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CYTOGENETICS (CL MARTIN, SECTION EDITOR)

Copy Number Variation in Congenital Heart Defects

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Abstract Congenital heart defects (CHDs) are the most common birth defect and a major contributor to mortality, morbidity, and healthcare costs throughout the world. Although improvements in surgical advances and cardiac care have increased the lifespan of individuals with CHDs, the underlying etiologies of disease remain elusive and there have been no interventions that decrease disease incidence. Genetic, epigenetic, and environmental factors all influence the development of CHDs, and an improved understanding of causation is a prerequisite for prevention. Genetic causes of CHDs include both structural chromosome abnormalities and single gene disorders. Copy number variation (CNV), or submicroscopic chromosomal deletions or duplications, has emerged as an important contributor to congenital genetic disorders, including CHDs, and has identified critical dosage sensitive genes important for cardiac development. Common CNVs associated with highly penetrant CHDs were first identified in genomic disorders such as 22q11.2 deletion syndrome and Williams–Beuren syndrome. More recently, research investigations and clinical diagnostic testing support a role for CNVs in CHDs with extracardiac abnormalities (ECAs) as well as isolated CHD. It is estimated that CNVs contribute to 3–25 % of CHDs with ECAs and 3–10 % of isolated CHDs. While somewhat less clear, new evidence suggests that there is an increase in rare, large, genic CNVs in patients with CHDs, indicating that the overall CNV burden may also be an important factor in disease. As genetic testing for CHDs moves forward, CNVs will play an important role in diagnosis and gene discovery.

Keywords Cardiovascular malformation · Dosage sensitive gene · Chromosome microarray · Genetic testing

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Introduction

Congenital heart defects (CHDs) are among the most common birth defects, affecting 8 in 1000 live births in the USA [1]. Congenital cardiovascular defects are the most common cause of infant death resulting from birth defects, and 24 % of infants who die of a birth defect have a heart defect [1]. The underlying causes of CHDs are varied and can include chromosome abnormalities, single gene disorders, environmental etiologies, or most commonly, multifactorial etiologies. The full impact of copy number variation (CNV) as a genetic mechanism in CHDs is not known with certainty, but both research investigations and clinical genetic testing indicate an important role that merits further investigation.

CHDs can occur as isolated findings, as part of a well-defined syndrome, or in conjunction with additional extracardiac anomalies (ECAs) not formally recognized as a syndrome. The designation of CHDs as isolated can be difficult since many important distinguishing features of syndromic conditions, such as developmental delay or dysmorphic features, may not be apparent at initial

evaluation. For the purposes of this review, "CHD with ECA" is used to describe patients with CHD and additional abnormalities or malformations not delineated as a well-characterized genetic syndrome. Likewise, the phrase "isolated CHD" is used to identify those patients in whom the only major phenotype at the time of diagnosis is a cardiovascular malformation.

In a scientific statement from the American Heart Association, Pierpont et al. cite four reasons it is important to determine the genetic cause of a child's congenital heart defect: (1) there may be other important organ system involvement; (2) there may be prognostic information for clinical outcomes; (3) there may be important genetic reproductive risks the family should know about; and (4) there may be other family members for whom genetic testing is appropriate [2]. As the number of individuals with CHD who are living to adulthood increases (about 1 in 150 adults in the USA has a congenital heart defect) [3], the reproductive implications are extended to the patient as well as family members. Thus, it is important to more precisely delineate disease-causing and disease-associated genetic contributions in order to begin to impact the incidence of CHDs.

The genetic etiology of CHDs can range from a single nucleotide variant (SNV) to complex genomic rearrangements to inheritance as a complex trait. Genetic copy number variations (CNVs) have emerged over the past two decades as an important cause of disease, including neuropsychiatric disorders [4–6] and developmental delay [7••]. CNVs are generally defined as genetic deletions or duplications that are not identifiable on traditional chromosome analysis. CNVs arise from recombination within the genome. One of the major mechanisms of CNV generation is through nonallelic homologous recombination and may be mediated by flanking low copy repeat regions or repeat regions from similar sequences in highly homologous genes. When this recombination occurs during meiosis, the result can be unequal distribution of genetic elements to the gametes, resulting in CNVs in the offspring. Nonallelic homologous recombination is most highly associated with large CNVs. Recombination can also occur among tandem arrays of variable numbers of tandem repeats (VNTR), typically leading to smaller CNVs [8, 9]. CNVs hold substantial potential to create variation throughout the genome and the de novo mutation rate of CNVs is higher than that of SNVs [10]. As opposed to SNVs, which are limited to four potential variations (A, C, G, T), CNVs can vary in size and gene content, allowing for more extensive genetic changes.

CNV Pathogenicity

CNVs occur relatively frequent, making up about 12 % of the genome of the average individual [8]. Many CNVs are common among the general population, and likely have little to no contribution to disease. Determining which CNVs contribute to disease has been a matter of intense investigation. Clinicians and researchers have converged on criteria to define CNV pathogenicity. In the context of an individual with a cardiovascular malformation, characteristics of a pathogenic CNV may include: (1) overlap with a known dosage sensitive CHD gene that causes a similar phenotype, (2) overlap with a known disease-associated region, (3) *de novo* CNV or segregation with phenotype within a family, (4) location in a gene-rich region, (5) large deletion or duplication size, and/or (6) rare (occurring in <1 % of healthy controls). Studies prioritize these attributes somewhat differently, but generally agree that pathogenic CNVs share a combination of the above qualities. In 2011, the American College of Medical Genetics (ACMG) released guidelines designed to assist in the clinical interpretation of potentially pathogenic CNVs [11]. It is important to note that the inheritance pattern of a CNV is not sufficient for making a determination of its pathogenicity. Many CNVs exhibit incomplete penetrance and/or variable expressivity. Therefore, the presence of a CNV in a healthy individual (including a parent) is not adequate to rule out a CNV as pathogenic, nor is the *de novo* nature of a CNV proof of pathogenicity. Vermeeschs et al. [12] illustrate this principle by showing *de novo* CNVs that were attributed as disease causing, but subsequent analysis identified that the phenotype resulted from point mutations in different genes [12–16]. Finally, when considering the gene content of a CNV, the ACMG warns against using data solely derived from model systems [11].

Mechanisms of CNV Effect

As discussed above, most CNVs are common in the healthy population and likely have little to no phenotypic consequence. However, when a dosage sensitive gene resides within the CNV region, deletion or duplication results in substantial effects on gene function. Disruption of critical genetic regulatory elements can also result in over- or under-expression of accompanying genes. Furthermore, CNVs do not respect genetic boundaries, and the rearrangement of DNA can result in the truncation or partial duplication of some genes, often resulting in a nonfunctional product. The mechanisms of CNVs in disease have been discussed in more depth elsewhere [17–20].

There is also evidence that some disease phenotypes are associated with higher numbers of total CNVs in the genome [21], indicating that the overall burden of CNVs may contribute to disease processes independent of any specific CNV. For example, a case–control study of patients with schizophrenia identified an increased number of novel rare

Table 1 CNVs associated with syndromic and non-syndromic CHD

Genomic region	Event	Common size (Mb)	Known syndrome	Heart defect	Other phenotypes	Ref.
1q21.1	Deletion	Variable (1.5–3.88)	1q21.1 del	LVOTO (40 %), septal (27 %), conotruncal (20 %)	DD, MC, autism	[23, 24•, 25•, 27••, 31, 40, 61••, 63, 70–72]
1q21.1	Duplication	Variable (0.42–4.27)	1q21.1 dup	PVS, TOF	DD, macrocephaly, Dys	[23, 24•, 25•, 27••, 31, 40, 61••, 63, 70–72]
1p36	Deletion	Variable (0.16–5.9)	1p36 del	Septal, PDA, ToF, Ebstein and other tricuspid valve; LVNC	CNS, Dys, DD, MC, Sz, hypotonia	[2, 28–30]
2q22-q23	Deletion[a]	Variable	Mowat–Wilson	Variable;	Hirschsprung, GU, DD, Dys, Sz, MC, CNS	[73]
4p16.3	Deletion	WHSCR, variable (1.4–1.9)	Wolf–Hirschhorn	Septal	DD, Dys, GR, Sz, MC	[2, 28–30]
5p15.2	Deletion	Variable	Cri du Chat	Septal, PDA, ToF	DD, Dys, GR, MC	[2, 28–30]
7q11.23	Deletion	1.5	Williams–Beuren	SVAS, PAS, arteriopathy	DD, Dys, GU, SS, hypercalcemia	[2, 28–30]
7q11.23	Duplication	1.5	7q11.23 dup	PDA, aortopathy	DD, Dys, behavioral abn	[74, 75]
8p23.1	Deletion	3.4	8p23.1 del	AVSD, septal, PS	DD, Dys, MC, GR, CDH	[76–78]
8p23.1-3	Duplication	3.4	8p23.1 dup	ToF, HLHS	DD, mild dys	[25•, 27••, 42, 61••]
11q24-25	Deletion	Variable (7–20)	Jacobsen	LVOTO	GR, trigonocpehaly, Dys, tcp	[2, 28–30]
17p11.2	Duplication	3.5	Potocki–Lupski	Aortopathy, septal, BAV	DD, Dys, apraxia	[79]
17p11.2	Deletion	3.5	Smith–Magenis	Variable	DD, Dys, behavioral abn, SS	[80]
22q11.2	Deletion	2.9	22q11.2 del (VCFS, DiGeorge)	Conotruncal	DD, Dys, GU, hypocalcemia, VPI	[2, 28–30]

Genomic region	Event	Size range (Mb)	Heart defect	Other phenotypes	Number reported	Ref.
2p22.3	Duplication	0.19–0.7	ToF/DORV, ASD		5	[31, 46]
5q35.3	Duplication	0.26–5.6	PS, ASD, Tri-At, ToF; dextrocardia, dextroversion, PFO	DD, Dys, MC, radial anomalies, SS,	6	[24•, 27••, 46, 81]
5q14.3	Deletion	2.03–3.06	AS	DD, SS, radial anomalies	1	[40]
5q14.3	Duplication	1.2–5.45	ToF	Mild DD	3	[24•, 25, 50]
8q24.1-3	Duplication	0.31–5.76	Heterotaxy, CoA	DD, Dys, GU, Sz	9	[26•, 40, 42]
9q34.3	Deletion	0.19–1.85	ToF, CoA, ASD	DD, Dys, autism, MC, hypotonia,	4	[23, 27••, 38, 40]
9q34.3	Duplication	0.23, 4	HLHS		1	[27••]
11q25	Duplication	0.34–0.69	VSD, CoA	hemifacial microsomia, eye anomalies	4	[31, 42, 47••]
15q11.2	Deletion	0.23–2.2	ToF		12	[24•]
16p11.2	Deletion	0.53	DORV		1	[47••]
16p11.2	Duplication	0.60–0.71	ToF, single ventricle		4	[25•, 61••]
16p13.11	Deletion	1.24	ASD		1	[27••]

Table 1 continued

Genomic region	Event	Size range (Mb)	Heart defect	Other phenotypes	Number reported	Ref.
16p13.11	Duplication	0.77–1.54	HLHS, TGA, VSD, ASD, CoA, ToF	nasal abnormalities, hypermobility, hypertonia, vocal cord palsy	8	[27••, 42, 61••, 82]
16q24.3	Deletion	0.14–1.8	Septal, AVSD	Dys, MC, Sz	4	[42]
17p13.3-13.2	Deletion	0.35–2.31	ToF, ASD, PDA	DD, Dys, GR, ptosis; Miller–Dieker syndrome region	7	[25•, 40, 42]
17p13.3-13.2	Duplication	0.24–0.54	ToF, heterotaxy	Miller–Dieker syndrome region	2	[25•, 26•]
18q23	Deletion	3.50	ToF		1	[25•]
18q23	Duplication	0.52–3.41	ToF, AVSD	Hydronephrosis, astigmatism	3	[25•, 42, 47••]
Xp11.2	Duplication	0.05–0.15	ToF		2	[25•]
Xp11.2	Unknown	Unk	LVOTO, aortopathy		2	[25•, 63]

ASD atrial septal defect, *AVSD* atrioventricular septal defects, *BAV* bicuspid aortic valve, *CNS* structural central nervous system anomalies, *CoA* coarctation of the aorta, *DD* developmental delay, *DORV* double outlet right ventricle, *Dys* dysmorphic features, *GR* growth retardation, *GU* genitourinary anomalies, *HLHS* hypoplastic left heart syndrome, *LVNC* left ventricular noncompaction cardiomyopathy, *LVOTO* left ventricular outflow tract obstructive defects, *MC* microcephaly, *PDA* patent ductus arteriosus, *PS* pulmonic stenosis, *PAS* pulmonary artery stenosis, *PVS* pulmonary valve stenosis, *SS* short stature, *SVAS* supravalvar aortic stenosis, *Sz* seizures, *TCP* thrombocytopenia, *TGA* transposition of the great arteries, *ToF* tetralogy of Fallot, *VPI* velopharyngeal insufficiency, *VSD* ventricular septal defect

[a] 15 % of Mowat–Wilson patients have deletions, the remainder have point mutations

CNVs in patients with adult-onset (15 %) or childhood-onset (20 %) disease compared with controls (5 %) [22]. Several studies have investigated the potential importance of CNV burden in CHDs. In studies that compared total numbers of CNVs in individuals with or without CHDs, the most common finding was that the genomes of individuals with CHDs were enriched for large, rare, and/or gene-containing CNVs [23, 24•, 25•, 26•, 27••]. This pattern was observed for both CHDs with ECAs and isolated CHDs. Interestingly, however, the number of total CNVs was frequently similar between groups with CHDs and control groups. This suggests that for CHDs, novel CNVs in large, gene-containing regions are more likely to collectively contribute to pathogenicity.

Analyses of CNVs in Congenital Heart Defects

Syndromic CHDs Caused by CNVs (Genomic Disorders)

Gene dosage is known to play a critical role in CHDs as exemplified by a number of genomic disorders which are strongly associated with CHDs. The most common genetic syndrome associated with CHD that is caused by a submicroscopic chromosome abnormality is 22q11.2 deletion syndrome. This multiple gene deletion syndrome occurs in approximately one in 4,000 livebirths and is most frequently caused by a common deletion from chromosome 22 mediated by nonallelic homologous recombination. Cardiovascular malformations are highly penetrant and include conotruncal malformations such as truncus arteriosus, tetralogy of Fallot, and interrupted aortic arch type B (Table 1). Additional CHD-associated deletion or duplication syndromes are shown in Table 1. Many of these syndromes are not identifiable by conventional chromosome analysis and require FISH or chromosome microarray analysis for diagnosis. However, a number of genomic disorders have variable breakpoints such that a subset is detectable by karyotyping. For example, approximately 60 % of patients with Wolf-Hirschhorn syndrome have deletions that are cytogenetically visible, whereas 40 % are submicroscopic chromosome deletions. Well-characterized genomic disorders featuring CHDs have been the subject of frequent reviews [2, 28–30].

It is worth noting that chromosome microarray analysis has increased the identification of syndromic disease in patient populations previously thought to have isolated CHDs. For example, Erdogan et al. [31] identified three individuals in their analysis of patients with reportedly isolated CHD who had syndromic CNVs for which the clinical manifestations were not yet fully present or appreciated. One patient harbored a 17p11.2 deletion, the critical region for Smith- Magenis Syndrome. A deletion

Table 2 Summary of diagnostic yield in studies of CNVs in CHD

Year and reference	# Patients	Subphenotypes	ECAs vs isolated	% with causal variants (%)	Overall CNV burden
CHDs with ECAs					
2007 Thienpont et al. [38]	60	Various	ECA	17.0	Not discussed
2008 Richards et al. [39]	20	Various	ECA	25.0	Similar to control
2010 Breckpot et al. [40]	150	Various	ECA	17.3	Not discussed
2011 Goldmuntz et al. [41]	58	Various	ECA	3–21	Not discussed
2013 Lalani et al. [42]	203	Various	ECA	3.9	Not discussed
2011 Fakhro et al. [26•]	262	Heterotaxy	Heterotaxy	14.5	2× rare variants versus control
Mixed isolated CHDs and CHDs with ECA					
2012 Tomita-Mitchell et al. [27••]	945	Various	Not specified	4.3	Enriched for large, rare CNVs
2012 Sorensen et al. [46]	402	Various	Both	3.2	Not discussed
2013 Connor et al. [47••]	121	Various	Both	7 + 22 VUS	Not discussed
2013 Bachman et al. [48•]	45	Various	Both	10.9[a]	Not discussed
Isolated CHDs					
2008 Erdogan et al. [31]	105	Various	Isolated	3	Not discussed
2013 Zhao et al. [49]	100 adults	Various- mild to moderate severity	Isolated	3[a]	Increased large, rare CNVs
Tetralogy of Fallot					
2009 Greenway et al. [23]	114 Trios	Tetralogy of Fallot	Isolated	10	No statistical difference
2012 Soemedi et al. [24•]	283 Trios + 2256 add'l	Various, most tetralogy of Fallot	Isolated	5	Increased rare, genic CNVs
2012 Derwinska et al. [50]	150	Various, most tetralogy of Fallot	Both	2.3	Not discussed
2012 Silversides et al. [25•]	433 adults	Tetralogy of Fallot	Both	9.9	Increased large, rare, genic CNVs
Hypoplastic left heart syndrome and other left-sided cardiac lesions					
2012 Payne et al. [60]	43	HLHS	Both	Not assessed	No difference in likely pathogenic CNVs
2013 Carey et al. [61••]	223	Single ventricle (72 % HLHS)	Both	13.9	Not discussed
2013 Warburton et al. [62••]	223 trios	HLHS and conotruncal defects	Both	5.6	Not discussed
2012 Hitz et al. [63]	67 families with 174 affected	Left-sided CHD	Not specified	10	Same as controls

[a] Percentages adjusted to include only those CNVs that were likely to be pathogenic

and duplication of 22q11.2 were identified in the other two patients. Because early diagnosis of a genetic syndrome allows patients to be monitored and treated proactively, identifying a genetic syndrome at an early age in patients with apparent isolated cardiovascular malformations provides an opportunity to improve management and outcome. Similarly, many CNVs listed in Table 1 have been identified both in patients with isolated CHD and in patients with syndromic CHD or CHD with ECA. For example, 8p23.1 deletion syndrome and 1q21.1 deletion syndrome are both phenotypically delineated. Characteristic syndromic features include intellectual disability, specific dysmorphic features, and microcephaly. However, a number of reports have also identified these CNVs in patients with isolated CHDs. Further investigation will be required to better understand whether this reflects differences in deletion or duplication sizes between patients, incomplete penetrance of ECA, or a need for deep phenotyping.

Congenital Heart Defects with Extracardiac Anomalies

CNVs have been shown to play an important role in the pathogenicity of complex birth defects [32–35], and the majority of CNV studies performed thus far in patients with CHDs analyze cohorts of patients with CHDs and ECAs. Table 1 highlights CNVs (either deletion or duplication) described in at least 4 patients with CHDs. While some analyses discussed below focused on one or a few subtypes of CHD, those here include patients with the full spectrum of CHDs. A summary of the findings and diagnostic yield from each study is shown in Table 2.

Approximately 22 % of CHDs are accompanied by extracardiac manifestations, including developmental delay, dysmorphic features, and/or additional developmental anomalies [36, 37]. Several studies have investigated the importance of CNVs in CHDs and extracardiac anomalies (ECAs) [38–43]]. Collectively, they estimate that CNVs contribute to approximately 3–25 % of cases of CHDs with ECAs, with several studies falling in the range of 17–20 % [38, 40, 41]. These data are consistent with a role for CNVs in patients with CHDs and extracardiac anomalies. As a point of reference, pathogenic CNVs have been identified in about 18.2 % of cases of autism spectrum disorder [44] and 19 % of patients with developmental delay/intellectual disability [45]. CNV analysis has been implemented as a first-line diagnostic tool in both of these populations. A significant proportion of the patients with ECAs in whom pathogenic CNVs were identified had a neurological abnormality (developmental delay, intellectual disability, autism, seizures, microcephaly, etc.). While neurological disorders did not completely segregate with CNVs (patients with neurological abnormalities were found to not have pathogenic CNVs, and patients without neurological disorders were found to have pathogenic CNVs), the presence of a neurological disorder with a CHD should prompt further evaluation and genetic testing.

Isolated Congenital Heart Defects

In most instances, CHDs occur in isolation, without any extracardiac abnormalities. While the above data demonstrated an important role for CNVs in CHDs with extracardiac anomalies, the question still remains whether CNVs contribute to isolated CHDs. One of the challenges in identifying isolated CHDs is that additional features may not have been identified, or may not have presented at the time of diagnosis (such as developmental delay). This is particularly true in instances where infants or young children are among the study population. There is a need for longitudinal studies with careful phenotyping for further investigation of this issue. Table 1 includes CNVs described in four or more patients with isolated CHDs or CHDs with ECA.

Several studies have investigated CNVs in mixed cohorts of patients: those with isolated CHD as well as patients with CHD and ECA. Tomita-Mitchell et al. [27••] performed CNV analysis on 945 patients and, after excluding those with chromosomal abnormalities associated with a known syndrome, identified potentially causative CNVs in 4.9 % of their population. Additionally, Sorensen et al. [46] analyzed CNVs specifically in regions of the genome known to contain CHD-associated genes and found likely causal variants in 3.2 % of their study cohort. This translates to 2.9 % of their isolated CHD cohort and 8.3 % of their patients with CHDs with ECAs. Connor et al. [47••] and Bachman et al. [48•] both analyzed the utility of CNV analysis in a clinical setting for infants with CHDs. Both studies reported a high rate of CNV detection in their patients, 29 % in the study by Connor et al., and 22 % in Bachman et al. These numbers did not include patients with positive results on classical cytogenetic testing, indicating the rate of genetic diagnoses in newborns with CHD is quite high. While the study cohort used by Connor was larger ($n = 121$ patients vs. $n = 45$), both studies included similar numbers of patients with apparently isolated CHDs (36 % in Connor, 28 % in Bachman). Interestingly, however, these two analyses detected different rates of CNVs in their isolated CHD population. In Connor et al., nearly half of the CNVs detected were in patients with apparently isolated CNVs, whereas in Bachman et al., all of the CNVs were in patients with CHD and ECAs. It is possible that differing patient populations, hospital practices, or definitions of "isolated" could account for some of the differences in results.

The majority of studies on isolated CHD have focused on specific subtypes of cardiovascular malformations. Only two studies published thus far have investigated patients with all types of isolated CHD. A study of 105 patients with varying

types of CHD performed by Erdogan et al. [31] found *de novo* pathogenic CNVs in 3 % of their patients with isolated CHD. Zhao et al. [49] examined CNVs in 100 Han Chinese adults with simple- to moderate-isolated CHD. They found large, rare CNVs in 39 % of their patients, compared with 21 % of the control group. Both of these numbers are substantially higher than those seen in other studies, possibly due to differences in study design. CNVs with genes implicated in cardiac development were present in three individuals. If we include only these three with the most likely pathogenic CNVs, these data agree with the study by Erdogan et al. [31]. Additional studies discussed below have focused on specific subtypes of cardiovascular malformations, and may be more informative regarding the utility of CNV analysis in isolated CHDs.

Tetralogy of Fallot

Tetralogy of Fallot (ToF) is a type of conotruncal cardiovascular malformation that is identified in both syndromic (notably 22q11.2 deletion syndrome) and nonsyndromic CHDs. Greenway et al. [23] focused exclusively on patients with isolated ToF and investigated 114 trios (the proband and both parents), identifying rare, *de novo*, gene-containing CNVs in 10 % of their patients. Soemedi et al. [24] analyzed 283 ToF trios and identified rare, *de novo*, gene-containing CNVs in 5 % of their cohort. When they combined this data with analysis of an additional 2,256 CHD patients, they estimate that rare genic deletions contribute approximately 4 % of the population-attributable risk of sporadic CHD. These two studies suggest that pathogenic CNVs contribute to approximately 4–10 % of isolated ToF.

Two other groups investigated the role of CNVs in ToF using cohorts that also included patients with ECAs. Derwińska et al. [50] analyzed 150 patients, 122 of whom had ToF, and found rare, gene-containing CNVs in eight of their patients. Based on gene content, they presumed that three of these would be pathogenic for CHDs. The three presumed pathogenic CNVs were identified in two patients with ToF and developmental delay and one patient with aortic arch anomalies. Additionally, the cohort analyzed by Silversides et al. [25•] analyzed 433 adults with ToF. Of these, they defined 57 as having "syndromic CHD" based on the presence of particular ECAs. This study identified rare, large (>500 kb) CNVs in 43 (~10 %) of their patients. These studies support the hypothesis that pathogenic CNVs play an important role in the pathogenesis of ToF.

Hypoplastic Left Heart Syndrome and Other Left-sided Cardiac Lesions

Hypoplastic left heart syndrome (HLHS) is among the most severe congenital heart defects, requiring multiple complex surgeries for survival, with 50–70 % survival at 5 years of age [51, 52]. While there is substantial evidence for the underlying genetic contribution to HLHS, and left ventricular outflow tract obstructive defects (LVOTO) [53–57], only a few genes have been identified as important contributors [58, 59]. Several studies have investigated the potential role of CNVs in the pathogenicity of HLHS.

In 2012, Payne et al. [60] analyzed 43 patients with HLHS, 10 of whom also demonstrated ECAs. While they found an increased number of small (~60 kb) CNVs in their HLHS patients, they did not detect any difference in likely pathogenic CNVs in their HLHS cohort as compared to their control population. Based on their data, they concluded that CNVs did not play a substantial role in the pathogenicity of HLHS. Subsequent to this study, however, additional reports indicate a potential important role for CNVs in HLHS and related disorders. Carey et al. [61••] investigated CNVs in 223 patients with isolated single ventricle defects, 76 % of whom had HLHS. They found putatively pathogenic CNVs in 13.9 % of their patients, compared with only 4.4 % of the control group. When comparing HLHS patients with putatively pathogenic CNVs to those without pathogenic CNVs at 14 months of age, the presence of a CNV was associated with worse neurodevelopmental and somatic growth outcomes. Warburton et al. [62••] also investigated the role of CNVs in patients with either conotruncal defects or HLHS. Among their 71 HLHS patients, they identified *de novo* CNVs in nine individuals (12.7 %). They found a similar percentage of *de novo* CNVs in patients with conotruncal defects. When filtered for regions and genes implicated in CHDs, CNVs potentially explained CHDs in 5.6 % of their total cohort. Interestingly, even though isolated CHD and CHD with ECA were included in this study, no differences in CNV number were identified between these two groups.

HLHS is often considered to be the more severe end of a spectrum of LVOTO defects that also includes aortic stenosis, bicuspid aortic valve, and coarctation of the aorta. Hitz et al. [63] performed CNV analysis on 67 families with left-sided cardiac defects, including 464 total individuals with 174 affected family members. After screening CNVs for gene content and inheritance pattern, they identified 17 individuals with CNVs that were likely pathogenic for CHD, accounting for about 10 % of their population. Thus, three of the four studies investigating CNVs in left-sided cardiac defects implicate CNVs in 10 % or more of the patients in their cohorts, indicating that this subgroup may benefit substantially from chromosome microarray analysis.

Heterotaxy Syndrome

Heterotaxy results when developmental processes regulating left–right patterning in the embryo are disrupted [64].

This results in organ misalignment and malformation. The developing heart is particularly sensitive to left–right patterning cues as it loops and partitions the different chambers. Congenital heart defects are the most common manifestation of heterotaxy, and frequently also the most severe. Fakhro et al. [26•] investigated the role of CNVs in patients with heterotaxy. In a cohort of 262 patients, 45 novel CNVs in 39 patients were identified. When compared to a control population, CNVs occurred at a higher rate in patients with heterotaxy (14.5 % of patients with heterotaxy had rare, genic CNVs vs 7.4 % of the control population). Several of these CNVs contained genes that have been shown in animal studies to be essential for left–right patterning, indicating that CNV analysis can also be a valuable tool in gene discovery.

CNVs in Fetal CHDs

Historically, prenatal genetic testing has relied on analysis of chromosomes by traditional karyotyping or fluorescence in situ hybridization (FISH). In 2009, Van den Veyver et al. analyzed the potential utility of CNV analysis in cases of advanced maternal age or abnormal ultrasound findings. They found pathogenic CNVs in 5 % of 300 cases and gained important new information by chromosome microarray in 2.3 % [65]. A few studies have investigated the utility of chromosome microarray analysis specifically for detection of CNVs in fetuses with CHDs. Schmid et al. [66] analyzed 12 fetuses who had CHDs and multiple anomalies with a normal chromosome analysis and FISH for 22q11.2 deletion. They reported causative CNVs in 3/12 fetuses (25 %) and CNVs of uncertain significance in another 25 %. Bao et al. [67] also performed an analysis on seven fetuses with particularly complex malformations (two aneuploid and five euploid). Two rare CNVs identified contained genes that were related to cardiac development. In a larger study, Yan et al. [68] analyzed 76 fetuses in the second trimester, all of whom had negative results on traditional G-band karyotype analysis and FISH for 22q11.2 deletion. Among their study cohort were 49 fetuses with apparently isolated CHDs and 27 with additional anomalies detectable by ultrasound analysis. Overall, they detected pathogenic CNVs in 6.6 % of their study population. An additional 5.3 % carried variants of unknown significance defined as *de novo* CNVs containing potentially important functional genes. Their data did not indicate a difference in CNV pathogenicity between patients with isolated CHD versus CHD with ECAs. As mentioned previously, however, the ability to detect ECAs early in life and particularly during gestation is limited may not be detectable using current fetal imaging technology. These studies indicate that pathogenic CNVs can be detected in fetuses both with isolated CHDs and CHD with ECAs. However, many CNVs are not fully understood and demonstrate evidence of variable penetrance and/or expressivity, indicating that CNV analyses need to be undertaken cautiously and should be paired with comprehensive genetic counseling in order to empower prenatal decision-making. A psychosocial analysis of 23 women who received prenatal CNV analysis as part of their prenatal care revealed that many women were unprepared for uncertain results and that this increased the psychological burden associated with the pregnancy [69]. Improvements in understanding the postnatal implications of CNVs will provide additional resources in the prenatal setting. Both pretest and posttest counseling is essential for prenatal chromosome microarray testing for CHD. Because different types of cardiovascular malformations carry different risks for association with syndromic versus isolated CHDs, genetic counselors with specific expertise in cardiovascular genetics are important resources.

Conclusions

CHDs are a complex set of disorders with varied causes that include genetic, epigenetic, environmental, and multifactorial. As genetic testing technology continues to expand, more opportunities are available to determine the genetic contributors to disease. CNVs have been increasingly appreciated as an important cause of birth defects, including CHDs. Studies published thus far estimate that CNVs contribute to disease burden in approximately 3–25 % of patients with CHDs with ECAs and 3–10 % of patients with isolated CHDs, indicating that CNV analysis can provide valuable diagnostic information in this patient population. Notably, many of these studies performed 22q11.2 FISH prior to CNV analysis and excluded patients with positive results suggesting that the actual yield of chromosome microarray testing in the CHD population is even higher. CNV analysis of patients with apparent isolated CHD may reveal a genetic abnormality consistent with a genetic syndrome [31] and allow for more proactive monitoring and treatment for these patients. There is a need for ongoing longitudinal studies and deep phenotyping in patients with CNVs and CHDs in order to determine the natural history, to better define the contribution to isolated versus syndromic CHDs, and to delineate the degree to which variable expressivity and incomplete penetrance play important roles. As with other genetic tests, proper counseling of patients and families before and after testing is essential. Additionally, while common CNVs have been identified in patients with CHDs (Table 1), most CNVs are associated with a variety of cardiovascular malformations, suggesting that CNVs may predispose an individual to CHD while other genetic and environmental factors fine-

tune the phenotype. Finally, determining which CNVs are pathogenic is a dynamic process, and new findings will likely influence our current understanding of CNV contribution to disease. Despite these limitations, copy number analysis has established itself as an important tool in the genetic diagnosis of CHDs, and can provide valuable information to patients and families with CHDs.

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Human and Animal Rights and Informed Consent All studies by SM Ware involving animal and/or human subjects were performed after approval of the appropriate institutional review boards. When required, written informed consent was obtained from all participants.

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