Genetics of Congenital Heart Disease: The Glass Half Empty

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Genetics of Congenital Heart Disease: The Glass Half Empty

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Genetics of Congenital Heart Disease
The Glass Half Empty

Akl C. Fahed, Bruce D. Gelb, J. G. Seidman, Christine E. Seidman

Abstract: **Congenital heart disease (CHD) is the most common congenital anomaly in newborn babies. Cardiac malformations have been produced in multiple experimental animal models, by perturbing selected molecules that function in the developmental pathways involved in myocyte specification, differentiation, or cardiac morphogenesis. In contrast, the precise genetic, epigenetic, or environmental basis for these perturbations in humans remains poorly understood. Over the past few decades, researchers have tried to bridge this knowledge gap through conventional genome-wide analyses of rare Mendelian CHD families, and by sequencing candidate genes in CHD cohorts. Although yielding few, usually highly penetrant, disease gene mutations, these discoveries provided 3 notable insights. First, human CHD mutations impact a heterogeneous set of molecules that orchestrate cardiac development. Second, CHD mutations often alter gene/protein dosage. Third, identical pathogenic CHD mutations cause a variety of distinct malformations, implying that higher order interactions account for particular CHD phenotypes. The advent of contemporary genomic technologies including single nucleotide polymorphism arrays, next-generation sequencing, and copy number variant platforms are accelerating the discovery of genetic causes of CHD. Importantly, these approaches enable study of sporadic cases, the most common presentation of CHD. Emerging results from ongoing genomic efforts have validated earlier observations learned from the monogenic CHD families. In this review, we explore how continued use of these technologies and integration of systems biology is expected to expand our understanding of the genetic architecture of CHD. (*Circ Res*. 2013;112:707-720.)**

Key Words: congenital abnormalities ■ genetics, medical ■ heart defects, congenital

Congenital heart disease (CHD) defines a large set of structural and functional deficits that arise during cardiac embryogenesis (Figure 1). CHD is the most common type of birth defect, accounting for one third of all major congenital

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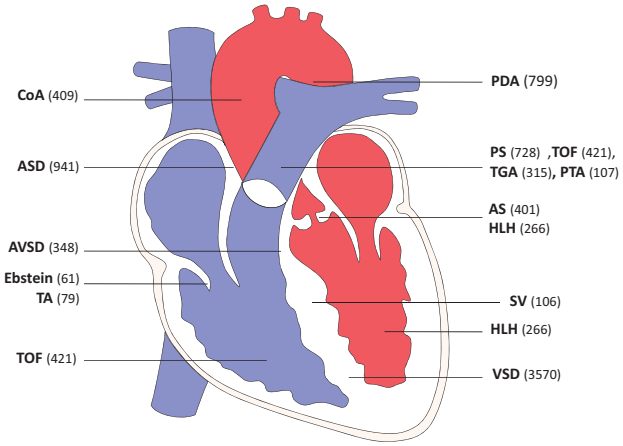

Figure 1. Locations of heart malformations that are usually identified in infancy, and estimated prevalence based on the CONCOR database.[9] Numbers indicate the birth prevalence per million live births. AS indicates aortic stenosis; ASD, atrial septal defect; AVSD, atrioventricular septal defect; CoA, coarctation of the aorta; Ebstein, Ebstein anomaly; HLH, hypoplastic left heart; MA, mitral atresia; PDA, patent ductus arteriosus, PS, pulmonary stenosis; PTA, persistent truncus arteriosus; TA, tricuspid atresia; TGA, transposition of the great arteries; SV, single ventricle; TOF, tetralogy of Fallot; and VSD, ventricular septal defect.

anomalies. Worldwide, 1.35 million infants are born with CHD each year. CHD is also identified in 10% of stillbirths[1] and is presumed to be a substantive cause of early fetal demise. The prevalence of CHD varies across countries and continents.[2] In North America, CHD occurs in 8.1 per 1000 live births,[3] whereas in Asia the prevalence is 9.3 per 1000 live births, a difference that is attributed, in part, to higher rates of parental consanguinity.[2] Because the full spectrum of congenital heart defects includes mild lesions that are clinically quiescent for decades (eg, bicuspid aortic valve with a population prevalence ranging from 0.5% to 0.9%),[4–6] the worldwide prevalence of all CHD may exceed these estimates.

Until recently, nearly half of the deaths because of CHD occurred during infancy, but with remarkable advances in prenatal diagnosis, corrective strategies, and longitudinal care, infantile mortality has substantially declined. Today, more than 75% of CHD children who survive the first year of life, including those with complex malformations, will live into adulthood.[7,8] Recent estimates define the prevalence of CHD in adults at approximately 3000 per million,[9] a figure that predicts that there are 21 million adults living with CHD. Moreover, this unique cardiovascular disease population has been increasing by almost 5% per year.[10] Life-long CHD can pose substantial physiological, emotional, and socioeconomic challenges for patients, families, and society. As such, discovery of the causes for CHD is not only a fundamental research endeavor, it is vital to the healthcare of this growing community.

Causes of CHD are often partitioned into genetic and nongenetic categories. Well-recognized nongenetic causes of CHD include environmental teratogens (dioxins, polychlorinated biphenyls, pesticides),[11] maternal exposures (alcohol, isotretinoin, thalidomide, antiseizure medications),[12] and infectious agents (eg, rubella).[13] Despite decades of international efforts to combat these factors, the compendium of nongenetic causes of CHD continues to increase and to diversify. Antiretroviral medications[14] that are taken by 8 million people worldwide,[15] and the epidemic of obesity[16] with associated phenotypes of diabetes mellitus[17] and hypercholesterolemia[18] are recognized as emerging risk factors for CHD.

The genetic landscape of CHD is also changing. The renowned pediatric cardiologist Dr Helen Taussig speculated that since "common cardiac malformations … occur in otherwise 'normal' individuals … these malformations must be genetic in origin."[19] Yet, discovery that gene mutations cause CHD began only decades after her death. Initial human genetics methodologies had poor resolution, which restricted analyses to inherited forms of CHD. Given the historical rates of poor reproductive fitness in CHD patients and high mortality, early genetic studies of familial CHD were often biased toward uncomplicated malformations such as atrial septal defects (ASDs) and ventricular septal defects (VSDs). The improved health of CHD patients and major advances in genomic technologies has shifted this paradigm. Contemporary methodologies provide robust opportunity for comprehensive genomic analyses of all CHD patients, including those with sporadic and complex malformations. An accompanying manuscript (The Congenital Heart Disease Genetic Network Study [CHD GENES]: Rationale, Design, and Early Results)[20] details approaches being spearheaded by the NIH Heart, Lung, and Blood Institute to study genetic pathogenesis in thousands of CHD patients. Comparable efforts are underway around the world.[21–23]

Deciphering the contributions of genetic and nongenetic causes of CHD has benefited from extensive model organism studies that have provided a wealth of insights into cardiac developmental biology. Molecular pathways have been identified that orchestrate formation of primordial cardiogenic fields that shape the cardiac crescent and linear heart tube, and which drive atrial, ventricular, inflow, and outflow tract morphogenesis.[24–27] Within these pathways, details have emerged about molecules that promote lineage specification, differentiation, cell growth, and migration, and that orchestrate temporal and spatial patterns of gene expression.[28–32] Positioning previously discovered and novel CHD genes onto this blueprint presents remarkable opportunities to further extend the knowledge base of cardiac embryogenesis and to fully understand the causes and mechanisms of CHD.

In this review, we examine historical and recent genetic discoveries in CHD, focusing on malformations identified in infancy (Figure 1) that inform developmental themes in heart development. In addition, we examine strategies (Figure 2) that can expand the discovery of new CHD genes and explore relationships between genetic and nongenetic pathogenesis. Given the current pace of human CHD genetics and genomics research, these efforts can only be considered a preview. Readers should expect that rapidly emerging data will provide a much fuller understanding of the genetic architecture of heart development and CHD.

Genetic Models of CHD

Familial CHD mutations occur as autosomal dominant, autosomal recessive, or X-linked traits that are expressed with high penetrance and with variable clinical manifestations.

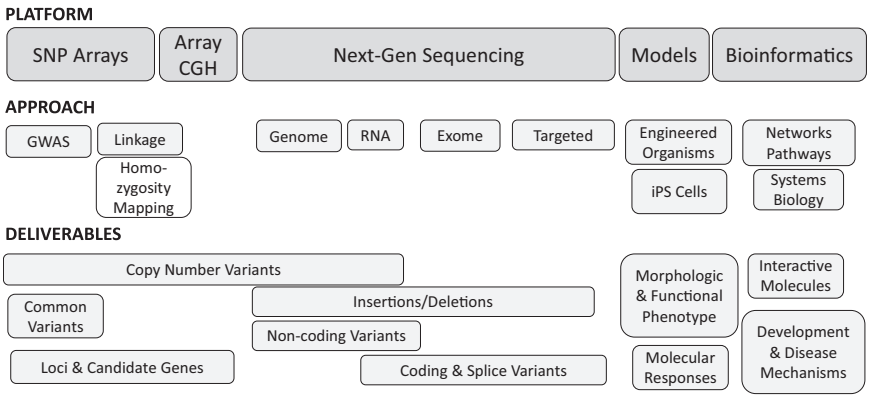

Figure 2. Strategies to define the genetic architecture of congenital heart disease (CHD) are illustrated by experimental platforms, approaches, and expected deliverables. Boxes that extend across categories indicate that multiple strategies can provide comparable data.

CHD is genetically heterogeneous. That mutations in different genes cause an identical malformation underscores the highly interdependent roles of molecules involved in heart development. Moreover, the spectrum of heart malformations that arise for an identical gene mutation implicates genomic context,[33,34] maternal–fetal environment,[12] cardiac biomechanics,[35] and other factors as important influences that impact the clinical consequences of CHD mutations.

Hundreds of autosomal dominant or X-linked mutations have been identified in familial forms of CHD. Information that has ensued from these discoveries is reviewed below. An evolutionary perspective of CHD mutations predicts that reduced reproductive fitness and early mortality would cause substantial negative selection that eliminates CHD mutations from human populations. If autosomal dominant or X-linked mutations make a significant contribution to the population prevalence of CHD, many must be new (de novo) mutations that initially result in sporadic CHD. Autosomal dominant de novo mutations should cause high recurrence rates in the offspring of sporadic CHD cases. However, a recent large analysis of 1.7 million Danes identified that only 2.2% of individuals with CHD had an affected first-degree relative,[36] data that challenges the model that dominant, de novo mutations are major contributors to CHD.

Autosomal recessive or somatic mutations and polygenic variants pose alternative genetic models to account for the population prevalence of CHD. In comparison with dominant gene mutations, far less is known about these genetic models in CHD. Epidemiological data that parental consanguinity (especially first-cousin marriages)[37–39] significantly increases CHD risk[40,41] provides compelling evidence that recessive mutations cause CHD. Discovery of CHD mutations in genetically closed populations[42,43] and ascertainment of the burden of compound recessive mutations in out-bred populations[44,45] should inform the contribution of recessive genes to CHD.

Somatic mutations in monogenic genes that arise during the early development of cardiac progenitor cells might cause some cases of CHD. Contemporary sequencing strategies provide estimates that in each generation a few (<10) de novo rare deleterious mutations occur.[46,47] Although these data cannot be extrapolated to estimates of the frequency of new somatic mutations in rapidly proliferating and differentiating cells, they indicate that de novo mutations occur not uncommonly and imply that analyses of somatic mutations in malformed cardiac tissues from CHD patients may be informative.

The population prevalence of CHD is not dissimilar from other common disorders. By extrapolation, an alternative genetic model for CHD is that multiple variants, which individually contribute small risks that can be maintained throughout evolution, collectively cause CHD. Genome-wide association studies of large cohorts (≥1000 cases) are typically used to explore the common disease–common variant hypothesis. The prevalence and viability of some forms of CHD (eg, ASD, VSD) and development of large CHD registries, such as CHD GENES, should enable testing of this genetic model. An alternative approach, which capitalizes on the evidence that rare monogenic mutations cause CHD, has been analyses of variants in candidate genes as polygenic risk factors of CHD. For example, an *NKX2-5* gene variant that has functional consequences when assayed by in vitro experiments, has been associated with CHD in 5 independent studies.[48] As 1% of the population carries this *NKX2-5* variant,[49] this variant may predispose to, but not directly cause, CHD. Associations with other CHD variants in selected loci[50,51] also support a polygenic model of CHD, and hint that genome-wide association studies in large CHD cohorts will be informative.

Recognizing these issues, below we review loci, genes, and mutations that cause CHD, and indicate how contemporary technologies continue to advance genetic models and mechanisms of CHD.

Structural Mutations in CHD

Chromosomal aneuploidy, the first recognized genetic cause of CHD, continues to be a major pathogenesis today (Table 1). CHD occurs in approximately 40% to 50% of trisomy 21 (1 in 600 births),[52] 20% to 50% of Turner syndrome (1 in 2500 female births),[53] and in almost all cases of both trisomy 13 and trisomy 18.[54] Although almost any cardiac malformation can occur with aneuploidy syndromes, prototypic lesions are observed in trisomy 21 (atrioventricular septal defect)[52] and Turner syndrome (coarctation of the aorta),[53] whereas other lesions (eg, transposition of the great arteries) are strikingly underrepresented. An important early conclusion from these genotype–phenotype observations was that cardiac malformations are not because of a global change in genomic content, but rather from altered dose of specific genes.

Table 1. Developmental Syndromes With Prominent CHD Phenotypes

Syndrome	Defect	Locus	Causal Gene(s)	Inheritance	Clinical Features	Most Common CHD	% With CHD	OMIM
Aneuploidy syndromes								
Down	Trisomy	Chr21	Unknown	Error in meiosis	Distinctive facial features; mental retardation; conductive hearing loss; CHD	AVC	40% to 50%	190685
Turner	Monosomy	ChrX	Unknown	Error in meiosis	Short stature; webbed neck; bowed arms; CHD	COA; BAV; dilation of ascending aorta; HLH; PAPVD without ASD	20% to 50%	
Patau	Trisomy	Chr13	Unknown	Error in meiosis	Orofacial clefts, postaxial polydactyly, microphthalmia, poor survival	ASD, VSD, PDA, polyvalvular disease	80% to 100%	
Edwards	Trisomy	Chr18	Unknown	Error in meiosis	Prenatal growth deficiency, distinctive facial features, distinctively clenched fingers, poor survival	ASD, VSD, PDA, polyvalvular disease	80% to 100%	
Abnormal chromosomal structural syndromes								
22q11 Deletion	Deletion	22p11.2	TBX1	De novo, AD (6% to 28% of cases)	Thymus aplasia or hypoplasia; parathyroid aplasia or hypoplasia; outflow tract abnormalities; distinctive facial features	TOF; IAA type B; TA; VSD; aortic arch abnormalies	80% to 100%*	188400
Williams-Beuren	Deletion	7q11.23	ELN	De novo, AD (minority of cases)	SVAS; mental retardation; distinctive facial features; hypercalcemia; renal disorders	SVAS; PAS: multiple arterial stenoses; AV and MV defects	80% to 100%*	194050
Cri-Du-Chat	Deletion	5p15.2	CTNND2	De novo	Microcephaly, severe psychomotor and mental retardation, distinctive facial features, high-pitched cry	VSD, PDA, ASD, TOF	10% to 55%	123450
Cat Eye	Inversion duplication	22q11	Unknown	De novo, AD	Coloboma of the iris, anal atresia, heart and renal malformations, periauricular tags or pits, high variability in the phenotype	TAPVR, TOF	>50%	115470
Jacobsen	Deletion	11q23	Unknown, JAM-3	De novo, AD. Inheritance of a folate-sensitive fragile site (FRA11B)	Growth and psychomotor retardation, trigonocephaly, strabismus, distinctive facial features, bilateral camptodactyly, hammertoes, isoimmune thrombocytopenia	HLH, LVOT defects	>50%	147791
1p36 Deletion	Deletion	1p36	DVL1	De novo	Mental retrdation, distinctive facial features, hearing loss, orofacial abnormalities, microcephaly	PDA, noncompaction cardiomyopathy,	43% to 70%	607872

(*Continued*)

Table 1. (Continued)

Syndrome	Defect	Locus	Causal Gene(s)	Inheritance	Clinical Features	Most Common CHD	% With CHD	OMIM
Single gene mutation syndromes								
Alagille	Single gene	20p12; 1p12	JAG1; NOTCH2	AD	Paucity of bile ducts and cholestasis; CHD; skeletal abnormalities; ocular disease; distinctive facial features	Peripheral pulmonary hypoplasia; PS; TOF	>90%	118450
Noonan	Single gene	12q24; 12p1.21; 2p21; 3p25.2; 7q34; 15q22.31; 11p15.5; 1p13.2; 10q25.2; 11q23.3; 17q11.2	PTPN11; KRAS; SOS1; RAF1; BRAF; MEK1; HRAS; NRAS; SHOC2; CBL; NF1	AD, AR	Distinctive facial features; short stature; webbed neck; pectus deformity; cubitus valgus; CHD	PS; ASD; VSD; PDA	80%	163950
Holt-Oram	Single gene	12q24	TBX5	AD	Upper limb deformities; cardiac septal defects	ASD; VSD; PDA	85%	142900
Char	Single gene	6p12	TFAP2B	AD	Distinctive facial features; PDA; limb deformities	PDA	100%*	169100
Ellis-van Creveld	Single gene	4p16	EVC; EVC2	AR	Skeletal dysplasia (short limbs; short ribs; postaxial polydactyly; dysplastic nails and teeth); CHD	ASD/ single atrium	60%	225500
Costello	Single gene	11p15.5	HRAS	De novo, AD	Distinctive facial features; short stature; failure to thrive; cardiac disease; developmental disabilities	PS; other structural heart disease; hypertrophy; rhythm disturbances	63%	218040
Cardiofaciocutaneous	Single gene	12p12.1; 7q34; 15q22.31; 19p13.3	KRAS; BRAF; MAP2K1; MAP2K2	De novo, AD	Distinctive facial features; mental retardation; cardiac defects	PS; ASD; HCM	71%	115150
CHARGE	Single gene	8p12; 7q21.11	CHD7; SEMA3E	AD	Choanal atresia; coloboma of the eye; CHD; mental retardation	TOF; ASD; VSD	85%	214800
Duane-radial ray syndrome DDRS (Okihiro syndrome)	Single gene	20q13.2	SALL4	AD	Forearm malformations, renal malformations, Duane ocular anomaly, CHD	VSD, PFO, TOF	….	607343
Kabuki syndrome	Single gene	12q13.12	MLL2	De novo, AD	Mental retardation, postnatal dwarfism, distinctive facial features, spinal deformities, cleft palate, recurrent otitis media	VSD, ASD, TOF, SV, COA, PDA, TGA, RBBB	31% to 55%	147920

AD indicates autosomal dominant; AR, autosomal recessive; and CHD, congenital heart disease.
*CHD is required for the diagnosis of the syndrome.

This concept gained more clarity with the development of methodologies to define subchromosomal changes in genome structure, denoted today as copy number variants (CNVs). CNVs are large deletions or amplifications of DNA segments that arise principally from inappropriate recombination, because of flanking region-specific repeat sequences or from highly homologous genes (such as ancestral duplication sites) that misalign during meiosis. CNVs that encompass millions of bases can be identified by cytogenetic analyses, often in combination with fluorescence in situ hybridization. Smaller CNVs (affecting as few as several hundred bases) can be detected using high-resolution array-comparative genomic hybridization or genomic microarrays that assess single nucleotide polymorphisms (SNPs) and copy number probes. These CNVs and insertions or deletions of size less than 100 bases (collectively denoted as indels) are identified by sequence-based approaches.

As CNVs alter the dosage of contiguous genes, they can produce syndromic CHD (Table 1). A 3-Mb CNV on chromosome 22q11 causing CHD, craniofacial abnormalities,

Table 2. Copy Number Variations (CNVs) Associated With Recurrent Cases of Nonsyndromic CHD

Locus	Size Range (Kbp)	No of Cases	Inheritance	CNV	No of Genes	Genes*	Phenotype	Reference(s)
1q21.1	418–3,981	21	De novo, inherited, n/a	Gain, loss	3–45	PRKAB2, FM05, CHD1L, BCL9, ACP6, GJA5, CD160, PDZK1, NBPF11, FMO5, GJA8	TOF, AS,CoA, PA, VSD	23, 63, 64, 66, 70
3p25.1	175–12,380	3	De novo, inherited	Gain	2	RAF J, TMEM40	TOF	63, 72
3q22.1–3q26.1	680–32134	3	Inherited, n/a	Gain, loss	0–300	FOXL2, NPHP3,FAM62C, CEP70, FAIM, PIK3CB, FOXL2, BPESC1	DORV, TAPVR, AVSD	69, 70, 71
4q22.1	45	2	De novo	Gain	1	PPM1K	TOF	63, 23
5q14.1-q14.3	4,937–5454	2	Inherited, de novo	Gain	41103	EDIL3, VCAN, SSBP2, TMEM167A	TOF	23, 64
5q35.3	264–1777	4	De novo, n/a	Gain	19–38	CNOT6, GFPT2, FLT4, ZNF879, ZNF345C, ADAMTS2, NSD1	TOF	23, 70
7q11.23	330–348	2	N/a	Gain	5–8	FKBP6	HLH, Ebstein's	70
8p23.1	67–12,000	10	N/a	Gain, loss	4	GATA4, NEIL2, FDFT1, CSTB, SOX7	AVSD, VSD, TOF, ASD, BAV	23, 70
9q34.3	190–263	3	De novo	Loss	2–9	NOTCH1, EHMT1	TOF, CoA, HLH	63, 70
11p15.5	256–271	2	N/a	Gain	13	HRAS	DILV, AS	70
13q14.11	555–1430	3	N/a, de novo	Gain	7	TNFSF11	TOF, TAPVR, VSD, BAV	64, 69
15q11.2	238–2,285	12	N/a	Loss	4	TUBGCP5, CYFIP1, NIPA2, NIPA1	CoA, ASD, VSD, TAPVD, complex left-sided malformations	23
16p13.11	1414–2903	3	N/a	Gain	11–14	MYH11	HLH	70
18q11.1–18q11.2	308–6118	2	N/a	Gain	1–28	GATA6	VSD	70
19p13.3	52–805	3	N/a, de novo	Gain, loss	1–34	MIER2, CNN2, FSTL3, PTBP1, WDR18, GNA11, S1PR4	TOF	64, 23
Xp22.2	509–615	2	N/a	Gain	2–4	MID1	TOF, AVSD	64

CHD indicates congenital heart disease; CNV, copy number variant; and HLH, hypoplastic left heart syndrome.
Only CNVs that have recurred in ≥1 CHD patient are listed. A more extensive list of CNVs in CHD is provided in Online Table I.
*Genes listed are encompassed by the CNV and were reported by the authors as candidate genes that are responsible for CHD.

neurocognitive disabilities, absent or hypoplastic thymus, hypocalcemia/hypoparathyroidism (velocardiofacial or DiGeorge syndrome, now denoted as 22q11 deletion syndrome) is the most common CHD CNV. Occurring in 1 in 4000 live births, chromosome 22q11 CNVs account for 15% of tetralogy of Fallot cases.[55] Although more than 30 genes are impacted by this CNV, sequence analyses of some of these candidates[56] and animal models[57] indicate that altered dose of one gene, *TBX1*, a T-box transcription factor that promotes cell proliferation in the secondary heart field,[58,59] from which the outflow tract and right ventricle develop,[60] accounts for most of the observed clinical features. CHD can also occur from a 1.5-Mb deletion on chromosome 7q11.23 that alters the dosage of over 25 genes and causes Williams–Beuren syndrome (supravalvar aortic stenosis, developmental delays, gregarious personality, elfin facies, and hypercalcemia).[61] Although disruption of the elastin gene (*ELN*) accounts for the cardiovascular abnormalities in Williams–Beuren syndrome and for isolated cases of nonsyndromic supravalvar aortic stenosis,[62] unidentified genes impacted by the chromosome 7q11.23 CNV account for other phenotypes in this syndrome.

Recent analyses have identified multiple CNVs that contribute to isolated (nonsyndromic) CHD (Table 2 and Online Table I). Large de novo CNVs (present in the probands but absent in both parents) have been reported in tetralogy of Fallot,[63,64] left-sided lesions (eg, aortic stenosis, bicuspid aortic valve, coarctation of the aorta,[65,66] hypoplastic left heart syndrome),[67] and other sporadic cases of CHD.[23,68–72] These studies estimate that 5% to 10% of sporadic, nonsyndromic CHD, in patients with normal karyotype and fluorescence in situ hybridization analyses, is because of a rare (≤1% population frequency) CNV.

Some CNVs encompass previously identified CHD genes or genes known to participate in heart development from the study of model organisms. For example, recurrent CNVs identified in CHD cases that occur at chromosome 8p23.1 impact the cardiac transcription factor *GATA4*,[23] and CNVs at chromosomes 20p12.2 and 9q34.3 impact members of the Notch signaling pathway, *JAG1* and *NOTCH1*.[63]

Other CNVs identified in CHD cases provide opportunities for the discovery of new disease genes, efforts that usually require multidisciplinary approaches (Figure 2). One strategy for defining the culprit gene is to demonstrate independent pathogenic mutations in CHD cases without the CNV, an approach that successfully identified *TBX1* as the critical gene responsible for chromosome 22q11 deletion syndromes[56] and *CHD7*[73] on chromosome 8q12.1 that causes CHARGE syndrome[74] (*c*oloboma of the eye, *h*eart defects, *a*tresia of the choanae, *r*etardation of growth and/or development, *g*enital and/or urinary abnormalities, and *e*ar abnormalities and/or deafness). Other approaches harness bioinformatic strategies to prioritize genes and capitalizing

on conserved pathways of heart development across species. A recent study of a recurrent CHD CNV at chromosome 6q24.3 to 25.1 that involved over 100 genes illustrates this strategy. Investigators annotated genes encoded within the critical CNV interval using datasets of cardiac developmental expression in the mouse. Selected candidate genes were then analyzed for dosage-sensitivity using morpholinos in zebrafish and monitoring cardiac development. This approach implicated *TAB2* that encodes transforming growth factor-β–activated kinase 1 (also known as MAP3K7 binding protein-2), a kinase complex member that participates in activation of nuclear factor κB and activator protein-1.[75] The identification of a chromosomal translocation [t(2;6)(q21;q25)] involving *TAB2* in a family with CHD provided further support that TAB2 participates in signal transduction during cardiac development.

Table 3. Genes That Cause Isolated CHD

Gene	Protein	Phenotypes*	OMIM
Transcription factors and cofactors			
ANKRD1	Ankyrin repeat domain	TAPVR	609599
CITED2	c-AMP responsive element-binding protein	ASD; VSD	602937
FOG2/ZFPM2	Friend of GATA	TOF, DORV	603693
GATA4	GATA4 transcription factor	ASD, PS, VSD, TOF, AVSD, PAPVR	600576
GATA6	GATA6 transcription factor	ASD, TOF, PS, AVSD, PDA, OFT defects, VSD	601656
HAND2	Helix-loop-helix transcription factor	TOF	602407
IRX4	Iroquois homeobox 4	VSD	606199
MED13L	Mediator complex subunit 13-like	TGA	608771
NKX2-5/NKX2.5	Homeobox containing transcription factor	ASD, VSD, TOF, HLH, CoA, TGA, DORV, IAA, OFT defects	600584
NKX2-6	Homeobox containing transcription factor	PTA	
TBX1	T-Box 1 transcription factor	TOF, (22q11 deletion syndromes)	602054
TBX5	T-Box 5 transcription factor	AVSD, ASD, VSD, (Holt Oram syndrome)	601620
TBX20	T-Box 20 transcription factor	ASD, MS, VSD	606061
TFAP2B	Transcription factor AP-2 beta	PDA, (Char syndrome)	601601
ZIC3	Zinc finger transcription factor	TGA, PS, DORV, TAPVR, ASD, HLH, VSD, Dextrocardia, L-R axis defects	300265
Receptors, ligands, and signaling			
ACVR1/ALK2	BMP receptor	AVSD	102576
ACVR2B	Activin receptor	PS, DORV, TGA, dextrocardia	602730
ALDH1A2	Retinaldehyde dehydrogenase	TOF	603687
CFC1/CRYPTIC	Cryptic protein	TOF; TGA; AVSD; ASD; VSD; IAA; DORV	605194
CRELD1	Epidermal growth factor-related proteins	ASD; AVSD	607170
FOXH1	Forkhead activin signal transducer	TOF, TGA	603621
GDF1	Growth differentiation factor-1	Heterotaxy, TOF, TGA, DORV	602880
GJA1	Connexin 43	ASD, HLH, TAPVR, (Oculodentodigital dysplasia)	121014
JAG1	Jagged-1 ligand	PAS, TOF, (Alagille syndrome)	601920
LEFTY2	Left-right determination factor	TGA, AVSD, IAA, CoA, L-R axis defects, IVC defects	
NODAL	Nodal homolog (TGF-beta superfamily)	TGA, PA, TOF, DORV, dextrocardia, IVC defect, TAPVR, AVSD	601265
NOTCH1	NOTCH1 (Ligand of JAG1)	BAV, AS, CoA, HLH	190198
PDGFRA	Platelet-derived growth factor receptor alpha	TAPVR	173490
SMAD6	MAD-related protein	BAV, CoA, AS	602931
TAB2	TGF-beta activated kinase	OFT defects	605101
TDGF1	Teratocarcinoma-derived growth factor 1	TOF, VSD	187395
VEGF	Vascular endothelial growth factor	CoA, OFT defects	192240
Structural Proteins			
ACTC	Alpha cardiac actin	ASD	102540
ELN	Elastin	SVAS, PAS, PS, AS, (Williams-Beuren syndrome)	130160
MYH11	Myosin heavy chain 11	PDA, Aortic Aneurysm	160745
MYH6	Alpha myosin heavy chain	ASD, TA, AS, PFO, TGA	160710
MYH7	Beta myosin heavy chain	Ebstein anomaly, ASD, NVM	160760

CHD indicates congenital heart disease; and TGF, transforming growth factor.
*Phenotypes in parentheses denote syndromes or extracardiac manifestations associated with gene mutations.

In addition to defining novel CHD genes, CNVs can be used to assess developmental networks using bioinformatic repositories of biological interactions and functional annotations, as well as gene–gene and protein–protein relationships. For example, bioinformatic analyses of rare CNVs identified in 2500 CHD cases[23] showed that CNVs impacted genes that were significantly enriched for participation in Wnt signaling, which regulates cellular processes involved in proliferation and differentiation. Although Wnt signaling in cardiac development has been identified in model organisms, this study provided the first evidence for this pathway in human CHD.[76,77]

Point Mutations in CHD

Discovery of genes with point mutations that caused CHD (Table 3) was initially undertaken in familial cases using classical linkage analyses to identify CHD loci, and sequence analyses of candidate genes or positional-cloned genes to define pathogenic mutations. Contemporary strategies bypass steps that define CHD loci and instead identify CHD mutations by direct next-generation sequencing at high read depths (≥20 reads per base) of the exome (the 1% of the genomic sequence that encodes protein) or the whole genome. These approaches identify tens of thousands of SNPs per exome[47] and multiple-fold more SNPs per genome. As most of these SNPs will be unrelated to CHD, extensive postsequencing filters are used to focus on novel or rare SNPs (occurring in ≤1% populations matched for race and ethnicity) that are predicted to have deleterious functional consequences (eg, nonsynonymous SNPs that alter evolutionarily conserved residues), and that occur in genes that are expressed during heart development. Additional evidence for pathogenicity of rare, deleterious nonsynonymous SNP includes (1) statistically significant cosegregation in familial CHD, (2) identification of recurrent deleterious nonsynonymous SNPs that arise de novo in unrelated cases of sporadic CHD, (3) genetic complementation (eg, CHD caused by deleterious nonsynonymous SNPs in genes that participate in different steps of a cardiac developmental pathway), and (4) recapitulation of CHD in model organisms.

A survey of the current compendium of definitive CHD gene mutations predicts that the mechanism by which these perturb heart development is through haploinsufficiency, or a reduction in the dosage of the encoded proteins. Haploinsufficiency occurs through gene inactivation (eg, nonsense or frameshift mutations), by altering gene expression (eg, noncoding regulatory mutations), or by encoding nonfunctional or loss-of-function (LOF) proteins (eg, missense mutations). CHD mutations that produce a gain in gene dosage (eg, duplications or noncoding regulatory mutations) or increase protein activity (eg, missense mutations that enhance protein function) are less common. The disproportionate numbers of haploinsufficiency/LOF CHD mutations may reflect inherent difficulties in recognizing sequence variants that increase gene expression or protein function. Alternatively, this imbalance may be biologically meaningful and indicate that a minimum threshold of expression of genes involved in heart development is more critical than excess levels.

Syndromic CHD Point Mutations

Point mutations that increase or decrease the dosage of genes functioning in developmental pathways that are broadly used in organogenesis cause syndromic CHD (Table 1). Alagille syndrome (tetralogy of Fallot, pulmonary (valve) stenosis, other CHD, cholestasis, skeletal abnormalities, distinctive facies, and ocular disease)[78,79] is caused by dominant mutations in the *JAG1* gene (which encodes the Notch receptor-1 ligand) in over 90% of cases or in the *NOTCH2* gene.[80] The broad mutational spectrum (frameshifts, nonsense, disrupted or cryptic splice signals, missense) in either gene reduces Notch signaling, a highly conserved pathway involved in lineage specification and cell-fate decision during development.

Holt-Oram Syndrome (ASDs, VSDs, conduction system disease, upper arm malformations) can arise from dominant LOF mutations in *TBX5*, a member of the T-box gene family,[81] that encode transcription factors that contain a conserved DNA-binding motif. T-box proteins function in regulating cell-fate decisions and early pattern formation, and different gene family members contribute to organogenesis.[82] *TBX5* is expressed in the upper limbs and heart.[81] CHD mutations have been identified that disrupt 5′-regulatory sequences, and that perturb residues in the T-box DNA-binding motif[83] are predicted to reduce the levels of functional TBX5 protein.

Noonan syndrome and related disorders (pulmonary [valve] stenosis, ASD, coarctation of the aorta, facial dysmorphism, short stature, pectus deformity, cubitus valgus, neck webbing, developmental delays) are caused by dominant gain-of-function mutations in 1 of 11 genes: *PTPN11*, SOS1, *RAF1*, *KRAS*, *BRAF*, *MEK1*, *MEK2*, *HRAS*, *NRAS*, *SHOC2*, and *CBL*. These genes encode molecules that function in the Ras/mitogen-activated protein kinase (RAS-MAPK) pathways signal transduction pathway[84,85] that communicates extracellular signals to the nucleus by modulating a GDP/GTP-regulated protein kinase cascade. The RAS-MAPK pathway is implicated in cell proliferation, differentiation, and survival by directly regulating transcriptional activation and indirectly by chromatin modification.[86]

Mutations in genes that cause syndromic CHD can occasionally produce isolated heart malformations.[56,62,87] Possible explanations for the absence of extracardiac manifestation might include subclinical phenotypes and tissue-specific mechanisms for dosage compensation.

Isolated CHD Point Mutations

The list of gene mutations that cause isolated CHD (Table 3) is rapidly expanding. Rather than providing details about each gene, below we discuss 3 broad functional categories, transcriptional regulation, signal transduction, and cardiac structural proteins, into which isolated CHD genes can be parsed.

The critical importance of transcriptional regulation of gene expression for normal heart development was first identified by the discovery of CHD mutations in *NKX2-5*,[34,88–90] *NKX2-6*,[42] *GATA4*,[70,91–93] *GATA5*,[94,95] *GATA6*,[96] *IRX4*,[97] *TBX20*,[98,99] and *ZIC3*.[100,101] CHD is caused by dominant mutations in each of these genes that are predicted to reduce physiological levels of the encoded protein by mutations that inactivate one allele, or cause LOF by disrupting DNA interactions[102] or perturbing the combinatorial interactions of transcription factors[34,91,103] and transcriptional cofactors (eg, *FOG2*, which encodes Friend of Gata-2).[104,105] Definitive evidence that haploinsufficiency of cardiac transcription factors causes CHD is predicated on independent LOF mutations that have been

identified in unrelated CHD cases and on mouse models with heterozygous gene deletions that recapitulate the cardiac malformations found in patients.[106–108]

Expression of cardiac transcription factors occurs in highly specified temporal–spatial patterns throughout development—a level of regulation that might predict there would be strong correlations between genotype and phenotype in CHD. In contrast, the clinical spectrum of malformations that arise from mutations in cardiac transcription factors is strikingly broad. Despite this generalization, the integration of insights from developmental biology has informed why some human CHD mutations produce specific clinical phenotypes. For example, LOF mutations in *ZIC3* cause cardiac laterality defects that are often accompanied by visceral heterotaxy, an association that is explained by evidence that *ZIC3* transcriptionally activates *NODAL*, a critical morphogen that is required for left–right patterning throughout the embryo.[109] LOF mutations in *GATA4* typically cause ASDs, but because a subset of these mutations disrupts GATA4–SMAD4 interactions that are critical for valve development, some patients have atrioventricular canal defects.[103] Mutations in *NKX2-5* and *TBX5* cause cardiac malformations that are associated with electrophysiological deficits, presumably because these transcription factors have been demonstrated to function in molecular specification of the myocytes in the conduction system.[110]

CHD occurs from LOF mutations in a variety of genes that encode molecules that participate in developmental signaling pathways. Establishment of a left–right axis during embryogenesis is predicated on a laterality signaling pathway that results in asymmetrical placement of organs about the midline as well as in cardiac looping.[111] Mutations in *ZIC3*, *NODAL*, and in *LEFTY2*, which encodes a molecule that restricts the expression of Nodal-responsive genes to the left side of embryos, disrupt normal laterality signals that direct cardiac looping and cause a spectrum of heart malformations.[112–118]

The Notch signaling pathway is implicated in multiple developmental processes. Mutations in *NOTCH1*, *NOTCH2*, and *JAG1* are all predicted to reduce ligand-induced signaling,[119] albeit with strikingly different consequences. As discussed above, mutations in *NOTCH2* and *JAG1* cause diverse phenotypes in Alagille syndrome. In contrast, *NOTCH1* mutations typically cause malformations of the aortic valve.[78,79,120] As Notch signaling participates in epithelial-to-mesenchymal transformation,[121] a process that is critical for normal valvulogenesis, Notch1-dependent signals appear to be particularly important in this cellular transformation process.

The identification of independent LOF mutations in developmental signaling factors in unrelated CHD cases and evidence for genetic complementation (eg, *NOTCH2* and *JAG1* cause Alagille syndrome) strongly supports the pathogenicity of these mutations. Although mice engineered to heterozygous LOF mutations in *Jag1*[122] or *Notch2*[123] lack CHD, homozygous deficiency of either gene causes an amalgam of defects and embryonic lethality. More recent studies of regional-specific depletion of these molecules[124,125] have demonstrated extensive abnormalities in cardiovascular morphogenesis,[126] therein substantiating that mutations in these genes are definitive causes of human CHD.

Genes that encode cardiac structural proteins comprise the smallest category and least definitive monogenic cause of CHD. LOF mutations in *ELN* (which encodes elastin) cause CHD in the context of Williams–Beuren syndrome (described above) and less commonly in isolated cases of supravalvar aortic stenosis.[62] Rare missense mutations and premature termination mutations in *MYH6*, *MYH7* (encoding the α and β cardiac myosin heavy chains, respectively), and *ACTC* (a cardiac actin) have been reported as rare causes of autosomal dominant ASDs,[127] Ebstein anomaly,[128] and other CHD.[129–132] As most missense mutations in cardiac sarcomere proteins cause human cardiomyopathy, and mice with haploinsufficiency of *MYH6*[133] or *ACTC*[134] have normal heart structure, the evidence that sarcomere protein genes mutations cause CHD is not definitive. Rare missense mutations in *MYH11* (encoding smooth muscle myosin heavy chain) are reported to cause dominant thoracic aortic aneurysm that is sometimes accompanied by patent ductus arteriosus.[135,136] As *MYH11*-null mice have delayed closure of the ductus arteriosus,[137] human *MYH11* mutations associated with patent ductus arteriosus may cause LOF.

Systems-Based Approach to CHD

Systems biology, which integrates complex datasets obtained from model organisms and humans into cogent pathways that operate in multidimensional spaces, provides new avenues to elucidate CHD.[138,139] Systems biological approaches capitalize on the conservation of heart development genes and processes across species,[140] molecular networks of heart development,[141] with genetic and environmental risks for CHD. Using bioinformatics and computational algorithms to elucidate molecular pathways and interactions in heart development and CHD, these strategies have the potential to predict the pathogenicity and consequences of individual CHD mutations.

Two examples illustrate new concepts that have emerged from systems-based analyses of CHD. A human dataset of CHD genes identified by CNVs, sequencing, or expression analyses[142] was used to construct a cardiac developmental network that was enriched for functional gene-ontogeny terms indicative of crucial biological processes. Twelve dysfunctional modules in these networks were perturbed by CHD that informed the clinical phenotypes found in CHD patients better than predictions based on existing pathways. These data also predicted CHD candidate genes based on guilt-by-association.[142]

Another approach built on developmental programs and functional molecular networks involved in distinct anatomic cardiac structures (eg, valves, septa, inflow, and outflow tracts).[141] Analyses of genetic and environmental risks for CHD in the context of these datasets showed significant convergence of these heterogeneous risk factors on these molecular networks.[143] An important conclusion from these analyses is that, although genetic and environmental factors involved in CHD impacted distinct genes involved in different pathways, these converged onto larger interaction network that collaborate to develop specific anatomic structures of the heart.[143]

The Glass Half Empty

The current repertoire of CHD genes can be epitomized by "the glass half empty." Collectively, these genes are still unable

to account for the population prevalence of CHD. Despite this limitation, this dataset has illuminated one important genetic mechanism for CHD: altered levels of developmental signaling molecules involved in cardiogenesis. Physiological levels can be perturbed by mutations that impact gene dosage, inactivate/enhance gene transcription, or that activate/inactivate a developmental pathway. This mechanism for disease is distinct from other cardiovascular genetic pathologies (eg, hypertrophic or dilated cardiomyopathy, long QT syndrome, or Marfan syndrome) that arise from mutations in structural proteins, with distinct and restricted functions in cardiovascular biology.

Discovery of other causes of CHD that affirm "the glass half empty" model may inform clinical insights. Instead of a monogenic mutation that alters gene dosage, CHD might arise from a two-hit model, as has been proposed in autism-spectrum disorders[144] based on finding CNVs that are inherited from an unaffected parent and a de novo CNV in affected children. Inherited genetic variants that predispose to heart malformations and that require a second genetic hit to cause CHD could account for the recurrence rates observed in the offspring of CHD patients[9] that are far less than recurrence rates for monogenic traits. A corollary to this hypothesis is that the collective burden or mitigating potential of altered levels of all molecules that participate in a common heart developmental pathway could account for variable clinical expression of CHD. Robust analyses of exomes, genomes, and RNA expression in malformed heart tissues have the potential to test these hypotheses.

Other mechanisms for regulating gene expression and levels of encoded proteins may also contribute to CHD. Histone modifications and chromatin remodeling have substantial roles in activating or silencing gene expression.[138,145,146] Recent studies[147] that demonstrated changing patterns of chromatin modification as mouse embryonic stem cells differentiate, and that correlate with the expression of cardiac developmental transcription factors implicate epigenetic pathogenesis for CHD.

MicroRNAs that regulate cardiac growth, remodeling and contribute to specific myocyte properties[148–150] might promote CHD by posttranscriptional regulation of protein levels. Mice engineered to lack microRNA-1 and -2 had decreased levels of the cardiac transcription factor *Hand2*[150] and heart malformations (VSDs) similar to those observed in Hand2-deficient mice.[151,152] Studies of malformed heart tissues from CHD patients[153–155] have demonstrated dysregulation of microRNAs, but to date there is no evidence that sequence variation in microRNAs nor altered levels directly cause CHD.

Greater understanding of the fundamental mechanisms that regulate cardiac gene and protein dosage can be expected to define new causes of CHD and to empower new therapies. Development of intrauterine fetal surgery over the past decade has fostered early interventions to attenuate critical heart defects.[156,157] In the future, these approaches might incorporate molecular interventions—to improve CHD. The considerable redundancy and complexity of transcriptional regulation poses multiple levels for compensatory interventions—epigenetic manipulations, targeting transcriptional partners, supplementing downstream molecules, or reducing posttranslational modifiers to increase protein levels.

So, although today the glass of CHD remains half empty, ongoing research efforts will soon change this scenario. Modern genomic technologies, experimental models, and system-based approaches hold the promise for a fuller understanding of causes and mechanisms of CHD. When combined with the development of new strategies to prevent or repair heart malformations, CHD patients worldwide can anticipate a full glass of life.

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Correction

In the *Circulation Research* article by Fahed et al (Fahed AC, Gelb BD, Seidman JG, Seidman CE. Genetics of congenital heart disease: the glass half empty. *Circ Res*. 2013;112:707–720. DOI: 10.1161/CIRCRESAHA.112.300853), the following items were incorrect:

On page 710, Table 1, Row 8 under the "Locus" column, the correct value is "22q11.2"

On page 714, 2nd column, last paragraph, the 4th line, references should be "*GATA4,*[91–93]…"

Reference 70 is: Tomita-Mitchell A, Mahnke DK, Struble CA, Tuffnell ME, Stamm KD, Hidestrand M, Harris SE, Goetsch MA, Simpson PM, Bick DP, Broeckel U, Pelech AN, Tweddell JS, Mitchell ME. Human gene copy number spectra analysis in congenital heart malformations. *Physiol Genomics*. 2012;44:518–541.

Reference 75 is: Thienpont B, Zhang L, Postma AV, et al. Haploinsufficiency of TAB2 causes congenital heart defects in humans. *Am J Hum Genet.* 2010;86:839–849.

These errors have been corrected in the online version of the article, which is available at http://circres.ahajournals.org/content/112/4/707.full.

(*Circ Res*. 2013;112:e182.)

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