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Chromosomal Imbalances in Patients with Congenital Cardiac Defects: A Meta-analysis Reveals Novel Potential Critical Regions Involved in Heart Development

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ABSTRACT

Objective. Congenital cardiac defects represent the most common group of birth defects, affecting an estimated six per 1000 births. Genetic characterization of patients and families with cardiac defects has identified a number of genes required for heart development. Yet, despite the rapid pace of these advances, mutations affecting known genes still account for only a small fraction of congenital heart defects suggesting that many more genes and developmental mechanisms remain to be identified.

Design. In this study, we reviewed 1694 described cases of patients with cardiac defects who were determined to have a significant chromosomal imbalance (a deletion or duplication). The cases were collected from publicly available databases (DECIPHER, ISCA, and CHDWiki) and from recent publications. An additional 68 nonredundant cases were included from the University of Michigan. Cases with multiple chromosomal or whole chromosome defects (trisomy 13, 18, 21) were excluded, and cases with overlapping deletions and/or insertions were grouped to identify regions potentially involved in heart development.

Results. Seventy-nine chromosomal regions were identified in which 5 or more patients had overlapping imbalances. Regions of overlap were used to determine minimal critical domains most likely to contain genes or regulatory elements involved in heart development. This approach was used to refine the critical regions responsible for cardiac defects associated with chromosomal imbalances involving 1q24.2, 2q31.1, 15q26.3, and 22q11.2.

Conclusions. The pattern of chromosomal imbalances in patients with congenital cardiac defects suggests that many loci may be involved in normal heart development, some with very strong and direct effects and others with less direct effects. Chromosomal duplication/deletion mapping will provide an important roadmap for genome-wide sequencing and genetic mapping strategies to identify novel genes critical for heart development.

Key Words. Heart Development; Chromosomal Imbalance; Congenital Heart Defects

Introduction

The high morbidity and mortality associated with severe congenital cardiac defects has stimulated the search for new strategies to decrease their frequency, reduce their severity and improve their treatment. While advances in surgical and medical care have substantially improved

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patient outcomes, recent studies suggest morbidity and mortality remains high for some of the most severe defects and that further improvements in outcomes may depend on the development of new therapeutic approaches.[1,2] However, our ability to reduce the incidence or severity of heart defects is limited by an incomplete understanding of their causes.[3] Previous studies have identified genes responsible for some types of congenital heart defects (CHDs).[4,5] To date, the identified genes appear to account for only a small percentage of the observed cases. This suggests that the pathophysiology of congenital heart disease is quite complex, potentially involving a large number of genes and genetic defects with variable levels of expressivity. Similar to other disorders demonstrating a complex pattern of inheritance, CHDs may often depend on the concurrent inheritance of multiple predisposing genetic factors that interact with epigenetic or environmental influences to result in impaired structural development.[6] The current challenge therefore is to identify such predisposing genetic factors that contribute to heart defects to aid development of new therapeutic strategies.

To meet this challenge, a number of approaches have been used to identify genes responsible for cardiac defects. In addition to the characterization of rare families with clear patterns of inheritance, targeted sequencing of genes determined to be potentially involved in heart development, either based on functional analysis in animal models or mapping chromosomal deletions/duplications in patients, has been successful in identifying causative mutations in patients with CHD.[3] In patients without significant chromosomal imbalances or clear familial inheritance, whole exome sequencing strategies have been utilized to identify disease-causing mutations in known or novel heart development genes.[7] Unfortunately, these nondirected sequencing strategies identify hundreds of novel genetic variants of unknown significance for each patient. Prioritizing sequence variants for further evaluation depends on (1) the identification of potentially pathogenic mutations using bioinformatics; (2) the identification and characterization of genes expressed in the tissues that contribute to cardiac development; and (3) the characterization of genetic and genomic abnormalities in other patients with congenital cardiac defects.

In this study, we sought to develop a roadmap for the prioritization of novel genetic variants in patients with heart defects by identifying genomic regions associated with congenital heart disease based on the mapping of chromosomal imbalances. Chromosomal imbalances occur approximately in 8–13% of patients with CHDs[8] and may represent important opportunities to localize and identify genes potentially involved in human cardiac development and the genesis of CHDs. In our review of publicly available databases and publications, we identified 1694 patients with a chromosomal imbalance (duplication or deletion) and congenital cardiac malformations. In addition, we identified 68 patients from our own institution. Patients with overlapping genomic abnormalities were grouped to identify minimal critical regions encompassing potential candidate genes involved in human cardiac development. We identified 79 chromosomal regions in which duplications or deletions have resulted in CHDs in five or more patients. Several of the regions identified have been noted previously to be involved in cardiac development including recurring deletions on chromosomes 7 and 22 (Williams and DiGeorge syndromes), and chromosomes 5 and 8 (involving the cardiac transcription factors NKX2-5 and GATA4). In addition, multiple sets of overlapping deletions and/or duplications were identified that may represent the locations of novel genes involved in cardiac development.

Methods

Database Search

The following three sources were used to identify patients for analysis: (1) the DECIPHER database (an international database containing genomic and phenotypic information on patients from over 150 centers in 29 countries)[9]; (2) the International Standards for Cytogenomic Arrays (ISCA) consortium; and (3) the Benchv3.3 beta database. For each database, a series of search terms were used to identify cases involving CHDs. For the DECIPHER database, the search term "heart" was used to identify patients with CHDs. For the ISCA database, the following search terms were used: atrial septal defect (ASD), AV canal defect, coarctation of the aorta (CoA), hypoplastic left heart, tetralogy of Fallot (TOF), ventricular septal defect (VSD), and heart. The Benchv3.3 database included a linked site for identified cases with CHDs (http://homes.esat.kuleuven.be/~bioiuser/chdwiki/index.php/CHD:Reports).[10]

Expansion of the Database Using Published Data

In addition, data were pooled from four recent publications examining the contribution of copy

number variation to the pathogenesis of CHDs.[11–14] The formats used for copy number variant (CNV) identification and verification are described in each publication. Only those cases in which a single or primary CNV could be identified were included in the database for this study. Cases with more than one CNV were included if the "secondary" CNVs were known to be common in the general population and were less than 100 kb in size. Patients with identical recorded phenotypes and identical genomic imbalances represented in multiple databases and/or publications were deemed to be the same patient and were recorded only once.

Mapping of CNVs

For each case, the deleted or duplicated intervals had been previously mapped to the human genome reference sequence using the NCBI36/hg18 or GRCh37/hg19 genome builds. All sequences were converted to the numbering of the GRCh37/hg19 genome build using the LiftOver program (http://genome.ucsc.edu/cgi-bin/hgLiftOver). For those sequences not successfully converted by the LiftOver program, approximate boundaries were determined using nearby genes and markers in the NCBI36/hg18 genome build and locating the corresponding markers/genes on the GRCh37/hg19 genome map. As those boundaries are less definite, they are represented in red in the composite database.

Grouping of Cases with Overlapping CNVs

There are many approaches to group the observed cases. The full database can be retrieved and resorted based on specific interests or scientific questions. We have presented one approach, but there are many alternatives. To identify regions of overlap, the cases were sorted by chromosome and then sequentially by proximal and distal boundary location (using the GRCh37/hg19 genome build). Eighty-one regions with at least five cases of overlapping duplications and/or deletions were identified by visual inspection. Proximal and distal "secondary" boundaries were determined based on the presence of at least one case with a breakpoint internal to the proposed boundary. This was done to ensure that an important candidate gene would not be excluded based on a single breakpoint in an individual case. The candidate region was identified using the UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly (http://genome.ucsc.edu/cgi-bin/hgGateway?hgsid=353279827). A list of genes in the region was recorded and examined for candidate genes known or suspected to have a role in human heart development. If a definite candidate was present, it was used to organize the overlapping cases in that all cases involving that locus were grouped prior to grouping the cases near but not involving the known cardiac gene. After regrouping based on candidate genes, a total of 79 candidate regions affected in five or more patients were identified.

Estimate of Percentage of Chromosomal Imbalances Associated with Congenital Cardiac Defects

For each of the 79 regions affected (included in a deletion or duplication) in five or more CHD patients, a candidate gene within the minimal region was identified and used to determine the copy number of that candidate region (recognizing that the selected gene may or may not be the one responsible for the observed cardiac defects). To determine if the incidence of cardiac defects associated with the gain or loss of that locus was in excess of what would be expected from a normal control population, we compared the rate of gain or loss of each locus in a population of patients referred to Signature Genomics for microarray testing with a diagnosis of a known cardiac defects (n = 1964) and in a control population without known heart disease (n = 8329). The analysis was restricted to this dataset as the other datasets were more subject to ascertainment bias making an accurate estimate of the denominator less reliable. The examined cases are a subset of the prenatal and postnatal cases submitted to Signature Genomics for clinical microarray-based comparative genomic hybridization from 2004 to 2013 (n = 58 980) and were tested using a variety of platforms, including targeted and whole-genome bacterial artificial chromosome arrays and whole-genome oligonucleotide-based arrays. This dataset includes all cases described in Cooper et al.[13] For each candidate region, the percentage of deletions and duplications that were associated with a reported cardiac defect was recorded. To determine if the observed difference between the CHD and control populations were statistically significant, a Wilcoxon signed rank test was used.

University of Michigan Patients

Retrospective chart review was approved by the Institutional Review Board for Human Subject Research at the University of Michigan. Chromosomal microarray analyses were conducted in the Michigan Medical Genetics Labo-

ratories at the University of Michigan using two oligonucleotide-based array platforms with whole genome coverage: a custom-designed EMArray Cyto6000 chip (Emory Genetics Laboratory, Decatur, GA, USA), implemented on the Agilent 44 K platform (Agilent Technologies, Inc., Santa Clara, CA, USA),[15] and the Illumina CytoSNP12 array. The procedures for array comparative genomic hybridization were performed according to the manufacturer's protocols as described in Quinonez et al.[16] The HumanCytoSNP-12 BeadChip assay (Illumina, San Diego, CA, USA), which targets the entire genome using 300 K probes, was performed according to the Infinium HD Ultra protocol provided by the manufacturer (Illumina). Briefly, 200 ng of genomic DNA was used for whole-genome amplification at 37°C for 20–24 hours. The amplified DNA was enzymatically fragmented, purified, loaded on the HumanCytoSNP-12 BeadChip, and allowed to hybridize at 48°C for 16–24 hours. Subsequently, the hybridizing DNA on the BeadChip was labeled by enzymatic single base extension and incorporated nucleotides were detected using fluorescently labeled antibodies. Stained BeadChips were scanned using a HiScan (Illumina). Data were generated with GenomeStudio (Illumina) and analyzed with Nexus Copy Number software version 6 (BioDiscovery, Hawthorne, CA, USA).

Results

The 1694 patients with congenital cardiac defects and chromosomal imbalances were identified in the databases and publications examined in this study, and 68 patients with congenital cardiac defects and chromosomal imbalances were included from our institution. The deletion or duplication was assigned to a specific chromosomal segment based on the available mapping data. The set of genes affected by the chromosomal imbalances were retrieved from the databases and used to develop a minimum overlapping set of genes for each locus. We identified 79 potential genetic loci that were involved in a minimum of five overlapping deletions or duplications (see Table 1 and Supporting Information Table S1).

Distribution of Insertions/Deletions Leading to CHDs

Chromosomal deletions and duplications associated with CHDs were not randomly distributed throughout the genome. There was clustering of CHD-associated genomic imbalances at particular chromosomal locations, including those involved in known cardiac syndromes and at the telomeres. Telomeric and subtelomeric deletions and duplications associated with congenital cardiac defects involved regions on distal chromosomes 1p, 1q, 2q, 3p, 3q, 4p, 5p, 5q, 6p, 8p, 9p, 9q, 10q, 11p, 11q, 12p, 14q, 15q, 16p, 16q, 17p, 17q, 18q, 19p, and 20q.

Chromosomal Duplication/Deletions Affecting Loci Known to Have a Role in CHD

As would be expected, there were a number of patients with chromosomal imbalances affecting regions previously determined to have role in heart development. Deletions affecting *TBX1* (DiGeorge syndrome) and elastin (Williams syndrome) were identified in 36 and 18 patients, respectively. These deletions may be relatively underrepresented in the databases as they have been well described and there is little incentive to catalogue additional patients with these deletions. Deletions affecting *NKX2-5* and *GATA4* were identified in 7 and 38 patients, respectively. Five of the patients with *NKX2-5* deletions were described as having ASDs with or without other cardiac abnormalities including atrioventricular block in three patients. There was a broad range of defects in patients haplosufficient for *GATA4* with the majority being described as atrial or ventricular septal defects and relatively frequently, in 12/38 patients, complete atrioventricular septal defects. This is consistent with a recent finding that copy number variants affecting *GATA4* were overrepresented in patients with atrioventricular septal defect (AVSD).[12]

Mutations in transforming growth factor-beta (TGFβ)-activated kinase (*TAB2*) have previously been noted in patients with CHDs.[17] Deletions of *TAB2* were noted in 11 patients and a duplication in one patient with a range of cardiac defects including ASDs and VSDs, as well as aortic and pulmonary stenosis. Several other genes previously noted to have deleterious mutations in patients with CHDs were affected by chromosomal imbalances in at least five patients including *CRELD1*, *CHD7*, *CREBBP*, *EHMT1*, *HAND2*, *and NOTCH1* (Table 1).

Chromosomal Insertions/Deletions Affecting Loci Suspected to Have a Role in CHD

Other chromosomal imbalances involved loci that contain genes that have been demonstrated in animal models to be involved in heart develop-

Table 1. Regions with Chromosomal Imbalances Noted in Five or More Patients with Congenital Heart Defects

Chrom	Location	Gene	Heart Defects	Chrom	Location	Gene	Heart Defects
1	934 342	HES4	ASD; CM; VSD	9	139 388 896	NOTCH1	CoA; VSD; ASD; TOF
1	8 420 172	RERE	VSD; PS; AVSD	9	140 513 444	EHMT1	VSD; ASD; TOF
1	104 159 999	AMY2A	VSD; ASD;TOF	10	47 658 233	ANTXRL	TOF; PA; VSD
1	147 228 332	GJA5	TOF; AS; ASD; SV; TGA; CoA; VSD	10	88 516 396	BMPR1A	AVSD; ASD
1	170 633 313	PRRX1	VSD	10	117 061 365	ATRNL1	PDA; VSD; TOF
1	245 912 642	SMYD3	VSD; PDA; TOF	10	135 340 867	CYP2E1	TOF; VSD
2	49 210 237	FSHR	TOF; HEART	11	50 368 318	LOC646813	PS; ASD
2	108 459 465	RGPD4	ASD; TOF; CoA	11	128 328 656	ETS1	VSD; ASD; PDA
2	110 935 964	NPHP1	TOF; HLHS; CoA	11	134 598 320	NKX6-2	TOF; CAT; HEART
2	175 212 878	CIR1	ASD, VSD, PDA, DORV	12	34 175 216	ALG10	TOF; PS; AVSD
3	1 134 620	CNTN6	AVSD; TOF	12	60 083 118	SLC16A7	ASD; PS; CoA; HLHS
3	8 775 486	CRELD1	ASD; AVSD	14	20 167 619	LOC100506393	TOF; PA; VSD; ASD
3	196 466 728	PAK2	ASD; PS; PDA	15	20 737 094	GOLGA6L6	TGA; ASD
4	53 227	ZNF595	PS; ASD; VSD; HLHS	15	22 051 853	POTEB	TOF; VSD; ASD
4	1 005 760	FGFRL1	ASD; VSD; TGA	15	22 546 565	REREP3	TOF; VSD; ASD; TGA
4	89 178 761	PPM1K	ASD; TOF	15	22 833 395	CYFIP1	CoA; VSD; ASD; TOF; CAT; TAPVR
4	91 048 684	CCSER1	ASD; PDA; CoA	15	32 322 686	CHRNA7	ASD; PDA; PS; HET
4	135 117 489	PABPC4L	TOF; TGA; PDA	15	55 495 164	RAB27A	CoA; AS; HLHS
4	174 447 652	HAND2	ASD; VSD; PS; TOF	15	100 106 133	MEF2A	HLHS; VSD; ASD; AVSD; PS
5	659 977	TPPP	HEART; ASD; TOF	16	3 775 056	CREBBP	ASD; PFO; PDA
5	37 812 779	GDNF	PDA; ASD	16	15 806 751	MYH11	ASD; VSD
5	70 330 951	GTF2H2	HEART; TOF; ASD	16	30 097 115	TBX6	ASD; VSD; CoA
5	172 659 107	NKX2-5	ASD; AVB; EBS	16	86 544 133	FOXC2	HLHS; IAA; AVSD; PA; LSVC
5	176 513 921	FGFR4	PDA; VSD	17	19 281 774	MAPK7	ASD; VSD; HLHS; HEART
6	292 101	DUSP22	ASD	17	17 584 787	AATF	HEART; ASD; AVSD
6	149 639 436	TAB2	ASD, VSD, PS, AS, CM	17	44 107 282	KANSL1	ASD; VSD; PDA; PS
6	161 768 590	PARK2	TOF; HLHS; AS	17	59 477 257	TBX2	ASD; PDA; BAV
6	168 707 584	DACT2	TOF; ASD; VSD	18	580 369	CETN1	AS; TOF; HEART
7	64 864 053	ZNF92	VSD; TOF; PS	20	13 976 146	MACROD2	TGA; ASD
7	73 442 427	ELN	SVPS; SVAS; VSD	21	28 208 606	ADAMTS1	AVSD; PS; AS
7	88 388 753	ZNF804B	CoA; TOF; SV	21	36 160 098	RUNX1	AVSD; PA; TGA
7	110 303 106	IMMP2L	TOF; TA;PS	22	19 744 226	TBX1	CAT; TOF; VSD; CoA; DORV
7	132 469 623	CHCHD3	AS; CoA; VSD	22	21 353 326	CRKL	TOF; ASD; VSD
7	143 970 523	ARHGEF34P	TGA; TOF; PA	22	22 113 947	MAPK1	CAT; TA; TOF
8	385 585	FBXO25	HEART; VSD	22	22 311 403	TOP3B	TOF; AS
8	7 272 385	DEFB4B	HEART; VSD; ASD; AVSD	22	25 747 385	LRP5L	TOF; ASD
8	11 561 717	GATA4	TOF; AVSD; VSD; BSVC	22	29 999 545	NF2	PS; VSD
8	61 591 324	CHD7	ASD, AVSD, IAA	X	585 079	SHOX	CoA; AS
9	214 865	DOCK8	TOF; ASD; VSD	X	7 866 804	PNPLA4	VSD; HEART; BAV
9	504 695	KANK1	TOF; PS				

Shown are the chromosome (Chrom) and approximate genetic location (Genome Reference Consortium Human Build 37 [GRCh37/hg19]), a gene within the minimal region of overlap and the observed spectrum of heart defects. Genes that have been previously identified with coding sequence mutations in patients with congenital cardiac defects are in red text; those determined to have a role in heart development based on an animal model (but without identified sequence variants in patients with heart defects) are in blue. Each locus spans multiple genes, and the location and listed gene are only intended to provide a landmark for the candidate interval. The boundaries of the critical region can be derived from the individual cases presented in Supporting Information Table S1. Heart defects listed include coarctation of the aorta (CoA), interrupted aortic arch (IAA), hypoplastic left heart syndrome (HLHS), atrial and ventricular septal defects (ASDs and VSDs), atrioventricular septal defect (AVSD), tetralogy of FALLOT (TOF), double outlet right ventricle (DORV), bicuspid aortic valve (BAV), truncus arteriosus (CAT), situs inversus (SI), heterotaxy (HET), supravalvar aortic and pulmonary stenosis (SVAS and SVPS), aortic stenosis or insufficiency (AS or AI), pulmonary stenosis or insufficiency (PS or PI), tricuspid stenosis or regurgitation (TS or TR), mitral stenosis or regurgitation (MS or MR), total or partial anomalous venous return (TAPVR or PAPVR), tricuspid atresia (TA), pulmonary atresia (PA), patent ductus arteriosus (PDA), left or bilateral superior vena cava (LSVC or BSVC), cardiomyopathy and left ventricular noncompaction (CM and LVNC), and Ebstein's anomaly (EBS). If the heart defect was not described, then "HEART" is listed.

ment but have not previously been implicated in causing human CHDs. These included imbalances involving *ETS1*, *HES4*, *MEF2A*, *MTHFR*, *TBX2*, or *TBX6* in five or more patients (Table 1).

Relative Risk of Congenital Cardiac Defects for Each Candidate Region

One of the major limitations of relying on database reporting for this type of analysis is the inability to assess risk of developing a cardiovascular abnormality if a particular gene or genetic region is involved in a chromosomal imbalance. To address this, we reviewed a large dataset of patients tested at a clinical microarray laboratory (Signature Genomics Inc., Spokane, WA, USA), which includes those reported by Cooper et al.[13] From that dataset, a rough estimate could be obtained of the relative risk of a CHD when a specific gene was involved in a chromosomal imbalance by comparing the gain or loss of that gene in a population of patients with known or suspected cardiac defects referred for copy number analysis com-

pared with a healthy control population. This analysis was performed for the 79 loci noted to be involved in chromosomal copy number variations in at least five patients.

For 66 of the 79 loci involved in chromosomal imbalances in five or more cases with CHD, the incidence of deletions and duplications in a control population of 8329 individuals without known CHDs was available. A total of 36 of these 66 loci were noted to have a significantly increased risk of CHDs compared with control when involved in a chromosomal duplication and/or deletion (Table 2). As noted above, 11 of the loci (including loci containing the *ELN*, *HAND2*, *GATA4*, *GJA5*, and *TBX1*) demonstrated significant elevations in CHD risk when the locus was duplicated and when it was deleted while the remaining 25 only achieved significance for a duplication or a deletion. For many loci, there were not enough occurrences of a duplication or deletion to calculate a meaningful statistic for that type of imbalance. Given the rarity of chromosomal loss or gain in the healthy population, it was difficult to exclude any of the identified loci as definitively not being associated with an increased risk of CHD with a gain or a loss. However, frequent chromosomal imbalances of loci on chromosome 7q31.1 (which includes *IMMPL2*) and on chromosome 6q26 (which includes *PARK2*) in the control population makes their involvement in CHD pathogenesis unlikely. Furthermore, several loci were only reported from a single database raising the possibility that the assignment of a chromosomal imbalance was an artifact of the testing format. In fact, this single data source artifact might account for six of the 12 loci for which imbalances were noted in five or more CHD cases, but no incidence data were available. These loci are also unlikely to be involved in CHD pathogenesis.

Table 2. Incidence of Reported Congenital Heart Defects in Patients with Chromosomal Imbalances Involving Selected Candidate Genes

	Signature Genomics			
	Cooper et al.[13]		DECIPHER	
	Deletion	Duplication	Deletion	Duplication
GATA4	9/21	5/29	11/11	2/8
NOTCH1	6/12	2/15	2/4	1/6
TBX1	88/324	15/172	6/15	5/22
GJA5	11/122	7/115	1/27	6/27
JAG1	1/2	1/6	2/2	1/2
CRELD1	1/12	2/13	2/10	2/3
CHRNA7	3/125	20/257	1/32	2/34
HEY1	0/2	0/3	2/2	0/1
HEY2	1/9	0/2	1/4	0/0
ATRNL1	1/3	0/8	5/7	0/0
KANSL1	8/47	1/10	2/18	0/9

All cases in the signature dataset and DECIPHER databases that involved the candidate gene were examined; shown are the number of cases with reported congenital cardiac defects over the total number of cases. Color coding of genes is as described for Table 1. Note that, even for genes such as *TBX1* that have been clearly linked to congenital heart defects, chromosomal imbalances involving those genes result in clinically recognized cardiac defects in only a subset of cases.

Other genes previously implicated in CHD pathogenesis, including the *NOTCH1*, *TAB2*, and *CHD7* genes, were more frequently involved in chromosomal imbalances in patients with CHD than in controls. In general, genes determined to be important for cardiac development were not commonly involved in chromosomal imbalances and when imbalances do occur they are commonly associated with congenital cardiac defects. Therefore, a complementary approach to assessing risk of CHD is to estimate penetrance of CHD in individuals with a gain or loss of a specific gene or locus (Table 3). Deletions of *GATA4* were noted to be associated with congenital cardiac defects in 9/21 patients in the Signature dataset[13] and 11/11 patients in the DECIPHER database. Interestingly, deletions involving *TBX1* resulted in reported cardiac abnormalities in 27% of the patients in the Signature dataset and in 40% of the patients in the DECIPHER database. This is comparable with the previously reported rate of cardiovascular abnormalities (approximately 25%) in patients with DiGeorge syndrome.[18] Chromosomal imbalances involving *NOTCH1*, which has been demonstrated to be responsible for cardiac defects in some families,[19] are also associated with an elevated risk of CHDs. The incidence of reported cardiac defects in patients with deletions of *NOTCH1* is approximately 50% based on the datasets from DECIPHER and from the Signature database.[13] Therefore, the frequency with which a chromosomal deletion involving a gene known to be involved in heart development results in a congenital cardiac defect varies and the penetrance is usually incomplete suggesting that other factors, either genetic or environmental, may be important for chromosomal imbalances to cause clinically significant congenital heart disease.

Refinement of Candidate Loci for CHD

How the data in the databases can be used to restrict the candidate regions for novel genes involved in human heart development and the pathogenesis of CHD is demonstrated with the following examples.

Table 3. Comparison of the Frequency of Observed Chromosomal Imbalances in Individuals with Congenital Heart Defects (n = 1964) Compared with Controls (n = 8329)

Gene	CHD Deletions	Control Deletions	*P* value	CHD Duplications	Control Duplications	*P* value
AATF	3/1964	2/8329	.051	3/1964	3/8329	.09
ADAMTS1	1/1964	0/8329	.19	8/1964*	0/8329	**<.0001**
ARHGEF5	4/1964	0/8329	**.001**	1/1964	0/8329	.19
ATRNL1	0/1964	8/8329	.37	0/1964	0/8329	N/A
BMPR1A	0/1964	0/8329	N/A	0/1964	1/8329	1.00
CCSER1	0/1964	2/8329	1.00	1/1964	4/8329	1.0
CETN1	5/1964	1/8329	**.001**	13/1964†	6/8329	**<.0001**
CHCHD3	4/1964	0/8329	**.001**	1/1964	0/8329	.19
CHD7	2/1964	0/8329	**.04**	0/1964	0/8329	N/A
CHRNA7	3/1964	4/8329	.13	17/1964	58/8329	.43
CIR1	1/1964	0/8329	.19	1/1964	0/8329	.19
CNTN6	2/1964	4/8329	.32	6/1964	19/8329	.61
CREBBP	2/1964	1/8329	.10	1/1964	7/8329	1
CRELD1	1/1964	1/8329	.35	1/1964	0/8329	.19
CRKL	46/1964	1/8329	**<.0001**	15/1964	8/8329	**<.0001**
CYP2E1	4/1964	6/8329	.11	70/1964	177/8329	**.0002**
DACT2	1/1964	0/8329	.19	0/1964	0/8329	N/A
DOCK8	3/1964	6/8329	.39	13/1964	29/8329	**.0498**
DUSP22	189/1964	27/8329	**<.0001**	219/1964	2/8329	**<.0001**
EHMT1	3/1964	3/8329	.09	3/1964	0/8329	**.01**
ELN	14/1964	0/8329	**<.0001**	2/1964	0/8329	**.04**
ETS1	11/1964	0/8329	**<.0001**	1/1964	0/8329	.19
FBXO25	5/1964	4/8329	**.02**	2/1964	2/8329	.17
FGFR4	0/1964	0/8329	N/A	2/1964	0/8329	**.04**
FGFRL1	4/1964	5/8329	.07	1/1964	1/8329	.35
FOXF1	1/1964	0/8329	.19	1/1964	0/8329	.19
FSHR	1/1964	2/8329	.47	0/1964	1/8329	1.00
GATA4	7/1964	0/8329	**<.0001**	4/1964	0/8329	**.001**
GDNF	0/1964	1/8329	1.000	2/1964	3/8329	.24
GJA5	6/1964	2/8329	**.001**	6/1964	3/8329	**.002**
HAND2	5/1964	0/8329	**<.0001**	2/1964	0/8329	**.04**
HES4	5/1964	1/8329	**.001**	1/1964	0/8329	.19
IMMP2L	15/1964	47/8329	.3	0/1964	5/8329	1.00
KANK1	9/1964	29/8329	.47	9/1964	9/8329	**.003**
KANSL1	0/1964	0/8329	N/A	1/1964	0/8329	.19
LOC100506393	0/1964	2/8329	1.00	3/1964	0/8329	**.01**
MACROD2	10/1964	8/8329	**.001**	2/1964	0/8329	**.04**
MAPK1	2/1964	1/8329	.10	4/1964	0/8329	**.001**
MAPK7	0/1964	0/8329	N/A	1/1964	0/8329	.19
MEF2A	0/1964	1/8329	1.00	2/1964	1/8329	.10
MTHFR	2/1964	0/8329	**.04**	0/1964	0/8329	N/A
MYH11	0/1964	3/8329	1.00	4/1964	12/8329	.53
NF2	0/1964	0/8329	N/A	2/1964	0/8329	**.04**
NKX2-5	0/1964	0/8329	N/A	1/1964	0/8329	.19
NKX6-2	0/1964	0/8329	N/A	4/1964	2/8329	**.01**
NOTCH1	4/1964	3/8329	**.03**	1/1964	1/8329	.35
NPHP1	10/1964	36/8329	.65	23/1964	32/8329	**.01**
PABPC4L	8/1964	22/8329	.29	2/1964	0/8329	**.04**
PAK2	0/1964	0/8329	N/A	3/1964	0/8329	**.01**
PARK2	5/1964	24/8329	.8	8/1964	29/8329	.69
PPM1K	0/1964	0/8329	N/A	0/1964	0/8329	N/A
PRRX1	1/1964	0/8329	.19	0/1964	0/8329	N/A
RAB27A	0/1964	2/8329	1.00	0/1964	1/8329	1.00
RGPD4	0/1964	1/8329	1.00	1/1964	0/8329	.19
RUNX1	1/1964	2/8329	.47	8/1964*	1/8329	**<.0001**
SLC16A7	0/1964	2/8329	1.00	0/1964	1/8329	1.00
SMYD3	5/1964	1/8329	**.001**	2/1964	3/8329	.24
TAB2	2/1964	0/8329	**.04**	0/1964	0/8329	N/A
TBX1	49/1964	5/8329	**<.0001**	10/1964	7/8329	**<.0001**
TBX2	1/1964	0/8329	.19	1/1964	0/8329	.19
TBX6	2/1964	3/8329	.24	3/1964	2/8329	.051
TPPP	5/1964	4/8329	**.02**	4/1964	17/8329	1.0
TUBGCP5	15/1964	22/8329	**.001**	16/1964	36/8329	**.03**
ZNF595	4/1964	0/8329	**.001**	5/1964	3/8329	**.01**
ZNF804B	3/1964	0/8329	**.01**	1/1964	5/8329	1.0
ZNF92	14/1964	52/8329	.66	8/1964	13/8329	**.04**

*All with trisomy 21.
†11 out of 13 with trisomy 18.
For each candidate region, the percentage of deletions and duplications that was associated with a reported cardiac defect was recorded. To determine if the observed difference between the CHD and control populations was statistically significant, a Wilcoxon signed-rank test was used. Statistically significant *P* values are represented in bold.
ND: ALG10; AMY2A; ANTXRL; DEFB4B; GOLGA6L6; GTF2H2; LOC6464813; LRP5L; POTEB; PNPLA4; REREP3; SHOX; TOP3B.

Locus at 1q24.2

In the databases, we identified seven individuals with deletions involving 1q24.2. The heart defects were variable but included VSD (3 patients), patent foramen ovale (PFO; 2 patients), heart defect not otherwise specified (1 patient) and ECG abnormality (1 patient) (Table 4A). We identified a primary critical region (CR), where the proximal and distal boundaries were each determined by a single case, that included nine genes. A secondary CR, defined by allowing the exclusion of single cases, included additional genes. None of the genes within the primary and secondary CRs has a known role in heart development or the pathogenesis of congenital cardiac defects. Although a risk calculation could not be performed using a gene (*PRRX1*) within the primary CR, the absence of chromosomal imbalances involving this region in healthy controls supports its potential role in CHD pathogenesis.

Locus at 2q31.1

Fourteen individuals with deletions (12 patients) or duplications (2 patients) involving 2q31.1 were identified (Table 4B). The heart defects were primarily septal defects including VSD[6] and ASD.[3] The primary CR contained six genes with a secondary CR containing another 2. Within the primary CR is the gene encoding for *CIR1* that has been reported to regulate the Notch signaling pathway and to be abundantly expressed in the heart[20] and the gene encoding for the SP3 transcription factor, which has been demonstrated in a mouse model to have an important role in heart development.[21] There was one case (Wiki389) in which the deletion did not significantly overlap with two other deletions in the region suggesting that (1) there is another cardiac gene located more proximally on chromosome 2, (2) there is an effect of the deletion on nearby genes, or (3) the deletion, in that case, is not directly responsible for the observed cardiac defect. Although a risk calculation could not be performed using a gene (*CIR1*) within the primary CR, the absence of chromosomal imbalances involving this region in healthy controls again supports its potential role in CHD pathogenesis.

Locus at 15q26.3

Thirteen individuals with deletions (9 patients) or duplications (4 patients) involving 15q26.3 were identified (Table 4C). A broad range of cardiac defects were noted but some less common heart defects appeared to be overrepresented including hypoplastic left heart syndrome (HLHS) in 3. CoA and ventricular septal defects were also relatively common. Of the genes in the primary and secondary CRs, the gene encoding the *MEF2A* transcription factor is an important candidate based on its high level of expression in the developing heart in animal models.[22]

DiGeorge Syndrome Region

Previous studies have noted that mutation affecting *TBX1* in human patients[23] or the targeted depletion of *Tbx1* in animal models[24–26] can reproduce many of the cardiac manifestations of DiGeorge syndrome including the development of conotruncal-type CHDs. However, it has also been noted that chromosomal imbalances involving chromosome 22q11 but not including *TBX1* can also be associated with heart defects.[27,28] Therefore, we examined the cases within the databases to determine if there was evidence that multiple genes on chromosome 22q11 might be involved in cardiac defects. We identified 85 chromosomal imbalances involving chromosome 22q11 in the database. Seventy-eight of the 85 involved at least one of three genes, *TBX1*, *CRKL*, and *MAPK1*, previously determined or suspected to have a role in human heart development and in the pathogenesis of CHDs. Of these, seven involved chromosomal imbalance of *TBX1* alone, 35 involved *TBX1* and *CRKL*, eight involved *CRKL* alone, four involved both *CRKL* and *MAPK1*, and 19 involved *MAPK1* alone (Table 5). Small (<300 kb) chromosomal imbalances involving chromosome 22q11 distal to *MAPK1* were noted in six patients from one database, but these may reflect nonpathogenic rearrangements or uncertain CNV ascertainment based on a single testing format.

Discussion

General Approach

Identification of the causes of CHDs has proved to be a challenging task. The underlying genetic abnormality responsible for a cardiac defect is known in only a very small minority of patients. Characterization of patients with chromosomal imbalances and associated congenital cardiac abnormalities is an attractive approach to identifying disease genes that has been successful in identifying *ELN* (elastin), *TBX1* and *GATA4* as genes involved in the pathogenesis of congenital cardiac defects. Unfortunately, there are currently a number of obstacles to this approach. Perhaps, most importantly is the lack of a centralized,

Table 4. Genes Contained within the Critical Regions for Three Loci

Table 4A. Critical Region on Chromosome 1q24.2 with Surrounding Genes. None of the Genes in the Primary or Secondary Critical Regions Are Strongly Implicated in Heart Development

	D 262739 DEL ECG ABNL	D 253856 DEL HEART	D 2572 DEL VSD	W 1505 DEL VSD	W 1506 DEL PFO/PDA	W 1546 DEL PFO	I nssv577229 DEL VSD
TBX19	X	X		X	X	X	X
XCL2	X	X		X	X	X	X
XCL1	X	X		X	X	X	X
DPT	X	X		X	X	X	X
ATP1B	X	X		X	X	X	X
NME7	X	X		X	X	X	X
SLC19A2	X	X		X	X	X	X
SELP	X	X		X	X	X	X
METTL18	X	X	X	X	X	X	X
SELL	X	X	X	X	X	X	X
SELE	X	X	X	X	X	X	X
KIFAP3	X	X	X	X	X	X	X
GORAB	X	X	X	X	X	X	X
PRRX1	X	X	X	X	X	X	X
FMO3	X	X	X	X	X	X	X
FMO2	X	X	X	X	X	X	X
FMO1	X	X	X	X	X	X	X
PRRC2C	X		X	X	X	X	X
VAMP4	X		X	X	X	X	X
METTL13	X		X	X	X	X	X
DNM3	X		X	X	X	X	X
PIGC			X	X	X	X	X
FASLG			X	X	X	X	X
TNSF18			X	X	X	X	X

Table 4B. Critical Region on Chromosome 2q31.1 with Surrounding Genes. *SP3* and *CIR1* Have Been Determined to Have a Role in Vertebrate Heart Development in Animal Models.

	W 1476 DEL VSD; PS	W 1475 DEL ASD	W 1474 DEL ASD; PDA	W 1481 DEL VSD	W 1501 DEL VSD	W 1502 DEL VSD	M 12 DEL Ao	W 389 DEL MS; MR	W 77 DEL VSD	W 78 DEL ASD; PDA	W 79 DEL VSD; DORV; PS	I nssv577738 DEL HEART	C 9900015 DUP HEART	D 258540 DUP HEART
PDK1			X	X	X	X	X	X	X				X	X
RAPGEF4			X	X	X	X	X	X	X				X	X
ZAK	X	X	X	X	X	X	X	X	X	X	X		X	X
CDCA7	X	X	X	X	X	X	X	X	X	X	X		X	X
SP3	X	X	X	X	X	X	X		X	X	X	X	X	X
OLA1	X	X	X	X	X	X	X		X	X	X	X	X	X
CIR1	X	X	X	X	X	X	X		X	X	X	X	X	X
SCRN3	X	X	X	X	X	X	X		X	X	X	X	X	X
GPR155	X	X	X	X	X	X	X		X	X	X	X	X	X
WIPF1	X	X	X	X	X	X	X		X	X	X	X	X	X
CHRNA1		X	X	X	X	X	X		X	X		X	X	X
CHN1		X	X	X	X	X	X		X	X		X	X	X

Table 4C. Critical Region on Chromosome 15q26.3 with Surrounding Genes. *MEF2A* Has Been Determined to Have a Role in Vertebrate Heart Development in Animal Models

	W 1338 DEL HLHS; CoA; VSD	D 251099 DEL CoA; VSD	D 270050 DEL VSD	W 1314 DEL AVSD	W 1313 DEL ASD; PS	W 3 DEL HLHS	W 5 DEL HLHS; VSD	W 1525 DEL VSD	D 259934 DEL VSD	C 9902150 DUP HEART	D 256144 DUP ASD	D 256144 DUP ASD	I nssv582685 DUP HEART
NR2F2	X	X		X		X	X		X	X	X	X	
ARRDC4	X	X		X		X	X	X	X	X	X	X	
IGF1R	X	X		X	X	X	X	X	X	X	X	X	
SYNM1	X	X		X	X	X	X	X	X	X	X	X	
TTC23	X	X	X	X	X	X	X	X	X	X	X	X	
LRRC28	X	X	X	X	X	X	X	X	X	X	X	X	X
MEF2A	X	X	X	X	X	X	X	X	X	X	X	X	X
LYSMD4	X	X	X	X	X	X	X	X	X	X	X	X	X
DNM1P	X	X	X	X	X	X	X	X	X	X	X	X	X
ADAMTS17	X	X	X	X	X	X	X	X	X	X	X	X	X
CERS3	X	X	X	X	X	X	X	X	X	X	X	X	X
LINS1	X	X	X	X	X	X	X	X	X	X	X	X	X
ASB7	X	X	X	X	X	X	X	X	X	X	X	X	X
LRRK1	X	X	X	X	X		X	X	X	X	X	X	X
PCSK6	X	X	X	X	X		X	X	X	X	X	X	X
TARSL2	X	X		X	X		X	X	X	X	X	X	X

The primary critical region, where all deletions overlap, is highlighted in light gray. The secondary critical region, where all but one deletion overlap, is highlighted in dark gray. The genes within the region and surrounding region are listed in the order that they occur on the chromosome. An X indicates that the gene is involved in the chromosomal imbalance in that patient. The patient and the database (C: Cooper et al.,[13] D: DECIPHER, I: ISCA, W: CHDWiki, and M: the University of Michigan) or publication from which they were gathered is listed along the top row. Along the second row is the designation of whether the rearrangement was a deletion or duplication. In the third row is the type of the cardiac defect observed if known (if unknown is listed as "HEART"). Nomenclature for heart defects is the same as for Table 1.

Note that there is one chromosomal imbalance that was in close proximity to but did not involve the genes in the primary critical region. "Ao" indicates dilatation of the aortic root in one patient.

Table 5. DiGeorge Syndrome Region on Chromosome 22q11.2 with Surrounding Genes

Database	Database ID	CHD	Chromo	Type	Genome Build	Boundary 1	Boundary 2	Locus
I	nssv579955	VSD	22	DEL	hg19	17019015	20718227	TBX1
I	nssv575290	HEART	22	DEL	hg19	18706001	20659606	TBX1
I	nssv575290	VSD	22	DEL	hg19	18890271	20659606	TBX1
W	1412	VSD	22	DEL	hg19	18894835	20659606	TBX1
C	9908014	HEART	22	DEL	hg19	18986906	20246906	TBX1
I	nssv577056	HEART	22	DEL	hg19	19771355	21123068	TBX1
I	nssv579962	CoA	22	DUP	hg19	19074579	19928090	TBX1
I	nssv1603215	HEART	22	DEL	hg19	18660553	21455556	TBX1/CRKL
I	nssv579970	VSD	22	DEL	hg19	18661724	21561514	TBX1/CRKL
I	nssv579984	HEART	22	DEL	hg19	18661724	21561514	TBX1/CRKL
TM	19	TOF	22	DEL	hg19	18805856	21721725	TBX1/CRKL
G	2573	TOF	22	DEL	hg19	18810089	21153772	TBX1/CRKL
G	2360	TOF	22	DEL	hg19	18895187	21463936	TBX1/CRKL
I	nssv579993	AI	22	DEL	hg19	18909032	21306115	TBX1/CRKL
I	nssv1603983	HEART	22	DEL	hg19	18919469	21456772	TBX1/CRKL
D	2213	HEART	22	DEL	hg19	18919469	21460658	TBX1/CRKL
D	256300	VSD	22	DEL	hg19	18919469	21460658	TBX1/CRKL
C	9882869	HEART	22	DEL	hg19	18919469	21460658	TBX1/CRKL
C	9893576	HEART	22	DEL	hg19	18919469	21460658	TBX1/CRKL
C	9883354	TOF	22	DEL	hg19	18919469	21460658	TBX1/CRKL
C	9883678	HEART	22	DEL	hg19	18919742	21440655	TBX1/CRKL
C	9883488	TOF	22	DEL	hg19	18919742	21440655	TBX1/CRKL
C	9881580	HEART	22	DEL	hg19	18919941	21440515	TBX1/CRKL
D	262934	HEART	22	DEL	hg19	18919941	21440515	TBX1/CRKL
I	nssv579999	TOF	22	DEL	hg19	18919942	21440514	TBX1/CRKL
C	9906174	HEART	22	DEL	hg19	18919942	21440514	TBX1/CRKL
C	9892257	CoA	22	DEL	hg19	18919942	21440514	TBX1/CRKL
I	nssv582251	ASD	22	DEL	hg19	18919942	21505417	TBX1/CRKL
I	nssv576304	VSD	22	DEL	hg19	18919942	21505417	TBX1/CRKL
I	nssv580013	HEART	22	DEL	hg19	18919942	21505417	TBX1/CRKL
I	nssv580012	HEART	22	DEL	hg19	18919942	21505417	TBX1/CRKL
I	nssv580007	CHF	22	DEL	hg19	18919942	21505417	TBX1/CRKL
I	nssv575367	ASD	22	DEL	hg19	18919942	21561514	TBX1/CRKL
I	nssv580006	HEART	22	DEL	hg19	18938161	21455556	TBX1/CRKL
I	nssv583020	HEART	22	DEL	hg19	18938161	21455556	TBX1/CRKL
I	nssv580004	PS	22	DEL	hg19	18938161	21455556	TBX1/CRKL
I	nssv582339	VSD	22	DEL	hg19	19023824	21798755	TBX1/CRKL
I	nssv580045	SAS	22	DUP	hg19	18627818	21940122	TBX1/CRKL
I	nssv580051	AVSD	22	DUP	hg19	18660553	21455556	TBX1/CRKL
I	nssv580052	HEART	22	DUP	hg19	18781534	21465835	TBX1/CRKL
C	9893158	HEART	22	DUP	hg19	18919942	21801604	TBX1/CRKL
D	1640	AVSD	22	DUP	hg19	19573160	21461017	TBX1/CRKL
D	251689	TOF	22	DEL	hg19	20330744	21465481	CRKL
D	254167	AAo	22	DEL	hg19	20659547	21561514	CRKL
TM	9	HEART	22	DEL	hg19	20733427	21505417	CRKL
D	768	ASD; PDA	22	DEL	hg19	20734244	21460658	CRKL
D	986	VSD	22	DEL	hg19	20743536	21462353	CRKL
TM	25	VSD	22	DEL	hg19	20754422	21440514	CRKL
D	262483	SAS	22	DUP	hg19	20763207	21461765	CRKL
I	nssv580061	TOF	22	DUP	hg19	20769981	21037727	CRKL
I	nssv580065	VSD	22	DEL	hg19	20372210	22548710	CRKL/MAPK1
W	1488	CAT	22	DEL	hg19	20976969	22484320	CRKL/MAPK1
S	B00BEJC	ASD	22	DEL	hg19	21075575	22467350	CRKL/MAPK1
I	nssv575749	HEART	22	DUP	hg19	21025653	22467351	CRKL/MAPK1
TM	21	CAT	22	DEL	hg19	21692108	23575642	MAPK1
S	B00B1CM	AVSD	22	DEL	hg19	21721591	22970128	MAPK1
W	98	AVSD	22	DEL	hg19	21721591	22970128	MAPK1
C	9895786	VSD	22	DEL	hg19	21798104	23739485	MAPK1
D	2366	HEART	22	DEL	hg19	21799773	23654237	MAPK1
S	B00BE7D	HEART	22	DEL	hg19	21808750	24643264	MAPK1
D	251833	HEART	22	DEL	hg19	21811979	24616199	MAPK1
D	251833	AI	22	DEL	hg19	21939922	24643109	MAPK1
D	250255	VSD; ASD; PDA	22	DEL	hg19	22095272	22834113	MAPK1
D	248709	CAT	22	DEL	hg19	22095272	22834113	MAPK1
I	nssv580074	CAT	22	DEL	hg19	22095272	22834113	MAPK1
I	nssv580080	CAT	22	DEL	hg19	22095272	22834113	MAPK1
TM	29	VSD; RAO	22	DEL	hg19	22095272	22834113	MAPK1

Table 5. *Continued*

Database	Database ID	CHD	Chromo	Type	Genome Build	Boundary 1	Boundary 2	Locus
C	9890170	BAV	22	DEL	hg19	22095272	22834113	MAPK1
W	21	CAT	22	DEL	hg19	22095272	22834113	MAPK1
W	18	CAT	22	DEL	hg19	22115848	23696229	MAPK1
W	24	CAT	22	DEL	hg19	22115848	23696229	MAPK1
W	23	VSD	22	DEL	hg19	22115848	23696229	MAPK1
W	22	TA	22	DUP	hg19	21934556	22381654	MAPK1
W	26	CAT	22	DEL	hg19	19031487	23071636	TBX1/CRKL/MAPK1
W	28	VSD	22	DUP	hg19	17397498	51178264	TBX1/CRKL/MAPK1
W	1003	VSD	22	DUP	hg19	17397498	51178264	TBX1/CRKL/MAPK1
W	27	DORV; SI	22	DUP	hg19	18546349	22336469	TBX1/CRKL/MAPK1
S	B00BDZ8	ECG	22	DUP	hg19	19174809	22264418	TBX1/CRKL/MAPK1
S	B00B0MB	CAT	22	DUP	hg19	19771355	19771891	
S	B00B15I	TA; TGA	22	DUP	hg19	22308883	22573637	TOP3B
S	B00BQP9	TOF	22	DUP	hg19	22311326	22573637	TOP3B
W	29	AS	22	DEL	hg19	22312383	22573637	TOP3B
W	25	TOF	22	DEL	hg19	22315312	22573637	TOP3B
S	B009FPG	BAV	22	DUP	hg19	22315312	22571854	TOP3B
S	B00B0D1	TOF	22	DUP	hg19	22315312	22573637	TOP3B

Data presented for each case includes the database source, the case ID number, the type of heart defect noted (CHD), the chromosome affected by the deletion or duplication (Chrom), the type of chromosomal imbalance (deletion [DEL] or duplication [DUP]) identification, the genome build of the annotated sequence involved (all hg19), and the approximate proximal (Boundary 1) and distal (Boundary 2) boundaries of the observed imbalance, and the candidate gene or genes involved in the imbalance (Locus). The chromosomal imbalances were divided into those involving just *TBX1*, *TBX1* and *CRKL*, *CRKL* only, *CRKL* and *MAPK1*, *MAPK1* only, and all three. Shading indicates involvement of more that one candidate gene. In six cases, a small chromosomal rearrangement occurred between *CRKL* and *MAPK1* but did not involve either gene. All of those cases did involve imbalance of the gene *TOP3B*, a gene not known to be involved in heart development. Only one chromosomal imbalance of 22q11 did not involve one of those four loci. Nomenclature for heart defects is the same as for Table 1. Sources listed include the following databases and publications: Cooper et al.[13] (C), DECIPHER (D), ISCA (I), CHDWiki (W), Tomita-Mitchell et al.[12] (TM), Greenway et al.[11] (G), Soemedi et al.[14] (S), and the University of Michigan (M).

searchable, and well-annotated database of well-phenotyped patients with clearly defined chromosomal imbalances. The obstacles to achieving this goal are not insurmountable and would yield enormous benefits for all patients with developmental defects, including heart defects.

Although there are very important limitations to the databases in their current state that restrict the ability to make conclusions based on these data, the database review suggested several important considerations with regard to human heart development and the pathogenesis of human congenital cardiac defects. Most importantly, the database review reinforces the observation that haploinsufficiency or duplication of even genes known to be critical for heart development only results in heart defects in a subset of cases. For instance, haploinsufficiency of *TBX1* led to clinically significant heart defects in 27% of patients in the Signature dataset and 40% in the DECIPHER database. The Signature dataset may have a lower ascertainment and reporting bias but also may under-report or under-detect heart defects. The DECIPHER database is more prone to ascertainment and reporting bias, which may inflate the estimated frequency of heart defects in patients with 22q11 deletions. Clearly, other factors are involved that help determine whether an individual with a genetic predisposition, such as *TBX1* haploinsufficiency, manifests a significant cardiac abnormality.

At least some of the clinical variability in cases of chromosome 22q11 deletion may be related to the extent of the deletion. In the database, as has been noted in previous studies, most commonly, cases (30 out of 85 reported) with a CHD and a chromosomal imbalance affecting chromosome 22q11 had an approximately 3 Mb deletion, which included both the *TBX1* and *CRKL* genes. A smaller 1.5 Mb deletion involving *TBX1* but not *CRKL* was noted in six patients in the database. The smaller deletion has been previously noted to account for DiGeorge syndrome in approximately 7% of cases and the larger deletion in 88%.[29,30] The smaller deletion has been noted to be sufficient to produce the full DiGeorge syndrome phenotype[31] including conotruncal heart defects,[32] but there has been noted to be an important difference in the rate at which the 1.5 Mb deletion and the 3 Mb deletion are passed to offspring.[33] The reduced rate of transmission of the larger deletion may indicate that the early developmental defects are more penetrant or more severe in the patients with the larger deletion. In our database, the patients with the smaller 1.5 Mb deletion had ventricular septal defects and unspecified heart abnormalities while those with the larger 3 Mb deletion, encompassing both *TBX1* and *CRKL*, demonstrated a range of heart defects including TOF (six cases), VSD (three cases), and pulmonary stenosis (one case). Conotruncal heart defects including truncus arteriosus (CAT) and TOF were

noted in cases involving deletions distal to the 1.5 Mb smaller DiGeorge syndrome deletion, including deletions involving (1) the distal part of the 3 Mb deletion region (and including *CRKL*) and/or (2) deletion of more distal 22q11 including *MAPK1*. The ability of 22q11 deletions that do not involve *TBX1* to result in conotruncal cardiac defects has been previously noted in cases involving the loss of both *CRKL* and *MAPK1*.[28] Therefore, the concurrent loss of 2 or more genes involved in cardiac development as occurs with the simultaneous loss of *TBX1* and *CRKL* in the larger and more common 3 Mb DiGeorge syndrome deletion or with the less common simultaneous loss of *CRKL* and *MAPK1* may enhance the penetrance of cardiac defects in patients with 22q11 deletions. This is supported by knockout studies in mice that demonstrated a higher penetrance and severity of cardiac defects in mice haplosufficient for both *TBX1* and *CRKL* compared to mice haplosufficient for either individually.[34]

CHD penetrance may also vary by disease locus. Chromosomal imbalances involving *GATA4* and *NKX2-5* are rare but, when they occur, seem to be associated with a high frequency of clinically significant cardiac defects. Few other loci appear to have comparable penetrance with regard to haploinsufficiency and the incidence of heart defects. However, disease genes that reside in regions that are relatively stable (i.e., very rarely involved in chromosomal imbalances) or in regions where chromosomal imbalances are associated with markedly reduced viability may be difficult to detect using this approach.

The generated composite database of each individual case (Supporting Information Table S1) will provide a useful starting point for the identification of novel genomic regions potentially involved in human heart development and the pathogenesis of congenital cardiac defects. Identification of such regions will facilitate the interpretation of genome-wide sequencing data, in the search for the genetic determinants of congenital cardiac defects. As cases are added to the database there may be further refinement of the region determined to be critical for heart development. Examples of how deletion/duplication mapping may be used to restrict candidate regions and identify potential candidate genes are described below.

Locus at 1q24.2

Deletions of 1q24 are relatively uncommon. There was a report of seven patients with deletion of 1q24.3-1q25 that overlaps minimally with the CHD CR defined in this study.[35] Cardiac defects were not noted to be an important feature of their observed phenotype. As noted above, none of the genes within the primary and secondary CRs has a known role in heart development or the pathogenesis of congenital cardiac defects. Of the genes in the primary CR, *GORAB*, and *PRRX1* are expressed in the heart, but loss of function in human patients or in animal models is not associated with heart defects.[36–38] Similarly, in the secondary CR, *VAMP4*, and *DNM3* are expressed the heart, but no role in cardiac development has been identified. In addition, there are a number of miRNAs that have been identified within the CRs at this locus. Most interesting is mir-214, which is encoded within the *DNM3* locus, is highly expressed in the heart and promotes myogenic differentiation.[39]

Locus at 2q31.1

Duplication of chromosome 2q31.1 has been noted in a 3 generation family with mesomelic dysplasia,[40] but deletions involving 2q31.1 are relatively uncommon.[41] The predominant cardiac lesions in patients with 2q31.1 deletions that have been recorded in the databases are defects in cardiac septation resulting in ASDs and VSDs. Within the primary CR on chromosome 2q31 is CBF1-interacting co-repressor (*CIR1*) (Table 4B). CBF1 (or RBPJ) is a transcriptional repressor whose activity is de-repressed by binding to the Notch intracellular domain, making it a key target in Notch-mediated transcriptional activation. CIR1's high level of expression in the heart and interaction with a signaling pathway critical for heart development make *CIR1* a likely candidate for the observed cardiac defects in these patients.[20] Other genes within the primary and secondary CRs that are expressed in the heart include *PDK1* (pyruvate dehydrogenase kinase), transcription factor *SP3*, and *ZAK*. *ZAK* encodes a kinase that activates MAPK1,[42] another kinase implicated in the pathogenesis of congenital cardiac defects,[28] and *SP3* encodes for a transcription factor that is required for normal heart development in the mouse.[21]

Locus at 15q26.3

A recurring deletion of chromosome 15q26.3 described by Rump et al.[43] was noted to be responsible for a distinct clinical phenotype that had been named Drayer syndrome. Common features include micrognathia, developmental delay, skeletal anomalies and growth failure. As noted above,

MEF2A, a transcription factor strongly expressed in smooth muscle throughout the body, including the smooth muscle of the developing heart tube, is a strong candidate for the heart defects associated with 15q26 deletions as has been previously proposed.[44] Another candidate within the region is *ADAMTS17*, which is expressed in the developing heart.[45] However, deleterious mutations of *ADAMTS17* in human patients cause ocular and ophthalmologic abnormalities but have not led to the development of CHDs.[45]

It is important to note that haploinsufficiency of this region did lead to heart defects with related etiologies, as two of the more commonly noted lesions with these deletions, HLHS and CoA, can occur together in families and are often defined as closely related left-sided obstructive lesions.[46] Therefore, a gene or genes within this region may be an important candidate(s) to consider when evaluating patients with left-sided obstructive lesions including HLHS.

DiGeorge Region

In several regions, nonoverlapping chromosomal imbalances are associated with cardiac defects, suggesting that more than one gene in the region participates in heart development. Given that (1) the "penetrance" of the cardiac phenotype in response to a chromosomal imbalance is variable and can be quite low even for imbalances involving a gene known to participate in heart development and (2) the incidence of heart defects in the general population is high enough that not all defects can be assumed to be "caused" by the observed imbalance, it can be very difficult to determine the precise extent of the candidate regions and the number of heart development genes involved. One region that may contain more than a single gene involved in heart development is chromosome 22q11, which includes the DiGeorge syndrome CR. As noted above, *TBX1*, which encodes for a T-box cardiac transcription factor, is within the CR and has been demonstrated in animal models to have an important role in heart development.[23,26,47] However, nonoverlapping chromosomal imbalances associated with heart defects have suggested the possibility of two additional 22q11 genes involved in heart development, and several lines of evidence support the potential involvement of the *CRKL* and *MAPK1* genes.[28,48,49] Review of the cases in the databases support the existence of at least three genes on chromosome 22q11 that contribute to cardiac development.

Limitations

As noted above, there are a number of important limitations to this study. The databases and publications included in this analysis do not share a common genotyping platform, do not have a consistent approach to phenotyping or phenotype reporting and are entirely voluntary. We have included a supplemental table that contains some basic information regarding each of the reported cases (Supporting Information Table S1). We recognize that our list of 79 loci involved in five or more rearrangements is incomplete and that there will be many instances where (1) multiple candidate genes have been encompassed by a single locus; (2) only a single candidate gene is present where there are several closely spaced candidate regions; (3) "false" loci have been created by database errors, random chance and genomic instability; and (4) "true" loci have been missed due to genomic stability (leading to very few cases of chromosomal imbalance for evaluation) and/or reduced viability of affected embryos. The list is meant to be a preliminary effort to consolidate what is known to date and it will need to be refined or replaced as case reporting becomes more standardized. The supplementary table will serve as a platform for identifying new candidate loci as additional cases are added to the database and will help to further refine existing loci.

Summary

Despite the limitations of this study, the database analysis demonstrates the potential value of this approach to restrict regions of interest for genes involved in developmental disorders including congenital cardiac defects. The low penetrance for observable cardiac defects associated with most chromosomal imbalances, the infrequent occurrence of chromosomal abnormalities in most genomic regions, and the relatively common occurrence of some types of cardiac defects (such as ASDs and VSDs) in the general population will make it difficult to define CRs based on a single patient or small cohorts. Therefore, there will be tremendous value in establishing standardized processes for reporting observed chromosomal imbalances and associated clinical phenotypes. The refined map of loci involved in human cardiac development will be essential as genome-wide sequencing approaches become more prevalent in the search for the genetic determinants of congenital cardiac defects.

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References

1 Ohye RG, Sleeper LA, Mahony L, et al. Comparison of shunt types in the Norwood procedure for single-ventricle lesions. *N Engl J Med*. 2010;362:1980–1992.
2 Petit CJ. Staged single-ventricle palliation in 2011: outcomes and expectations. *Congenit Heart Dis*. 2011;6:406–416.
3 Pierpont ME, Basson CT, Benson DW Jr, et al. Genetic basis for congenital heart defects: current knowledge: a scientific statement from the American Heart Association Congenital Cardiac Defects Committee, Council on Cardiovascular Disease in the Young: endorsed by the American Academy of Pediatrics. *Circulation*. 2007;115:3015–3038.
4 McCulley DJ, Black BL. Transcription factor pathways and congenital heart disease. *Curr Top Dev Biol*. 2012;100:253–277.
5 Wolf M, Basson CT. The molecular genetics of congenital heart disease: a review of recent developments. *Curr Opin Cardiol*. 2010;25:192–197.
6 Millan MJ. An epigenetic framework for neurodevelopmental disorders: from pathogenesis to potential therapy. *Neuropharmacology*. 2013;68:2–82.
7 Zaidi S, Choi M, Wakimoto H, et al. De novo mutations in histone-modifying genes in congenital heart disease. *Nature*. 2013;498:220–223.
8 Ferencz C, Neill CA, Boughman JA, Rubin JD, Brenner JI, Perry LW. Congenital cardiovascular malformations associated with chromosome abnormalities: an epidemiologic study. *J Pediatr*. 1989;114:79–86.
9 Firth HV, Richards SM, Bevan AP, et al. DECIPHER: database of chromosomal imbalance and phenotype in humans using ensemble resources. *Am J Hum Genet*. 2009;84:524–533.
10 Barriot R, Breckpot J, Thienpont B, et al. Collaboratively charting the gene-to-phenotype network of human congenital heart defects. *Genome Med*. 2010;2:16.
11 Greenway SC, Pereira AC, Lin JC, et al. De novo copy number variants identify new genes and loci in isolated sporadic tetralogy of Fallot. *Nat Genet*. 2009;41:931–935.
12 Tomita-Mitchell A, Mahnke DK, Struble CA, et al. Human gene copy number spectra analysis in congenital heart malformations. *Physiol Genomics*. 2012;44:518–541.
13 Cooper GM, Coe BP, Girirajan S, et al. A copy number variation morbidity map of developmental delay. *Nat Genet*. 2011;43:838–846.
14 Soemedi R, Wilson IJ, Bentham J, et al. Contribution of global rare copy-number variants to the risk of sporadic congenital heart disease. *Am J Hum Genet*. 2012;91:489–501.
15 Baldwin EL, Lee JY, Blake DM, et al. Enhanced detection of clinically relevant genomic imbalances using a targeted plus whole genome oligonucleotide microarray. *Genet Med*. 2008;10:415–429.
16 Quinonez SC, Hedera P, Barr M, et al. Maternal intrachromosomal insertional translocation leads to recurrent 1q21.3q23.3 deletion in two siblings. *Am J Med Genet A*. 2012;158A:2591–2601.
17 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.
18 Bassett AS, Chow EW, Husted J, et al. Clinical features of 78 adults with 22q11 Deletion Syndrome. *Am J Med Genet A*. 2005;138:307–313.
19 Garg V, Muth AN, Ransom JF, et al. Mutations in NOTCH1 cause aortic valve disease. *Nature*. 2005;437:270–274.
20 Hsieh JJ, Zhou S, Chen L, Young DB, Hayward SD. CIR, a corepressor linking the DNA binding factor CBF1 to the histone deacetylase complex. *Proc Natl Acad Sci U S A*. 1999;96:23–28.
21 van Loo PF, Mahtab EA, Wisse LJ, et al. Transcription factor Sp3 knockout mice display serious cardiac malformations. *Mol Cell Biol*. 2007;27:8571–8582.
22 Subramanian SV, Nadal-Ginard B. Early expression of the different isoforms of the myocyte enhancer

factor-2 (MEF2) protein in myogenic as well as non-myogenic cell lineages during mouse embryogenesis. *Mech Dev*. 1996;57:103–112.
23 Yagi H, Furutani Y, Hamada H, et al. Role of TBX1 in human del22q11.2 syndrome. *Lancet*. 2003;362:1366–1373.
24 Lindsay EA, Vitelli F, Su H, et al. Tbx1 haploinsufficieny in the DiGeorge syndrome region causes aortic arch defects in mice. *Nature*. 2001;410:97–101.
25 Merscher S, Funke B, Epstein JA, et al. TBX1 is responsible for cardiovascular defects in velo-cardio-facial/DiGeorge syndrome. *Cell*. 2001;104:619–629.
26 Jerome LA, Papaioannou VE. DiGeorge syndrome phenotype in mice mutant for the T-box gene, Tbx1. *Nat Genet*. 2001;27:286–291.
27 Momma K. Cardiovascular anomalies associated with chromosome 22q11.2 deletion syndrome. *Am J Cardiol*. 2010;105:1617–1624.
28 Breckpot J, Thienpont B, Bauters M, et al. Congenital heart defects in a novel recurrent 22q11.2 deletion harboring the genes CRKL and MAPK1. *Am J Med Genet A*. 2012;158A:574–580.
29 Shaikh TH, Kurahashi H, Saitta SC, et al. Chromosome 22-specific low copy repeats and the 22q11.2 deletion syndrome: genomic organization and deletion endpoint analysis. *Hum Mol Genet*. 2000;9:489–501.
30 Saitta SC, Harris SE, Gaeth AP, et al. Aberrant interchromosomal exchanges are the predominant cause of the 22q11.2 deletion. *Hum Mol Genet*. 2004;13:417–428.
31 Morrow B, Goldberg R, Carlson C, et al. Molecular definition of the 22q11 deletions in velo-cardio-facial syndrome. *Am J Hum Genet*. 1995;56:1391–1403.
32 Rauch A, Zink S, Zweier C, et al. Systematic assessment of atypical deletions reveals genotype-phenotype correlation in 22q11.2. *J Med Genet*. 2005;42:871–876.
33 Adeyinka A, Stockero KJ, Flynn HC, Lorentz CP, Ketterling RP, Jalal SM. Familial 22q11.2 deletions in DiGeorge/velocardiofacial syndrome are predominantly smaller than the commonly observed 3Mb. *Genet Med*. 2004;6:517–520.
34 Guris DL, Duester G, Papaioannou VE, Imamoto A. Dose-dependent interaction of Tbx1 and Crkl and locally aberrant RA signaling in a model of del22q11 syndrome. *Dev Cell*. 2006;10:81–92.
35 Burkardt DD, Rosenfeld JA, Helgeson ML, et al. Distinctive phenotype in 9 patients with deletion of chromosome 1q24-q25. *Am J Med Genet A*. 2011;155A:1336–1351.
36 Hennies HC, Kornak U, Zhang H, et al. Gerodermia osteodysplastica is caused by mutations in SCYL1BP1, a Rab-6 interacting golgin. *Nat Genet*. 2008;40:1410–1412.
37 Sergi C, Kamnasaran D. PRRX1 is mutated in a fetus with agnathia-otocephaly. *Clin Genet*. 2011;79:293–295.
38 Martin JF, Bradley A, Olson EN. The paired-like homeo box gene MHox is required for early events of skeletogenesis in multiple lineages. *Genes Dev*. 1995;9:1237–1249.
39 Liu J, Luo XJ, Xiong AW, et al. MicroRNA-214 promotes myogenic differentiation by facilitating exit from mitosis via down-regulation of proto-oncogene N-ras. *J Biol Chem*. 2010;285:26599–26607.
40 Cho TJ, Kim OH, Choi IH, et al. A dominant mesomelic dysplasia associated with a 1.0-Mb microduplication of HOXD gene cluster at 2q31.1. *J Med Genet*. 2010;47:638–639.
41 Theisen A, Rosenfeld JA, Shane K, et al. Refinement of the region for split hand/foot malformation 5 on 2q31.1. *Mol Syndromol*. 2010;1:262–271.
42 Gotoh I, Adachi M, Nishida E. Identification and characterization of a novel MAP kinase kinase kinase, MLTK. *J Biol Chem*. 2001;276:4276–4286.
43 Rump P, Dijkhuizen T, Sikkema-Raddatz B, et al. Drayer's syndrome of mental retardation, microcephaly, short stature and absent phalanges is caused by a recurrent deletion of chromosome 15(q26.2→qter). *Clin Genet*. 2008;74:455–462.
44 Tonnies H, Schulze I, Hennies H, Neumann LM, Keitzer R, Neitzel H. De novo terminal deletion of chromosome 15q26.1 characterised by comparative genomic hybridisation and FISH with locus specific probes. *J Med Genet*. 2001;38:617–621.
45 Morales J, Al-Sharif L, Khalil DS, et al. Homozygous mutations in ADAMTS10 and ADAMTS17 cause lenticular myopia, ectopia lentis, glaucoma, spherophakia, and short stature. *Am J Hum Genet*. 2009;85:558–568.
46 Hinton RB, Martin LJ, Rame-Gowda S, Tabangin ME, Cripe LH, Benson DW. Hypoplastic left heart syndrome links to chromosomes 10q and 6q and is genetically related to bicuspid aortic valve. *J Am Coll Cardiol*. 2009;53:1065–1071.
47 Vitelli F, Morishima M, Taddei I, Lindsay EA, Baldini A. Tbx1 mutation causes multiple cardiovascular defects and disrupts neural crest and cranial nerve migratory pathways. *Hum Mol Genet*. 2002;11:915–922.
48 Fagerberg CR, Graakjaer J, Heinl UD, et al. Heart defects and other features of the 22q11 distal deletion syndrome. *Eur J Med Genet*. 2013;56:98–107.
49 Guris DL, Fantes J, Tara D, Druker BJ, Imamoto A. Mice lacking the homologue of the human 22q11.2 gene CRKL phenocopy neurocristopathies of DiGeorge syndrome. *Nat Genet*. 2001;27:293–298.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. All 1762 cases collected for this study are presented. Data presented for each case includes the database source, the case ID number, the type of heart defect noted (CHD), the chromosome affected by the deletion or duplication (Chrom), the type of chromosomal imbalance (deletion [DEL] or duplication [DUP]) identification, the genome build of the annotated sequence involved (all hg19), and the approximate proximal (Boundary 1) and distal (Boundary 2) boundaries of the observed imbalance, and the candidate gene or genes involved in the imbalance (Locus). Loci highlighted in red were affected in 5 or more patients with heart defects. For each case, the chromosome (Chrom) and approximate genetic location (Genome Reference Consortium Human Build 37 [GRCh37/hg19]), a gene within the minimal region of overlap, the observed heart defects and the source of the case (database or publication) are shown. The gene listed is not the only one within the critical region but is a representative one and, if one is present, will be the gene within the interval determined to have a role in heart development. Identification of all genes in the primary and secondary critical regions would require further mapping as noted in Tables 4 and 5. Heart defects listed include coarctation of the aorta (CoA), interrupted aortic arch (IAA), hypoplastic left heart syndrome (HLHS), atrial and ventricular septal defects (ASDs and VSDs), atrioventricular septal defect (AVSD), tetralogy of Fallot (TOF), double outlet right ventricle (DORV), bicuspid aortic valve (BAV), truncus arteriosus (CAT), situs inversus (SI), heterotaxy (HET), supravalvar aortic and pulmonary stenosis (SVAS and SVPS), aortic stenosis or insufficiency (AS or AI), pulmonary stenosis or insufficiency (PS or PI), tricuspid stenosis or regurgitation (TS or TR), mitral stenosis or regurgitation (MS or MR), total or partial anomalous venous return (TAPVR or PAPVR), tricuspid atresia (TA), pulmonary atresia (PA), patent ductus arteriosus (PDA), left or bilateral superior vena cava (LSVC or BSVC), cardiomyopathy and left ventricular noncompaction (CM and LVNC), and Ebstein's anomaly (EBS). If the heart defect wasn't described then "HEART" is listed. Sources listed include the following databases and publications: Cooper et al.[13] (C), DECIPHER (D), ISCA (I), CHDWiki (W), Tomita-Mitchell et al.[12] (TM), Greenway et al.[11] (G), Soemedi et al.[14] (S), and the University of Michigan (M).