.

## Other Important Cardiovascular Disorders with A Genetic Basis

While the focus of this special issue is on emerging topics in congenital heart defects, the genetic basis of a number of other cardiac disorders including cardiomyopathy, inherited arrhythmias, atrial fibrillation, coronary artery disease, and hyperlipidemia is rapidly evolving. We briefly discuss relevant issues related to use of genetic testing in clinical practice and refer the reader to several excellent recent reviews for more comprehensive overviews of these topics [131-140].

Genetic testing is becoming increasingly incorporated into clinical practice in the assessment of heritable arrhythmias such as long QT syndrome (LQTS), Brugada syndrome, Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC), and Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) and cardiomyopathies such as Hypertrophic Cardiomyopathy (HCM), Dilated Cardiomyopathy (DCM), Left Ventricular Noncompaction (LVNC), and Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC). HCM, DCM, LVNC, and restrictive cardiomyopathy (RCM) all occur in childhood with a bimodal age distribution (Figure 5A-D) and the underlying causes of cardiomyopathy are more heterogeneous than in adults. Genetic syndromes, neuromuscular disease, inborn errors of metabolism, mitochondrial disorders, and mutations in genes encoding structural components of the cardiomyocyte including the sarcomere and cytoskeleton all contribute to the genetic heterogeneity [141,142]. Recent reviews highlight the genetic basis and pathogenesis of these disorders [143,144]. Heart Failure Society of America guidelines have been developed to guide cardiac surveillance and genetic testing in cardiomyopathy, and similar guidelines exist to guide testing for other autosomal dominantly inherited cardiovascular disorders [140,145]. These guidelines recommend that clinical genetic testing should be incorporated into routine patient care and highlight the importance of



**Figure 4:** Cardiac magnetic resonance imaging of a child with heterotaxy syndrome depicting a midline liver with symmetric hepatic venous drainage.





**Figure 5:** A) Cardiac magnetic resonance imaging of an adolescent with dilated cardiomyopathy depicting a dilated left ventricle in end-diastole with thinning of the left ventricular septum and free wall. B) Cardiac magnetic resonance imaging of an adolescent with hypertrophic cardiomyopathy depicting a thickened left ventricle with below normal left ventricular end diastolic dimension. C) Cardiac magnetic resonance imaging of a child with left ventricular noncompaction depicting trabeculations in the left ventricle with deep recesses. D) Echocardiogram of an adolescent with restrictive cardiomyopathy depicting severe dilation of both atria with preserved left ventricular size and thickness.

genetic counseling for patients and at risk family members with regard to inheritance, family screening recommendations, genetic testing options, and management implications of results [140,146,147].

## Future Directions

### The role of the geneticist in pediatric cardiology

As the technology for evaluation of the human genome continues to improve, there is an increasing need for professionals to apply and interpret genetic testing in a thoughtful and clinically meaningful way. Pediatric cardiologists and geneticists need cross discipline training in order to provide the best care to patients with congenital heart disease. The increased availability of genetic testing provides an opportunity to improve diagnostic yield and precision and to deliver more sophisticated recurrence risk information. Subsequently, there is a clear need for longitudinal clinical studies in which careful phenotyping and outcome measures are evaluated in the context of a known genetic diagnosis. *A priori* knowledge of a patient's genotype should be used for the development of more sensitive measures of disease and to improve the ability to monitor disease progression.

Studies of large cohorts are required in order to begin to identify genetic and environmental modifiers of disease and determine whether risk stratification is feasible.

## Epigenetics

Epigenetics refers to a functionally relevant modification of the genome that does not change the nucleotide sequence. Examples of epigenetic changes include DNA methylation, histone modification, and microRNAs. These modifications function to alter the expression of genes or determine allele-specific inheritance. These modifications play an important role in the development of cardiac hypertrophy and heart failure, and a role in cardiac development is being increasingly recognized [148-150]. In addition, epigenetics underlie cellular reprogramming that can occur, for example, in induced pluripotent stem cells (iPS) in which DNA methylation of pluripotency genes is required to alter cell fate.

Recently, the effect of maternal diet and nutritional status on the epigenome has been identified [151,152]. Likewise, transcriptional changes in the fetus are speculated to result from environmental effects such as maternal folate status and maternal hyperglycemia via epigenetic mechanisms [153,154]. Epigenetic changes are therefore important candidates to explain the biological mechanisms underlying gene x environment interactions. Future studies are required to determine whether epigenetic mechanisms integrate these gene x environment influences to dysregulate cardiac morphogenesis.

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