# A Comprehensive Clinical Genetics Approach to Critical Congenital Heart Disease in Infancy

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**Objective** To investigate the frequency of genetic diagnoses among infants with critical congenital heart disease (CHD) using a comprehensive cardiovascular genetics approach and to identify genotype–phenotype correlations.

**Study design** A retrospective chart review of patients evaluated by cardiovascular genetics in a pediatric cardiac intensive care unit from 2010 to 2015 was performed. Infants with CHD who were <1 month of age were included. CHD was classified using structured phenotype definitions. Cardiac and noncardiac phenotypes were tested for associations with abnormal genetic testing using $\chi^1$ and Fisher exact tests.

**Results** Genetic evaluation was completed in 293 infants with CHD, of whom 213 had isolated congenital heart disease (iCHD) and 80 had multiple congenital anomalies. Overall, the yield of abnormal genetic testing was 26%. The multiple congenital anomalies cohort had a greater yield of genetic testing (39%) than the iCHD cohort (20%) (OR 2.7). Using a non-hierarchical CHD classification and excluding 22q11.2 deletion and common aneuploidies, right ventricular obstructive defects were associated with abnormal genetic testing ($P = .0005$). Extracardiac features associated with abnormal genetic testing included ear, nose, and throat ($P = .003$) and brain ($P = .0001$) abnormalities. A diagnosis of small for gestational age or intrauterine growth retardation also was associated with abnormal genetic testing ($P = .0061$), as was presence of dysmorphic features ($P = .0033$, OR 3.5). Infants without dysmorphia with iCHD or multiple congenital anomalies had similar frequencies of abnormal genetic testing.

**Conclusions** The present study provides evidence to support a comprehensive cardiovascular genetics approach in evaluating infants with critical CHD while also identifying important genotype–phenotype considerations. *(J Pediatr 2020;227:231-8)*.

The incidence of severe congenital heart disease (CHD) requiring expert cardiologic care is 2.5 to 3 in 1000.[1] It is estimated that up to one-quarter of CHD with or without extracardiac anomalies has an identifiable genetic etiology, including copy number variation,[2-7] chromosomal,[8,9] or single gene.[8] Isolated, nonsyndromic CHD is thought to account for 70% of all CHD and is considered multifactorial in the absence of an identifiable genetic cause. The American Heart Association has cited reasons to pursue genetic testing in the setting of CHD, including possible involvement in other organ systems, prognostic information for clinical outcomes, genetic reproductive risks for the family, and consideration of genetic testing for additional family members when appropriate.[10,11] Genetic testing is also known to have personal utility for patients and families.[12] Positive genetic testing can be used to confirm a genetic etiology for an individual's CHD, whereas negative genetic testing, although not ruling out a genetic cause, allows for risk stratification to a lower recurrence risk and likely lower risk of medical complications associated with genetic syndromic disease.

Early identification of a genetic syndromic condition allows for optimization of outcomes through proactive medical management and by initiation of appropriate therapy and neurodevelopmental services in patients at risk for developmental delay or intellectual disability.[13,14] Neurodevelopmental delays are also frequently associated with genetic diagnoses in children with CHD[13]; however, these delays will not be appreciated in a newborn. The STATseq study of research-based whole-genome sequencing in

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|---|---|---|---|
| CCHMC | Cincinnati Children's Hospital Medical Center | IUGR | Intrauterine growth retardation |
| CHD | Congenital heart disease | ROH | Regions of homozygosity |
| CICU | Cardiac intensive care unit | RVOTO | Right ventricular outflow tract obstruction |
| CNV | Copy number variant | SGA | Small for gestational age |
| ENT | ear–nose–throat | VSD | Ventricular septal defect |
| FISH | Fluorescent in situ hybridization | VUS | Variant of uncertain significance |
| iCHD | Isolated congenital heart disease | | |

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infants and children in neonatal and pediatric intensive care units found that phenotypes of known syndromes were less differentiated in infancy.[15,16] Of the 3 recurrent conditions identified, Noonan syndrome and CHARGE syndrome (Coloboma of the eye, Heart defects, Atresia of the choanae, Retardation of growth and development, and Ear abnormalities and deafness) are commonly associated with CHD but were not recognized in infants in the study.[17]

Although standard of care guidelines recommend genetic testing in infants with CHD,[11,18] practice variation exists. Within the pediatric setting, recommendations have been made to implement algorithms for genetic services, including genetic testing among infants with CHD based on cardiac lesion and presence of extracardiac anomalies.[19] This type of protocol has been reported to increase the rate of diagnosis for genetic conditions and reduce cost to patients.[20] Overall yields of genetic testing range from 18% to 36%. Genetic testing modality, CHD lesions, and additional extracardiac features are noted to influence the yield of genetic testing.[20-22] These studies differed in their ascertainment of patients and inclusion criteria as well as their use of genetic testing modalities.

We investigated the yield of genetic diagnosis among infants with critical isolated congenital heart disease (iCHD) and multiple congenital anomalies using a standardized algorithm and comprehensive cardiovascular genetics approach and to identify genotype–phenotype correlations that highlight phenotypic features that should increase suspicion for a genetic condition.

## Methods

This retrospective chart review included patients with critical CHD as defined by required admission to the cardiac intensive care unit (CICU) at Cincinnati Children's Hospital Medical Center (CCHMC) from April 2010 to June 2015 for observation and/or intervention. Approval from the CCHMC institutional review board was obtained. To ensure a comprehensive cardiovascular genetics approach, the CCHMC CICU uses an algorithm to incorporate genetic services for patients with CHD as well as other types of genetic heart disease, as outlined in **Figure 1** (available at www.jpeds.com).[19,20] Cardiovascular genetic counseling consultations were placed at the time of admission for all infants younger than 1 month of age with CHD as part of the standing admission orders, assuring that all individuals with CHD were ascertained for genetic services. Although infants older than 1 month of age did obtain genetic services, they were not included in the study cohort. At CCHMC, all infants admitted to the CICU with CHD have head and renal ultrasounds to assess for any anomalies. The study population was ascertained using an Epic query (Epic Systems Corp, Verona, Wisconsin) for consultation requests generated by the CICU for either a cardiovascular genetics consult (which may also include genetic counseling) or a cardiovascular genetic counseling consult. Typically, patients with multiple congenital anomalies received a cardiovascular genetics consult, whereas patients with iCHD started with a cardiovascular genetic counseling consult for assessment, risk stratification, and testing as outline by the algorithm. Patients were eligible for this study if they had CHD and were seen by a genetics provider during CICU stay.

Infants were defined as having iCHD if they had CHD with no additional birth defects or extracardiac abnormalities. Extracardiac features were defined as an abnormality in at least 1 non-cardiac organ system: gastrointestinal, ribs/vertebrae, renal, hepatobiliary, spleen, ear–nose–throat (ENT), genitourinary, limb, brain, and intrauterine growth retardation/small for gestational age (IUGR/SGA). Dysmorphic features were not included as an extracardiac feature because they were only recorded for those who had a geneticist evaluation. Infants with CHD in addition to another extracardiac feature were defined as having multiple congenital anomalies. Patients who received genetic services for cardiac diagnoses other than iCHD or multiple congenital anomalies, including cardiomyopathy, aortopathy, and arrhythmia, were noted for volume accounting but were excluded from the remainder of the study. All patients meeting the aforementioned inclusion criteria were included in the full retrospective chart review.

Clinical data were obtained from the existing electronic medical record for each eligible patient and entered into a REDCap (Research Electronic Data Capture) database hosted at CCHMC.[23] Data collected included demographics, echocardiography and other imaging results, clinical notes, family history, prenatal history, genetic testing results, and geneticists' evaluation (including dysmorphology examination). Only genetic testing associated with the genetic services provided in the CICU encounter were included in analysis. Prenatal testing was noted when documented in the patient's chart; however, it was not confirmed through maternal chart review and thus we cannot comment on prenatal genetic evaluation or diagnosis.

Cardiac phenotype data were collected by review of echocardiography reports. Each patient's first complete echocardiogram performed at CCHMC was reviewed. Additional cardiac imaging and clinical records were reviewed as necessary when diagnoses were uncertain or information was incomplete. Detailed (or "level I") and broad (or "level III") cardiac diagnoses were recorded for each patient. The list of CHD diagnoses that were recorded was derived from the cardiac phenotype axis of the Botto cardiac classification system.[24] Level III categories of Aortopathy, Arteriopathy, Coronary anomaly, and Cardiomyopathy were also added, as previously described.[25] The level of detail in cardiac phenotyping was further increased by recording level I diagnoses that were not systematically included in the original description of the Botto system, such as left-sided superior vena cava, otherwise specified valve malformations such as valve dysplasia, and presence of ventricular hypoplasia in patients without hypoplastic left heart syndrome. Patients were allowed to have more than one level I diagnosis recorded. Level I diagnoses that were the combinations of 2 level I diagnoses in the Botto system were also recorded individually. For

example, in a patient with the Botto level I diagnosis of coarctation of the aorta and ventricular septal defect (VSD), the VSD also would have been recorded and specified (eg, perimembranous VSD). Level I diagnoses that may have been excluded in the Botto system also were recorded (eg, an atrial septal defect in a patient with tetralogy of Fallot) to completely characterize each patient's phenotype. The level III classification was recorded for each level I diagnosis. Thus, patients were allowed to have more than 1 level III diagnosis recorded. In addition to this non-hierarchical phenotyping, the level I diagnoses were used to aggregate each patient's CHD lesions into a single CHD type. This classification was based on a hierarchical method that applied the Botto system in previous genetic epidemiology studies.[26,27] In the present study, the level III diagnosis category of Complex included only patients with single ventricle (double-inlet left ventricle) and was therefore labeled as single ventricle in tables for clarity.

Genetic testing included in the study cohort included chromosome analysis, fluorescence in situ hybridization (FISH) for 22q11.2, single-nucleotide polymorphism microarray (chromosome microarray), and any molecular testing that may have included disease-specific gene panels or single gene testing. Although molecular testing was sent to a variety of clinical laboratories, all of the cytogenetic testing was completed at CCHMC. Due to the nature of evolving interpretation of genetic test results, all abnormal (variant of unknown significant [VUS], likely pathogenic, or pathogenic) chromosome microarray results were re-reviewed at the time of manuscript preparation for a possible change in interpretation by the CCHMC cytogenetics laboratory. All molecular testing results classified as VUS were reinterpreted by the laboratories who performed the initial testing to assure up-to-date interpretation.

The associations between categorical clinical/phenotype variables and abnormal genetic testing were tested using $2 \times 2$ cross tables. Pearson $\chi^2$ testing was used when all values in the cross table were 5 or greater. When at least one value was less than 5, the Fisher exact 2-tail test was used. Unadjusted $P$ values were tabulated. $P$ values were adjusted for multiple testing using the Bonferroni correction when multiple independent variables were tested for the same dependent variable. Reported $P$ values used a threshold of $<.05$ for statistical significance. Statistical analyses were performed using JMP statistical software package (SAS Institute, Cary, North Carolina).

## Results

The CICU at CCHMC admitted 2391 unique patients between April 1, 2010, and June 30, 2015. Among these patients, 316 were infants <1 month of age referred for cardiovascular genetics consultations (genetics and/or genetic counseling) during their inpatient stay. The indications for genetics evaluation across all ages were iCHD (249), multiple congenital anomalies including CHD (95), cardiomyopathy (32), arrhythmia (15), aortopathy/concern for connective tissue disorder (2), and other (10) (**Figure 2**; available at www.jpeds.com). All infants <1 month of age at the time of consultation with iCHD or multiple congenital anomalies who had a genetics and/or genetic counseling consultation were included for study (n = 293; **Table I** [available at www.jpeds.com]). Among these, 204 (70%) patients had prenatal diagnosis of CHD and 21 (7%) patients had family history of CHD.

**Table II** summarizes the overall rates and yields of genetic testing. There were 245 patients (84%) who had at least one genetic test completed postnatally. Testing rates were similar between patients with iCHD (82%) or multiple congenital anomalies (86%). When genetic testing was not completed, this was most often due to family declination. Among all patients tested, the overall yield of positive testing was 26%. Testing yields were greater in patients with multiple congenital anomalies than iCHD ($P = .001$) (OR 2.7 and 95% CI 1.5-4.9). The cohort included 23 patients who tested positive for the following common syndromes: 22q11.2 deletion (13), Down syndrome (7), Turner syndrome (2), and trisomy 13 (1). Among patients who did not have one of these common diagnoses, the testing yield was slightly lower (18%). Again, the yields were greater in multiple congenital anomalies than iCHD groups ($P = .0007$) with an OR 3.3 (CI 1.6-6.6). Although testing yields were lower in iCDH, the 12% testing yield in iCHD is clinically significant.

Genetic testing included chromosome analysis, chromosome microarray, 22q11.2 FISH, and molecular analysis. **Figure 3** (available at www.jpeds.com) summarizes the testing strategies and results. Of the 245 patients who had genetic testing, 155 (63%) had 1 type of genetic testing, 76 (31%) had 2 types, 11 (4%) had 3 types, and 3 (1%) had all 4 types. Two types of genetic testing were ordered together as the initial testing for 49 patients (20%). Chromosome microarray was the most common initial test

**Table II. Rates and yields of genetic testing**

| Groups | No. with genetic testing (%) | No. with abnormal genetic testing results (%) | Testing yield |
|---|---|---|---|
| All (N = 293) | 245 (84%) | 63 (22%) | 63/245 = 26% |
| iCHD (N = 205) | 169 (82%) | 33 (16%) | 33/169 = 20% |
| Multiple congenital anomalies (N = 88) | 76 (86%) | 30 (34%) | 30/76 = 39% |
| Excluding T21/T13/TS/22q11 (N = 270) | 222 (82%) | 40 (15%) | 40/222 = 18% |
| iCHD (N = 191) | 155 (81%) | 19 (10%) | 19/155 = 12% |
| Multiple congenital anomalies (N = 79) | 67 (85%) | 21 (27%) | 21/67 = 31% |

*22q11*, 22q11.2 deletion syndrome; *T13*, trisomy 13; *T21*, trisomy 21; *TS*, Turner syndrome.

(n = 182). Second-, third-, and fourth-line testing primarily consisted of chromosome microarray (n = 21) or molecular testing (n = 22). First-line testing had a yield of 21%, second-tier testing had a yield of 26%, none of the third-line testing was positive, and both fourth-line tests were positive. None of the patients had multiple molecular panels. Among the 182 patients who did not have any positive testing results, 123 (68%) had only 1 test completed.

**Table III** (available at www.jpeds.com) summarizes yields for each type of genetic testing. Chromosome analysis was abnormal in 13 patients, including aneuploidies (9), large deletions (2), and translocations (2). Chromosome microarray was abnormal in 30 patients. Five of these chromosome microarray abnormalities helped to define abnormal chromosome analysis findings (3 were sent together with chromosome analysis and 2 were sent as follow-up testing). Syndromic diagnoses identified by chromosome microarray included 22q11.2 deletion (3) and Turner syndrome (1). Two patients had regions of homozygosity (ROH) identified on chromosome microarray that led to further molecular testing that identified pathogenic sequence variants (*DNAH11* and *CFC2*) within the ROH. The 19 other chromosome microarray abnormalities included 5 pathogenic copy number variants (CNVs) and 14 CNVs determined to be VUS. There were 10 patients with 22q11.2 deletion identified by FISH; one of these was also detected by chromosome analysis that was sent concurrently with FISH. There were 17 patients with abnormal molecular analysis. Autosomal-dominant syndromic diagnoses included Noonan syndrome due to variants in *PTPN11* (4) or *KRAS* (2), CHARGE syndrome due to variants in *CHD7* (6), Alagille syndrome due to variant in *JAG1* (1), branchio-oto-renal syndrome due to variant in *EYA1* (1), and Rubenstein–Taybi syndrome due to variant in *CREBBP* (1). As referenced previously, molecular analysis in concert with chromosome microarray identified autosomal recessive causes of CHD associated with primary ciliary dyskinesia (*DNAH11*) and the molecular cause of heterotaxy syndrome (*CFC1*). In addition, a clinical genetic diagnosis was established for 3 patients who had phenotypes consistent with Kabuki syndrome, Holt–Oram syndrome, or Noonan syndrome, despite normal molecular testing for these conditions. All abnormal testing results are tabulated in **Table IV** (available at www.jpeds.com).

We initially tested for association between abnormal genetic testing and CHD class using a non-hierarchical CHD classification method, which permitted each patient to be classified with multiple different level III CHD types. Using this classification method, the most common lesion represented was septal defects (n = 144) with a genetic testing yield of 22% (32/144). Atrioventricular septal defect lesions had the highest yield of abnormal genetic testing (13/31, 42%) (**Table V**). As described earlier, 23 patients were diagnosed with 22q11.2 deletion or an aneuploidy commonly associated with CHD. Genotype–phenotype associations for these syndromes are well established and clinically integrated. For instance, many cardiac centers routinely screen patients with conotruncal defects for 22q11.2 deletion using chromosome microarray or FISH. Also, patients with one of these aneuploidy syndromes often are diagnosed prenatally or soon after birth based on external features and CHD phenotypes. Therefore, to study the impact of genetic evaluations in patients with CHD beyond these relatively common and well-characterized syndromes, further analyses excluded these 23 patients. In this analysis right ventricular obstructive defect (RVOTO) was significantly associated with abnormal genetic testing (OR 3.4, CI 1.7-7.0; $P = .0005$) (**Table VI**). The association was statistically significant with Bonferroni correction for multiple comparisons consisting of 11 separate tests (corrected $P = .0055$).

We next tested for associations between specific level I CHD lesions and abnormal genetic testing, limiting the analysis to CHD lesions present in at least 10% of patients tested. For example, a secundum atrial septal defect was present in 63 (28%) and pulmonary valve stenosis/hypoplasia in 34 (15%) patients (**Table VII**; available at www.jpeds.com). There were nominally significant associations between abnormal genetic testing and pulmonary valve stenosis/

**Table V. Frequency of abnormal genetic testing for different CHD types**

| CHD types | No. | Patients with any abnormal genetic test, n (%) | No. of abnormalities by genetic test | | | |
|---|---|---|---|---|---|---|
| | | | Chromosome analysis | 22q11 FISH | Chromosome microarray | Molecular |
| All | 245 | 63 (26) | 13 | 10 | 30 | 17 |
| Septal defect | 144 | 32 (22) | 5 | 2 | 18 | 13 |
| LVOTO | 139 | 35 (25) | 6 | 4 | 19 | 11 |
| Conotruncal defect | 105 | 33 (31) | 4 | 10 | 11 | 9 |
| RVOTO | 96 | 32 (33) | 6 | 3 | 14 | 13 |
| Laterality | 63 | 16 (25) | 3 | 0 | 9 | 6 |
| Arteriopathy | 42 | 17 (40) | 3 | 6 | 6 | 4 |
| AVSD | 31 | 13 (42) | 6 | 0 | 8 | 2 |
| Aortopathy | 26 | 10 (38) | 4 | 3 | 2 | 2 |
| APVR | 17 | 6 (35) | 0 | 0 | 5 | 3 |
| Coronary | 12 | 1 (8) | 0 | 0 | 0 | 1 |
| Single ventricle | 10 | 3 (30) | 0 | 0 | 3 | 0 |

*APVR*, anomalous pulmonary venous return; *LVOTO*, left ventricular outflow tract obstruction.

**Table VI. Genetic testing yields for different CHD types**

| CHD types | No. with genetic testing (N = 222) | No. with abnormal genetic testing (%) | OR (95% CI) | *P* value |
|---|---|---|---|---|
| Septal defect | 138 | 26 (19) | 1.16 (0.57-2.37) | .6827 |
| LVOTO | 129 | 25 (19) | 1.25 (0.62-2.53) | .5341 |
| Conotruncal defect | 88 | 16 (18) | 1.02 (0.51-2.05) | .9590 |
| RVOTO | 90 | 26 (29) | **3.42 (1.67-7.02)** | **.0005** |
| Laterality | 59 | 12 (20) | 1.23 (0.58-2.61) | .5883 |
| Arteriopathy | 33 | 8 (24) | 1.57 (0.65-3.79) | .3467 |
| AVSD | 25 | 7 (28) | 1.93 (0.75-5.00) | .1680 |
| Aortopathy | 20 | 4 (20) | 1.15 (0.36-3.65) | .7644* |
| APVR | 17 | 6 (35) | 2.74 (0.95-7.92) | .0538 |
| Coronary | 12 | 1 (8) | 0.40 (0.05-3.18) | .6985* |
| Single ventricle | 10 | 3 (30) | 2.03 (0.50-8.21) | .3917* |

Data excludes patients with 22q11.2 deletion (13), Down syndrome (7), trisomy 13 (1), or Turner syndrome (2).
*Fisher exact test.
Bold indicates statistically significant.

hypoplasia ($P$ = .02) or specified pulmonary valve malformation (eg, dysplastic) ($P$ = .03). However, Bonferroni correction (15 CHD lesions were separately tested) determined that these associations were not statistically significant.

Finally, each patient's set of CHD lesions was classified into a single CHD type using a hierarchical classification method from the previous studies of Oyen et al that applied the Botto system.[26,27] None of the CHD types arising from this classification method was significantly associated with abnormal genetic testing (**Table VIII**; available at www.jpeds.com).

Recognizing that the overall rates of genetic testing were similar between iCHD and multiple congenital anomalies groups but yields were greater in patients with multiple congenital anomalies (**Table II**), we next sought to further elucidate the association of non-cardiac phenotype(s) on genetic testing yield. Non-cardiac congenital abnormalities were grouped by organ or body system (**Table IX**; available at www.jpeds.com). The most frequent groups were gastrointestinal (n = 15), ribs/vertebrae (n = 15), and renal (n = 14). Among the 9 groups of non-cardiac congenital abnormalities, ENT abnormalities (OR 5.2, CI 1.6-17.0; $P$ = .003) and brain abnormalities (OR 31.9, CI 3.7-273.8; $P$ = .0001) were significantly associated with abnormal genetic testing after Bonferroni correction for 9 tests. In addition, a diagnosis of IUGR or SGA was present in 13 (6%) patients and was significantly associated with abnormal genetic testing (OR 4.5, CI 1.4-14.1; $P$ = .0061).

Among the whole cohort, 162 (55%) patients had a physical examination by a geneticist. A geneticist examined all 88 patients in the cohort who had multiple congenital anomalies. Among the total 162 with genetics examination, 88 (54%) were documented by the geneticist to have dysmorphic features. Genetic testing was completed in 144 (89%) patients seen by a geneticist and was abnormal in 56 (yield 39%). All 23 patients who tested positive for 22q11.2 deletion (n = 13), Down syndrome (n = 7), trisomy 13 (n = 1), or Turner syndrome (n = 2) were examined by a geneticist. Among these, only 9 (39%) met criteria for multiple congenital anomalies when not considering the presence of dysmorphic features. Of the 14 without multiple congenital anomalies, 9 had 22q11.2 deletion and 5 had Down syndrome. Thirteen of these 14 patients had dysmorphic features documented by the geneticist. The one patient without multiple congenital anomalies or dysmorphic features had 22q11.2 deletion.

A physical examination was completed by a geneticist for 121 of the 222 patients (55%) who did not have one of the common genetic syndromes and who underwent genetic testing. Patients with CHD classification of laterality defects (88%) were frequently examined whereas those with left ventricular outflow tract obstruction were less frequently examined (29%) (complete list in **Table X** [available at www.jpeds.com]). Forty-seven (39%) had 1 genetic test, 60 (50%) had 2 genetic tests, 11 (9%) had 3 genetic tests, and 3 (2%) had 4 genetic tests, totaling 212 separate tests (1.8 tests per patient). Genetic testing results were abnormal in 33 (27%) patients examined by a geneticist. Four patients had abnormal chromosomes and chromosome microarray defining the chromosome abnormality, and 2 had chromosome microarray with ROH and positive molecular testing with a heterotaxy panel. Otherwise, 12 had chromosome microarray abnormality and 15 had abnormal molecular testing. In contrast, genetic testing results were abnormal in only 7 of the 101 patients (7%) who had genetic testing sent without ever being examined by a geneticist. Ninety (89%) had 1 test and 11 (11%) had 2 tests, totaling 112 tests (1.1 tests per patient). Examination by a geneticist was significantly associated with abnormal genetic testing (OR 5.0, CI 2.1-12.0; $P < .0001$). A clinical diagnosis was also established by a geneticist for 5 patients. Three of these patients were given a clinical diagnosis of a genetic syndrome (Kabuki syndrome, Holt–Oram syndrome, Noonan syndrome) and 2 were given a diagnosis of diabetic embryopathy. Overall, 38 (31%) patients evaluated by a geneticist without a common syndrome were identified as having a genetic diagnosis by either genetic testing or clinical evaluation.

The frequency of dysmorphic features and genetic testing abnormalities was investigated in this cohort of patients that was evaluated by a geneticist (**Figure 4**). Of the 121 patients evaluated, 54 (45%) had iCHD and 55% had multiple congenital anomalies. In the iCHD group, 30 patients were noted to have dysmorphic features, of whom 9 (30%) had abnormal genetic testing. Twenty-four patients in the iCHD were not noted to have dysmorphic features and only 3 (13%) had abnormal genetic testing. Although the frequency of abnormal genetic testing was higher in the dysmorphic group with iCHD, it did not reach statistical significance ($P$ = .12). In the multiple congenital anomalies group, genetic testing was abnormal in 14 (21%) patients who were noted to have dysmorphic features and 7 (10%) without. This is statistically significant ($P$ = .0053) with OR 4.6 [1.5-13.8]. Considering

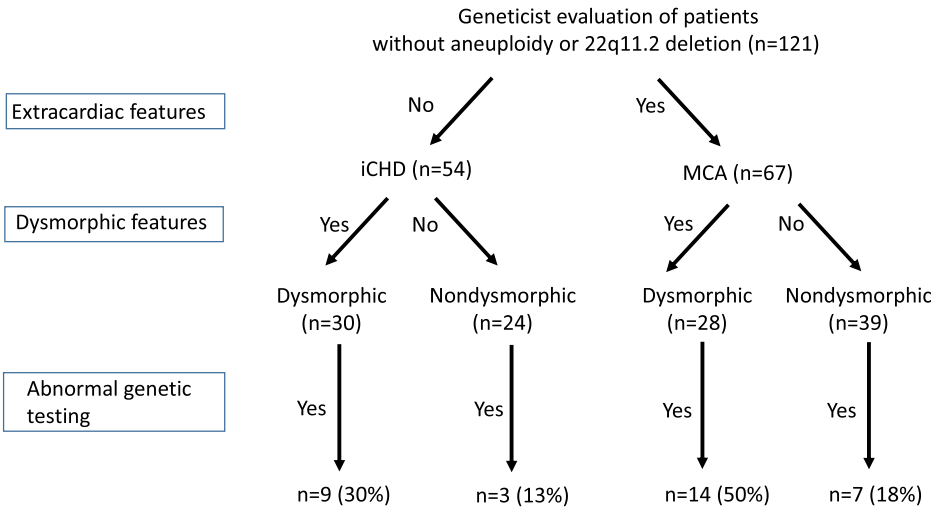


**Figure 4.** Geneticist evaluation of patients without aneuploidy or 22q11.2 deletion (N = 121).

patients with dysmorphic features in both iCHD and multiple congenital anomalies groups, genetic testing was abnormal in 23 (40%). Thus, the identification of dysmorphic features on geneticist evaluation was significantly associated with abnormal genetic testing (OR 3.5, CI 1.5-8.2; $P = .0033$).

## Discussion

Within our overall cohort, 26% of infants with CHD had genetic testing that was abnormal. Infants with multiple congenital anomalies had a greater yield (39%) than infants with iCHD (20%). Other centers have reported similar yields (25-36%) among their CHD cohorts using a similar approach.[20,21] Abnormal testing yield differed for iCHD and multiple congenital anomalies across most testing modalities. Chromosome testing had the greatest abnormal yield within both the iCHD and multiple congenital anomalies groups (32%). The proportion of infants tested by chromosome analyses was approximately 20% of those tested using the more sensitive chromosome microarray modality; thus, the high diagnostic yield likely reflects the fact that chromosome analysis was primarily ordered in infants in whom there was a high suspicion of aneuploidy. Molecular testing had the second greatest yield in the multiple congenital anomalies group (31%) compared with the iCHD group in which 22q11.2 FISH (25%) had the second greatest yield. These results suggest that infants with multiple congenital anomalies may benefit from additional expertise of a genetics evaluation to help guide appropriate molecular genetic testing. Ahrens-Nicklas et al also reported the presence of dysmorphic facial features as a significant factor increasing overall genetic diagnosis yield in their cohort, however the presence of extracardiac anomalies did not reach significance.[21] In contrast, ENT anomalies and brain anomalies were found to be associated with abnormal genetic testing in our cohort. In previous studies, renal abnormalities were reported in 28% of infants with CHD and head abnormalities were seen in 22% using ultrasound.[22] This is greater than what was found in our cohort, where 10% had an abnormal head and/or renal ultrasound. In our cohort, more than 80% of infants with an abnormal head ultrasound had an abnormal genetic test, the most significant factor associated with positive genetic testing in this study with an OR of 31.9. More than one-half of infants in our cohort with an abnormal renal ultrasound also had abnormal genetic testing. There were 3 infants with both head and renal abnormalities on screening ultrasound and all had an abnormal genetic test. Although this is limited evidence, our data do seem to support the practice of completing head and renal ultrasounds in infants with critical CHD as genetic testing yields are increased when a brain and/or renal anomaly is identified which may helpful in guiding genetic testing approach.

Our study also demonstrated an association between infants with IUGR/SGA and abnormal genetic testing. This association suggests the value of early genetics consultation in infants with history of IUGR/SGA. This is especially important because smaller infants are more technically complex when considering cardiac surgery and discussions about a potential syndromic cause of CHD can optimize management strategies.

This study investigated CHD phenotype associations with abnormal genetic testing using both hierarchical and non-hierarchical cardiac classification methods. Using non-hierarchical classification, we demonstrated that RVOTO lesions are associated with abnormal genetic testing. These results suggest that a hierarchical/single classification approach may obscure some genotype–phenotype associations, such as RVOTO which have been reported to make a genetic diagnosis less likely.[21] When considering cardiac lesion as a guide for genetic testing yield, perhaps a traditional view of the heart, where a single dominant phenotype raises suspicion

for a particular genetic cause, does not apply to infants with complex heart disease (ie, multiple lesion types). This seems to be especially true outside of the classic syndromes and highlights the need for complete cardiac phenotyping and more dynamic classification systems in infants with complex lesions. This finding also suggests that highly detailed phenotyping is helpful. For instance, we observed a possible association for pulmonary valve malformation (eg, dysplastic, bicuspid, redundant) and abnormal genetic testing, which likely contributed to the larger RVOTO association.

We restricted our analyses of dysmorphic features to those patients who were evaluated by a geneticist to better standardize the phenotyping. This limitation of the study results from its retrospective nature and the variability in documentation of dysmorphology by non-geneticists and would benefit from additional investigation and standardization. Dysmorphic features were identified both in infants with iCHD as well as multiple congenital anomalies. Infants with dysmorphic features, regardless of cohort, were more likely to have a positive genetic testing result than those classified as nondysmorphic. Patients underwent molecular testing based on a differential generated in response to their specific cardiac features, dysmorphic features, and/or extra cardiac features. Most would not have fulfilled clinical criteria for a diagnosis in infancy and the examination findings combined with molecular testing were required for diagnosis. Five patients who did fulfill clinical criteria for a syndromic diagnosis were given etiologic diagnoses despite normal genetic testing. Geneticists' involvement in the evaluation of infants with CHD may identify those at higher risk for whom additional genetic testing, or outpatient longitudinal follow-up with genetics in the event of normal genetic testing, may be beneficial. The genetic testing yield in infants without dysmorphia was relatively similar between the multiple congenital anomalies group (18%) and the iCHD group (12.5%), suggesting some baseline rate of syndromic diagnoses in infants with CHD regardless of presentation. Thus, infants without dysmorphia with isolated CHD have identifiable genetic diagnoses.

It is important to consider that clinical genetic testing in this cohort was not universal, as some families declined testing. The cohort was limited in racial and ethnic diversity. Another limitation of our study is that genetic testing has rapidly evolved in the last few years. For example, in 2010, 23% of patients had a FISH for 22q11.2 deletion, whereas in 2015 only 6% had FISH testing. This is likely due to the fact that FISH was being replaced by microarray technology and genotype–phenotype correlations for atypical 22q11.2 deletion sizes were emerging, suggesting the utility of more comprehensive assessment by chromosome microarray. Additionally, molecular genetic testing now can identify CNVs, whereas it could not at the time of this study. Exome sequencing and genome sequencing were not used for clinical care during the course of this study; however, both tests are now being incorporated into clinic care at some institutions. Previous studies have demonstrated that the likelihood of identifying a pathogenic or likely pathogenic variant for CHD through exome sequencing/genome sequencing ranges from 10% to 43%.[28-30] This range can be explained by practice variation among centers, variability in study design, and applied criteria for variant interpretation. For example, the Pediatric Cardiac Genetics Consortium completed exome sequencing in 1213 CHD parent–offspring trios that identified de novo mutations in 20% of patients with CHD, extracardiac features, and neurodevelopmental disabilities compared with 2% of patients with iCHD.[31] Although the variant interpretation process used in this study provides important insight into CHD gene discovery, it does not meet clinical standards and thus cannot directly inform yield in a clinical setting. Our study and others suggest that involvement of a geneticist improves diagnoses yields among patients with CHD; however, genetics providers are not always an available resource locally, suggesting that innovations in healthcare delivery, such as the incorporation of facial recognition/artificial intelligence and telegenetics services, may be beneficial. Standardized incorporation of exome sequencing/genome sequencing could be considered in the future as a means to provide rapid and comprehensive genetics evaluation for infants with CHD, as it has been shown to be a cost effective approach for critically ill infants with phenotypes beyond CHD.[32] Many institutions lack the infrastructure required for exome sequencing/genome sequencing that includes the consent process, complex results interpretation, and possibility of secondary findings. When available, it is strongly recommended that a geneticist or genetic counselor be used to guide exome sequencing/genome sequencing use. ■

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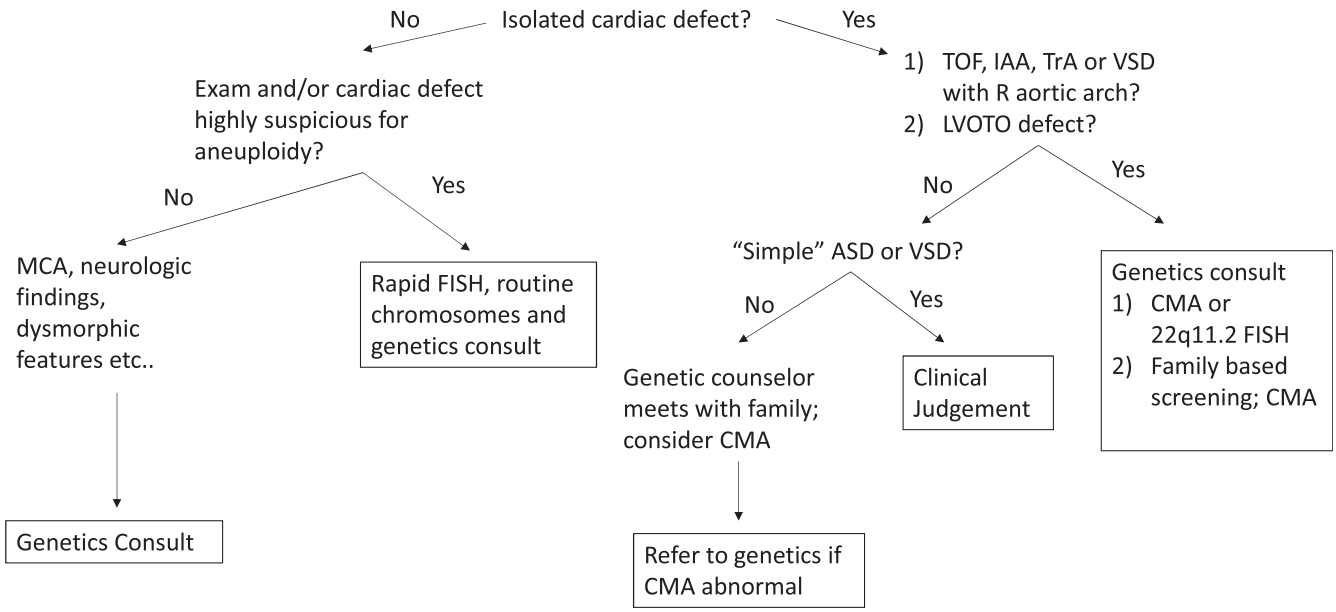


**Figure 1.** Genetic evaluation and testing algorithm for infants with critical CHD. *ASD*, atrial septal defect; *CMA*, chromosome microarray; *IAA*, interrupted aortic arch; *MCA*, multiple congenital anomalies; *LVOTO*, left ventricular outflow tract obstruction; *TOF*, tetralogy. of Fallot; *TrA*, truncus arteriosus.

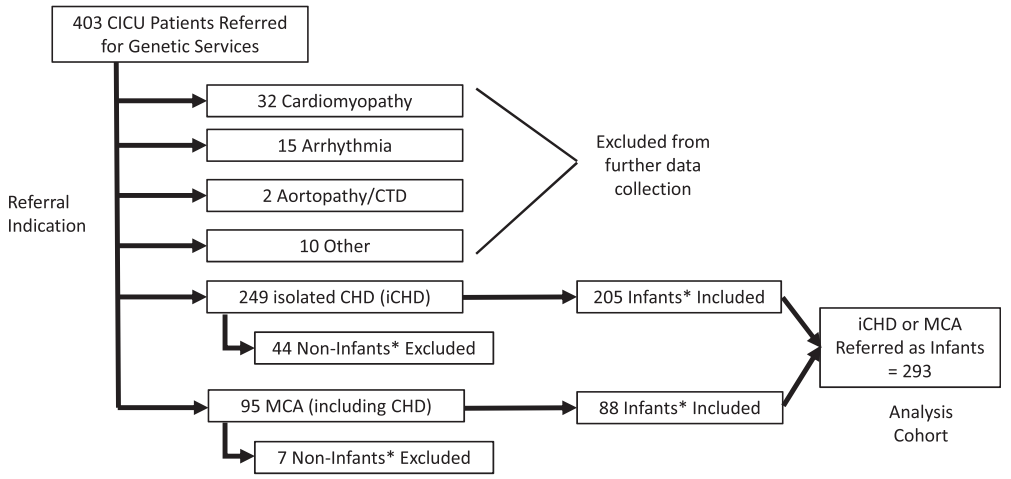


**Figure 2.** Indications for cardiovascular genetics consultation among patients admitted to the. CICU. *Infants defined as less than one month of age at the time of consultation. *CTD*, connective tissue disorder.

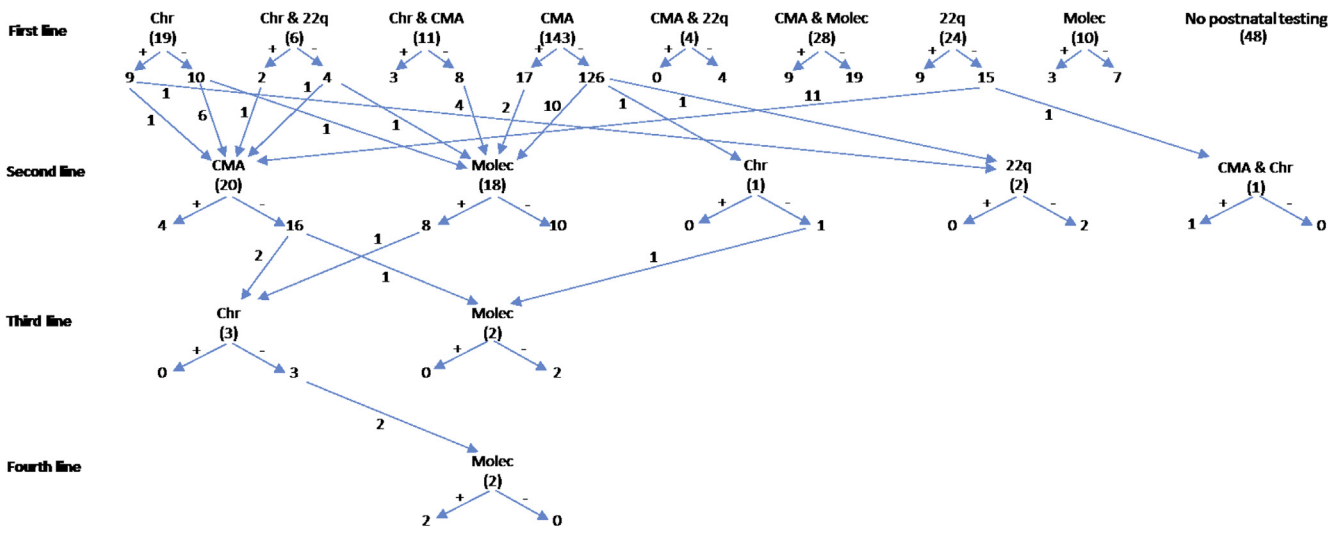


**Figure 3.** Completed genetic testing in CHD and multiple congenital anomalies cohort. *22q*, deletion Fluorescence In Situ Hybridization 11.2 FISH; *Chr*, chromosomes; *Molec*, molecular.

**Table I. Characteristics of infants with CHD and age <1 month (N = 293)**

| Characteristics | n (%) |
|---|---|
| Sex | |
| Male | 193 (66) |
| Female | 100 (34) |
| Race | |
| White | 239 (82) |
| Black | 45 (15) |
| Asian | 4 (1) |
| Other | 5 (2) |
| Ethnicity | |
| Not Hispanic | 284 (97) |
| Hispanic | 9 (3) |
| Current vital status | |
| Alive | 221 (75) |
| Deceased | 63 (22) |
| Unknown | 9 (3) |

**Table III. Yields for different genetic testing types**

| | All | | | Multiple congenital anomalies | | | iCHD | | |
|---|---|---|---|---|---|---|---|---|---|
| Groups | No. sent | No. abnormal | Yield | No. sent | No. abnormal | Yield | No. sent | No. abnormal | Yield |
| Chromosome analysis | 41 | 13 | 32% | 22 | 7 | 32% | 19 | 6 | 32% |
| FISH 22q11.2 | 38 | 10 | 26% | 10 | 3 | 30% | 28 | 7 | 25% |
| Chromosome microarray | 210 | 30 | 14% | 60 | 13 | 22% | 150 | 17 | 11% |
| Molecular | 62 | 17 | 27% | 39 | 12 | 31% | 23 | 5 | 22% |

Eight patients had 2 abnormal test results where 1 test result clarified the other. These tests are counted in both categories.

**Table IV. Abnormal genetic testing results**

| Study IDs | iCHD/ multiple congenital anomalies | Cardiac phenotype | Result | Interpretation |
|---|---|---|---|---|
| Chromosome analysis | | | | |
| 47* | Multiple congenital anomalies | HLHS + VSD<br>ASD nos<br>Possible aberrant RSCA | 46,XX,der(1)t(1;4)(p36.3;q25) | Pathogenic |
| 279* | Multiple congenital anomalies | CoA-VSD<br>PM VSD<br>AV thickened<br>Mitral valve thick and redundant<br>Pulmonary valve thickened<br>Tricuspid valve thick and redundant<br>Arch hypoplasia | 46,XX,del(2)(q36.3q37.1) | Pathogenic |
| 148* | Multiple congenital anomalies | Dilated AscAo<br>Balanced CAVC<br>PA-VSD (non-TOF)<br>LSVC<br>Sec ASD | 46,XX,der(8)t(5;8)(p15.2;p23.1) pat | Pathogenic |
| 384* | Multiple congenital anomalies | Inlet VSD<br>R arch<br>PS | 47,XY,+8[8]/46,XY[12] | Pathogenic |
| 331 | Multiple congenital anomalies | Balanced CAVC<br>PA-VSD (TOF anatomy)<br>LSVC | 47,XX,+13 | Pathogenic (trisomy 13) |
| 58 | iCHD | Balanced complete AVSD<br>CoA-VSD<br>Sec ASD<br>Distal transverse arch hypoplastic | 47,XX,+21 | Pathogenic (trisomy 21) |
| 114 | iCHD | Root dilation<br>TOF<br>Probably discontinuous Pas vs severe proximal LPA stenosis | 47,XY,+21 | Pathogenic (trisomy 21) |
| 248* | iCHD | Balanced CAVC<br>CoA-VSD<br>Hypoplastic arch | 47,XY,+21 | Pathogenic (trisomy 21) |
| 277 | Multiple congenital anomalies | LVDCAVC<br>LSVC<br>RV hypoplasia<br>No RSVC<br>Dysplastic AV valve leaflets | 47,XY,+21 | Pathogenic (trisomy 21) |
| 295 | iCHD | Root dilation<br>TOF<br>TV thickened with redundant chordae | 47,XX,+21 | Pathogenic (trisomy 21) |
| 359 | Multiple congenital anomalies | CAVC (LV dominant)<br>RV hypoplasia<br>Dysplastic pulmonary valve | 46,XX,+21,der(21;21)(q10;q10) | Pathogenic (trisomy 21) |

(*continued*)

**Table IV. Continued**

| Study IDs | iCHD/ multiple congenital anomalies | Cardiac phenotype | Result | Interpretation |
|---|---|---|---|---|
| 15* | iCHD | Type B IAA<br>Aberrant SCA<br>Conoventricular VSD<br>AS<br>BAV<br>Sec ASD<br>Sub AS | 46,XYdel(22)(q11.2q11.2) | Pathogenic (22q11.2 deletion syndrome) |
| 321 | iCHD | BAV<br>CoA-IVS<br>LSVC | 45,X | Pathogenic (Turner syndrome) |
| Chromosomal microarray analysis | | | | |
| 34 | iCHD | CoA-VSD<br>AS<br>BAV<br>PM VSD | arr[GRCh37] 22q11.21(18891398_21463730)x3 | Pathogenic (22q11.2 deletion syndrome) |
| 48 | iCHD | PA-VSD (TOF)<br>Discont PAs<br>LV hypoplasia<br>AP collaterals<br>Midline abdominal aorta | arr[GRCh37] 22q11.21(17269490_19796715)x1 | Pathogenic (22q11.2 deletion syndrome) |
| 294 | iCHD | Truncus<br>Sec ASD<br>Mildly thickened trileaflet truncal valve | arr[GRCh37] 22q11.21(18640300_21608479)x1 | Pathogenic (22q11.2 deletion syndrome) |
| 150 | iCHD | CoA-VSD<br>ASD<br>RVDCAVC<br>LV hypoplasia<br>Sec ASD<br>BAV<br>Dysplastic AV<br>LSVC | arr[GRCh36] Xp22.33q28(262_154899943)x1 | Pathogenic (Turner syndrome) |
| 248* | iCHD | Complete balanced AVCD<br>CoA-VSD<br>Hypoplastic arch | arr[GRCh37] 21p11.2q22.3(10824040_48090629)x3 | Pathogenic |
| 47* | multiple congenital anomalies | HLHS + VSD<br>ASD, nos<br>Possible aberrant RSCA | arr[GRCh37] 4q25q35.2(109970465_190915650)x3 | Pathogenic |
| 279* | multiple congenital anomalies | CoA-VSD<br>PM VSD<br>Thickened AV<br>Thick and redundant MV<br>Thickened PV<br>Thick and redundant TV<br>Arch hypoplasia | arr[GRCh37] 2q36.3q37.1(229119155_234050398)x1 | Pathogenic |
| 384* | multiple congenital anomalies | Inlet VSD<br>R arch<br>PS | arr[GRCh37] 8p23.3q24.3(213-146,264,218)x2-3 | Pathogenic |

(*continued*)

**Table IV. Continued**

| Study IDs | iCHD/ multiple congenital anomalies | Cardiac phenotype | Result | Interpretation |
|---|---|---|---|---|
| 148* | multiple congenital anomalies | Complete balanced AVCD<br>PA-VSD (non-TOF)<br>LSCV<br>Sec ASD | arr[GRCh36]<br>5p15.33p15.2(66648_8920419)x3,<br>8p23.3p23.1(213_11,898,254)x1 | Pathogenic |
| 144* | multiple congenital anomalies | TAPVR<br>Sec ASD<br>Mesocardia<br>RPA moderately hypoplastic | arr[GRCh37] 1p31.1(72309009_79066593)x2 hmz, 2q11.2q14.1(101161050_118403937)x2 hmz, 2q22.2q24.1(142754084_156420492)x2 hmz, 2q24.2q31.1(162107295_170978340)x2 hmz, 3p22.3p13(35167376_70304462)x2 hmz, 5p15.2q11.2(10072247_54187813)x2 hmz, 6p12.3p12.2(47223077_52343899)x2 hmz, 6q23.2q25.2(132533363_155366098)x2 hmz, 8q21.3q22.1(88244548_97221895)x2 hmz, 9p24.3p21.1(1872957_33186156)x2 hmz, 10p13q21.1(13208063_60230128)x2 hmz, 10q23.1q25.3(85521252_117217957)x2 hmz,11p11.2q12.1(45565539_56675823)x2 hmz,13q14.13q21.1(47292611_56578765)x2 hmz,15q12q13.3(27740007_33055904)x2 hmz,16p13.3p12.2(7704203_22947652)x2 hmz,20p12.1q13.13(16787314_49726467)x2 hmz | VUS—12.7% regions of homozygosity (indicative of close familial relationship between parents) |
| 379* | multiple congenital anomalies | Atrial isomerism<br>Dextrocardia<br>RVDCAVC<br>LV hypoplasia<br>L-looped ventricle<br>DORV (side by side with aorta leftward)<br>SubPS<br>TAPVR+RVOTO<br>LSVC<br>Common atrium | arr[GRCh37]<br>7p21.1p15.1(16974692_30970344)x2 hmz | VUS |
| 137 | iCHD | CoA-VSD<br>BAV<br>Parachute MV<br>Musc VSD<br>ASD, nos<br>LSVC | arr[GRCh37]<br>20q13.33(59497040_62431738)x3 | Pathogenic |
| 61 | multiple congenital anomalies | Atrial isomerism<br>L-looped ventricle<br>PA-VSD (nonTOF)<br>LSVC<br>TAPVR+RVOTO<br>LVDCAVC<br>RV hypoplasia<br>DORV (aorta left and anterior to PA)<br>LV trabeculations | arr[GRCh37]<br>17p12(14101029_15449627)x1 | Pathogenic (unrelated to cardiac phenotype) |

(*continued*)

**Table IV. Continued**

| Study IDs | iCHD/ multiple congenital anomalies | Cardiac phenotype | Result | Interpretation |
|---|---|---|---|---|
| 401 | iCHD | Root dilation<br>DORV (TOF type)<br>Musc VSD<br>Dysplastic and redundant TV<br>LV hypoplasia<br>LSVC<br>Sec ASD | arr[GRCh36]<br>5p15.33p15.31(66648_7175604)x1,<br>8p23.3p21.2(213_26130535)x3 | Pathogenic |
| 78 | multiple congenital anomalies | Tricuspid valve stenosis/hypoplasia<br>RV hypoplasia<br>d-TGA+RVOTO<br>ASD, nos<br>Musc VSD<br>AS<br>CoA-VSD<br>Severe arch hypoplasia | arr[GRCh37]<br>5q23.2q34(123730483_167621784)x3 | Pathogenic |
| 393 | multiple congenital anomalies | PA-VSD (TOF)<br>BAV<br>R arch<br>Sec ASD | arr[GRCh36]<br>Xp22.33p22.11(262_22215611)x1,<br>Xp22.11q28(22217004_154894859)x2,<br>Y,22q11.1q11.21(14430822_18692668)x1 | Pathogenic |
| 30 | iCHD | PS<br>Dysplastic PV<br>TS<br>Thickened/dysplastic TV | arr[GRCh37]<br>5p13.1(38777383_39021044)x1 | VUS |
| 36 | iCHD | LV hypoplasia<br>AS<br>CoA-IVS<br>MS<br>ASCA<br>SubAS<br>Hypoplastic arch | arr[GRCh37]<br>19p13.3(374160_1380367)x3 | VUS |
| 182 | iCHD | AS<br>Thickened AV leaflets<br>CoA-IVS<br>Arch hypoplasia | arr[GRCh37]<br>15q11.2(22652330_23272733)x1 | VUS |
| 207 | iCHD | Conoventricular VSD<br>AS<br>CoA-VSD<br>Sec ASD<br>Arch hypoplasia | arr[GRCh37]<br>1q21.1q21.2 (146501348_147843733)x1 | VUS |
| 227 | iCHD | DILV-L-malposition<br>Sec ASD<br>MA<br>Sub PS | arr[GRCh37]<br>16p11.2(29647342_30200975)x1 | VUS |
| 239 | iCHD | Truncus | arr[GRCh37]<br>1q21.1q21.2(146089254_147826789)x1 | VUS |
| 278 | iCHD | RV hypoplasia<br>Common atrium<br>TAPVR | arr[GRCh37]<br>1p36.32(2449711-4473263)x3 | VUS |
| 307 | iCHD | CoA-IVS | arr[GRCh37]<br>15q13.3(29806023_30303141)x1 | VUS |

(*continued*)

**Table IV.** Continued

| Study IDs | iCHD/ multiple congenital anomalies | Cardiac phenotype | Result | Interpretation |
|---|---|---|---|---|
| 317 | iCHD | CoA-IVS<br>BAV<br>AS<br>Closely spaced mitral papillary muscles<br>MS | arr[GRCh37]<br>7p15.3(21294396_23528927)x3 | VUS |
| 325 | iCHD | Single ventricle, os (no identifiable LV)<br>RVDAVC<br>DORV (side-by-side with aorta rightward)<br>PS<br>PV bicuspid and thickened<br>Sec ASD<br>R arch | arr[GRCh37]<br>17q21.31(44211338-44326245)x1-2 | VUS |
| 328 | iCHD | HLHS<br>ASD nos | arr[GRCh37]<br>6p22.1p21.33(27623511_30649134)x2 hmz,<br>6p21.31p21.2(33864998_39723709)x2 hmz | VUS |
| 346 | iCHD | DILV, nos<br>PS<br>Sub PS<br>PV dysplastic | arr[GRCh37]<br>13q12.3(27886795_28398922)x3 | VUS |
| 64 | Multiple congenital anomalies | MA<br>LV hypoplasia<br>AS<br>CoA-VSD<br>PV slightly thickened, mildly dysplastic<br>TAPVR + LVOTO<br>DORV (NRGV)<br>Hypoplastic arch<br>No discernible LV cavity | arr[GRCh37]<br>15q23(68815034_70018990)x1 | VUS |
| 230 | Multiple congenital anomalies | LVDAVCD<br>RV hypoplasia<br>L-looped ventricle<br>PA-VSD (non-TOF)<br>R arch<br>LSVC<br>Common atrium<br>Abdominal situs inversus with levocardia<br>Anterior and leftward aorta<br>Pulmonary venous return to confluence before entering common atrium | arr[GRCh37]<br>2q14.3q22.1(123225623_138447427)x2 hmz,<br>8p21.3p12(19989194_32119175)x2<br>hmz,19p13.12q12(14893513_30050668)x2 hmz | VUS |
| FISH 22q11 | | | | |
| 2 | iCHD | Type B IAA<br>Aberrant SCA<br>Conoventricular VSD<br>AS<br>Sub AS<br>BAV<br>ASD vs PFO | ish del(22)(q11.2q11.2)(TUPLE1-) | Pathogenic (22q11.2 deletion syndrome) |

(*continued*)

**Table IV. Continued**

| Study IDs | iCHD/ multiple congenital anomalies | Cardiac phenotype | Result | Interpretation |
|---|---|---|---|---|
| 15* | iCHD | Type B IAA<br>Aberrant SCA<br>Conoventricular VSD<br>AS<br>BAV<br>Sec ASD<br>Sub AS | ish del(22)(q11.2q11.2)(HIRA-) | Pathogenic (22q11.2 deletion syndrome) |
| 256 | iCHD | TOF<br>Root dilation<br>AscAo dilation<br>STJ dilation<br>Right arch<br>Aberrant SCA<br>AP collaterals | ish del(22)(q11.2q11.2)(TUPLE1-) | Pathogenic (22q11.2 deletion syndrome) |
| 300 | multiple congenital anomalies | Root dilation<br>STJ dilation<br>TOF-APV<br>Redundant TV<br>Right arch | ish del(22)(q11.2q11.2)(TUPLE1-) | Pathogenic (22q11.2 deletion syndrome) |
| 315 | iCHD | Type B IAA<br>Aberrant SCA<br>BAV<br>Conoventricular VSD<br>SubAS<br>AS | ish del(22)(q11.2q11.2)(TUPLE1-) | Pathogenic (22q11.2 deletion syndrome) |
| 350 | iCHD | Truncus<br>Bicuspid truncal valve with thickened cusps | ish del(22)(q11.2q11.2)(TUPLE1-) | Pathogenic (22q11.2 deletion syndrome) |
| 355 | multiple congenital anomalies | TOF | ish del(22)(q11.2q11.2)(TUPLE1-) | Pathogenic (22q11.2 deletion syndrome) |
| 370 | iCHD | Type B IAA<br>Conoventricular VSD<br>AS<br>BAV<br>PV thickened | ish del(22)(q11.2q11.2)(TUPLE1-) | Pathogenic (22q11.2 deletion syndrome) |
| 374 | iCHD | DORV (doubly committed)<br>PS<br>Right arch<br>ASD nos | ish del(22)(q11.2q11.2)(TUPLE1-) | Pathogenic (22q11.2 deletion syndrome) |
| 390 | iCHD | TOF-APV<br>R arch | ish del(22)(q11.2q11.2)(TUPLE1-) | Pathogenic (22q11.2 deletion syndrome) |
| Molecular analysis | | | | |
| 31 | Multiple congenital anomalies | d-TGA-IVS+LVOTO<br>PV bicuspid and dysplastic and prolapsing<br>PS<br>Sec ASD<br>LSVC | *CHD7* sequencing | *CHD7*<br>Pathogenic |

(*continued*)

**Table IV.** Continued

| Study IDs | iCHD/ multiple congenital anomalies | Cardiac phenotype | Result | Interpretation |
|---|---|---|---|---|
| 139 | Multiple congenital anomalies | Root dilation<br>STJ dilation<br>DORV (TOF-type)<br>PS<br>PV thickened, bicuspid<br>Sub PS<br>PFO vs ASD<br>Likely aberrant RSCA | *CHD7* sequencing | *CHD7*<br>Pathogenic |
| 157 | Multiple congenital anomalies | DORV-TGA type<br>MS<br>LV hypoplasia<br>PS<br>Sub PS<br>Sec ASD<br>R arch<br>Side-by-side great arteries | *CHD7* sequencing | *CHD7*<br>Pathogenic |
| 163 | Multiple congenital anomalies | PS<br>d-TGA-VSD<br>PM VSD<br>PV bicuspid<br>Sec ASD<br>MS<br>Deficient mitral anterolateral papillary muscle and posterior leaflet)<br>LSVC<br>R arch<br>Aberrant SCA<br>TV mildly redundant | *CHD7* sequencing | *CHD7*<br>Pathogenic |
| 402 | Multiple congenital anomalies | Type B IAA<br>Conoventricular VSD<br>Sub AS<br>AS<br>Aberrant SCA<br>Sec ASD<br>Deficient mitral posteromedial papillary<br>TV septal leaflet shortened/tethered | *CHD7* sequencing | *CHD7*<br>Pathogenic |
| 309 | Multiple congenital anomalies | Dextrocardia<br>TS<br>RV hypoplasia<br>d-TGA-VSD + RVOTO<br>VSD nos<br>LV trabeculations<br>TAPVR + RVOTO<br>LSVC<br>Coronary anomaly (LAD off RCA off anterior facing sinus)<br>ASD nos<br>Arch hypoplasia | *CHD7* sequencing | *CHD7*<br>VUS |

(*continued*)

**Table IV. Continued**

| Study IDs | iCHD/ multiple congenital anomalies | Cardiac phenotype | Result | Interpretation |
|---|---|---|---|---|
| 90 | iCHD | AS<br>BAV<br>AV dysplastic<br>PS<br>PV dysplastic<br>AscAo dilation | Noonan panel | *PTPN11*<br>Pathogenic |
| 162 | Multiple congenital anomalies | PS<br>PV dysplastic<br>Sub AS<br>AV dysplastic<br>Musc VSD<br>Outlet VSD | Noonan panel | *PTPN11*<br>Pathogenic |
| 106 | Multiple congenital anomalies | AS<br>Asymmetric septal hypertrophy<br>Musc VSD<br>ASD nos<br>LSVC | Noonan panel | *KRAS*<br>Pathogenic |
| 284 | iCHD | Balanced CAVC Parachute "mitral" valve variant<br>DORV-TOF type | Noonan panel | *PTPN11*<br>Pathogenic |
| 403 | Multiple congenital anomalies | Tri atresia-IVS<br>PA-IVS<br>ASD nos<br>AV thickened | Noonan panel | *PTPN11*<br>Likely-Pathogenic |
| 386 | iCHD | PS<br>ASD nos<br>PV dysplastic, bicuspid | Noonan panel | *KRAS*<br>VUS |
| 357 | iCHD | PA-IVS<br>ASD nos<br>TS<br>RV hypoplasia<br>AP collaterals | *JAG1* sequencing | *JAG1*<br>Pathogenic |
| 358 | Multiple congenital anomalies | CoA-IVS<br>BAV<br>MS<br>TV dysplastic<br>Anterior mitral leaflet moves abnormally and hinges at its midpoint and papillary muscles closely spaced | *CREBBP* sequencing | *CREBBP*<br>Pathogenic |
| 144* | Multiple congenital anomalies | TAPVR<br>Sec ASD<br>Mesocardia<br>RPA moderately hypoplastic | Heterotaxy panel | *CFC1*<br>Pathogenic |

(*continued*)

**Table IV. Continued**

| Study IDs | iCHD/ multiple congenital anomalies | Cardiac phenotype | Result | Interpretation |
|---|---|---|---|---|
| 379* | Multiple congenital anomalies | Atrial isomerism<br>Dextrocardia<br>RVDCAVC<br>LV hypoplasia<br>L-looped ventricle<br>DORV (side by side with aorta leftward)<br>Sub PS<br>TAPVR + RVOTO<br>LSVC<br>Common atrium | *DNAH11* sequencing | *DNAH11*<br>Pathogenic |
| 146 | iCHD | CoA-VSD<br>Conoventricular VSD<br>AS<br>Sub AS<br>BAV<br>Sec ASD | Branchio-oto-renal panel | *EYA1*<br>Pathogenic |

*AP*, aortopulmonary; *AS*, aortic stenosis; *AscAo*, ascending aorta; *ASD*, atrial septal defect; *AV*, aortic valve; *AVSD*, atrioventricular septal defect; *BAV*, bicuspid aortic valve; *CAVC*, complete atrioventricular canal; *CoA*, coarctation of the aorta; *DILV*, double-inlet right ventricle; *DORV*, double-outlet right ventricle; *HLHS*, hypoplastic left heart syndrome; *IAA*, interrupted aortic arch; *IVS*, intact ventricular septum; *LAD*, left anterior descending; *LPA*, left pulmonary artery; *LSVC*, left superior vena cava; *LV*, left ventricular; *LVDAVCD*, left ventricular dominant complete atrioventricular canal defect; *LVOTO*, left ventricular outflow tract obstruction; *MA*, mitral atresia; *MS*, mitral stenosis; *Musc*, muscular; *MV*, mitral valve; *nos*, not otherwise specified; *NRGV*, normally related great vessels; *os*, otherwise specified; *PA*, pulmonary atresia; *PFO*, patent foramen ovale; *PM*, primary muscular; *PS*, pulmonary stenosis; *PV*, pulmonary valve; *R*, right; *RCA*, right coronary artery; *RPA*, right pulmonary artery; *RSCA*, right subclavian artery; *RV*, right ventricular; *RVOTO*, right ventricular outflow tract obstruction; *RVDCAVC*, right ventricular dominate complete atrioventricular canal defect; *SCA*, subclavian artery; *Sec ASD*, secundom atrial septal defect; *STJ*, sinotubular junction; *Sub AS*, subaortic stenosis; *Sub PS*, sub-pulmonary stenosis; *TAPVR*, total anomalous pulmonary venous return; *TGA*, transposition of the great arteries; *TOF*, tetralogy of Fallot; *TS*, tricuspid stenosis; *TV*, tricuspid valve.
*Multiple abnormal genetic tests.

**Table VII. Genetic testing yields for the most frequent CHD lesions**

| CHD lesions | Total no. with genetic testing (N = 222) | No. with abnormal genetic testing (%) | OR (95% CI) | *P* value |
|---|---|---|---|---|
| Secundum ASD | 63 | 11 (17) | 0.95 (0.44-2.04) | .8917 |
| ASD, nos | 48 | 9 (19) | 1.06 (0.47-2.42) | .8815 |
| Left SVC | 45 | 10 (22) | 1.40 (0.63-3.13) | .4112 |
| Aortic valve stenosis/hypoplasia | 43 | 11 (26) | 1.78 (0.81-3.93) | .1752 |
| CoA with VSD | 41 | 7 (17) | 0.92 (0.38-2.26) | .8217 |
| Pulmonary valve malformation, os | 36 | 11 (31) | 2.38 (1.06-5.37) | .0325 |
| Pulmonary valve stenosis/hypoplasia | 34 | 11 (32) | 2.62 (1.15-5.96) | .0181 |
| Muscular VSD | 28 | 5 (18) | 0.99 (0.35-2.78) | .9811 |
| CoA with IVS | 27 | 5 (19) | 1.04 (0.37-2.93) | .9424 |
| Mitral valve malformation, os | 27 | 7 (26) | 1.72 (0.67-4.39) | .2540 |
| RV hypoplasia | 27 | 6 (22) | 1.35 (0.51-3.60) | .5442 |
| Right aortic arch | 26 | 5 (19) | 1.09 (0.39-3.10) | .8640 |
| HLHS with IVS | 23 | 1 (4) | 0.19 (0.02-1.42) | .0868* |
| BAV | 23 | 6 (26) | 1.71 (0.63-4.66) | .2876 |
| LV hypoplasia | 23 | 5 (22) | 1.30 (0.45-3.74) | .6611 |

*SVC*, superior vena cava.
*Fisher exact test.

**Table VIII. Genetic testing yields for CHD types defined using a hierarchical classification method of CHD**

| CHD classes | No. with genetic testing (N = 222) | No. with abnormal genetic testing (%) | *P* value |
|---|---|---|---|
| Conotruncal defect | 66 | 11 (17) | .7333 |
| LVOTO | 56 | 8 (14) | .4007 |
| RVOTO | 27 | 5 (19) | .9424 |
| Laterality | 24 | 3 (13) | .5822* |
| LVOTO + septal defect | 18 | 4 (22) | .7480* |
| Conotruncal defect + AVSD | 9 | 4 (44) | .0578* |
| APVR | 6 | 2 (33) | .2955* |
| AVSD | 6 | 1 (17) | 1* |
| Other | 3 | 0 | 1* |
| SV | 4 | 1 (25) | .5510* |
| RVOTO + septal defect | 2 | 1 (50) | .3286* |
| Septal defect | 1 | 0 | 1* |

*SV*, single ventricle.
Data exclude patients who did not undergo genetic testing.
*Fisher exact test.

**Table IX. Genetic testing yields among patients with non-cardiac abnormalities**

| Organ systems | Total no. (%) (N = 222) | No. with abnormal genetic testing (%) | OR (95% CI) | *P* value |
|---|---|---|---|---|
| All multiple congenital anomalies | 67 (30) | 21 (31) | 3.26 (1.61-6.61) | .0007 |
| Gastrointestinal | 15 (7) | 4 (27) | 1.73 (0.52-5.73) | .4829* |
| Ribs/vertebrae | 15 (7) | 4 (27) | 1.73 (0.52-5.73) | .4829* |
| Renal | 13 (6) | 5 (38) | 3.11 (0.96-10.06) | .0481 |
| Hepatobiliary | 13 (6) | 3 (23) | 1.39 (0.37-5.32) | .7082* |
| Spleen | 13 (6) | 5 (38) | 3.11 (0.96-10.06) | .0481 |
| ENT | 12 (5) | 6 (50) | **5.18 (1.57-17.00)** | **.0030** |
| Genitourinary | 8 (4) | 3 (38) | 2.87 (0.66-12.54) | .1577* |
| Limb | 8 (4) | 3 (38) | 2.87 (0.66-12.54) | .1577* |
| Brain | 7 (3) | 6 (86) | **31.9 (3.73-273.79)** | **.0001*** |
| IUGR/SGA | 13 (6) | 6 (46) | **4.47 (1.41-14.14)** | **.0061** |

Data exclude patients with 22q11.2 deletion (13), Down syndrome (7), trisomy 13 (1), or Turner syndrome (2).
*Fisher exact test.
Bold indicates statistically significant.

**Table X. Frequency of CHD types among patients who had an examination by a geneticist**

| CHD types | No. with an examination by a geneticist (%) |
|---|---|
| All (N = 222) | 121 (55) |
| Conotruncal defect (N = 66) | 38 (58) |
| LVOTO (N = 56) | 16 (29) |
| RVOTO (N = 27) | 15 (56) |
| Laterality (N = 24) | 21 (88) |
| LVOTO + septal defect (N = 18) | 10 (56) |
| AVSD (N = 6) | 5 (83) |
| Conotruncal defect + AVSD (N = 9) | 8 (83) |
| APVR (N = 6) | 3 (50) |
| Single ventricle (N = 4) | 1 (25) |
| Other (N = 3) | 1 (33) |
| RVOTO + septal defect (N = 2) | 2 (100) |
| Septal defect (N = 1) | 1 (100) |