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

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Abstract

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 non-cardiac phenotypes were tested for associations with abnormal genetic testing using chi-squared and Fisher's exact tests.

Results: Genetic evaluation was completed in 293 infants with CHD, of whom 213 had isolated CHD (iCHD) and 80 had multiple congenital anomalies (MCA). Overall the yield of abnormal genetic testing was 26%. The MCA cohort had a greater yield of genetic testing (39%) than the iCHD cohort (20%) (odds ratio 2.7). Utilizing a non-hierarchical CHD classification and excluding 22q11.2 deletion and common aneuploidies, right ventricular obstructive defects were associated with abnormal genetic testing (p=0.0005). Extracardiac features associated with abnormal genetic testing included ear, nose and throat (p=0.003) and brain (p=0.0001) abnormalities. A diagnosis of small for gestational age or intrauterine growth retardation was

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also associated with abnormal genetic testing (p=0.0061), as was presence of dysmorphic features (p=0.0033, odds ratio 3.5). Nondysmorphic infants with iCHD or MCA had similar frequencies of abnormal genetic testing.

Conclusion: 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.

Keywords

chromosome microarray; copy number variant; genetic syndrome; genetic testing; cardiovascular genetics

Introduction

Congenital heart disease (CHD) is the most common birth defect, affecting ~1% of livebirths (1, 2). The incidence of severe CHD requiring expert cardiologic care is 2.5 to 3/1,000 (2). It is estimated that up to one quarter of CHD with or without extracardiac anomalies has an identifiable genetic etiology including copy number variation (3–8), chromosomal (9, 10) or single gene (9). 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 4 specific reasons to pursue genetic testing in the setting of CHD. These reasons include 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 (11, 12). Genetic testing is also known to have personal utility for patients and families (13). Positive genetic testing can be used to confirm a genetic etiology for an individual's CHD, whereas negative genetic testing, while 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 (14, 15). Although identification of newborns with aneuploidies is often straightforward, many genetic syndromes associated with CHD can be very challenging to diagnose, especially in a critically ill newborn. In some cases, dysmorphic features are not yet readily evident and, lacking other major anomalies, patients appear to have isolated CHD (iCHD). In other cases, multiple congenital anomalies (MCA) are noted but a specific diagnosis is not made.

Neurodevelopmental delays are also frequently associated with genetic diagnosis in children with CHD (14), however these delays may not be appreciated in a newborn. It is notable, for example, that the STATseq study of research-based whole genome sequencing in infants and children in neonatal and pediatric intensive care units found that phenotypes of known syndromes were less differentiated in infancy (16, 17). Of the 3 recurrent conditions

identified, Noonan syndrome and CHARGE syndrome are commonly associated with CHD but were not recognized in infants in the study (18).

Although standard of care guidelines recommend genetic testing in infants with CHD (12, 19), 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 (20). This type of protocol has been reported to increase the rate of diagnosis for genetic conditions and reduce cost to patients (21). Several single-institution studies have reported yield of genetic evaluation, genetic testing and/or screening for extracardiac features among infants with critical CHD. Overall yields of genetic testing range from 18%−36%. Genetic testing modality, CHD lesion and additional extracardiac features are noted to influence the yield of genetic testing (21–23). These studies differed in their ascertainment of patients and inclusion criteria as well as their use of genetic testing modalities. As such, the field has been hindered by lack of good data from a comprehensive, standardized cardiovascular genetics approach without significant ascertainment bias.

Additional evidence is needed to support the thoughtful use of genetic testing for both iCHD and CHD associated with MCA. Our study sought to investigate the yield of genetic diagnosis among infants with critical iCHD and MCA using a standardized algorithm (20) and comprehensive cardiovascular genetics approach. It also sought to identify genotype-phenotype correlations that highlight phenotypic features that should increase suspicion for a genetic condition.

Methods

Study Population

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 utilizes an algorithm to incorporate genetic services for patients with CHD as well as other types of genetic heart disease, as outlined in Figure 1, online (20, 21). Cardiovascular Genetic Counseling Consultations were placed at the time of admission for all infants less 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. While 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 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 MCA 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 one non-cardiac organ system: gastrointestinal, ribs/vertebrae, renal, hepatobiliary, spleen, ear, nose and throat (ENT), genitourinary, limb, brain, and intrauterine growth retardation/small for gestational age (IUGR/SGA). Dysmorphic features were not included as an extracardiac feature since they were only recorded for those who had a geneticist evaluation. Infants with CHD in addition to another extracardiac feature were defined as having MCA. Patients who received genetic services for cardiac diagnoses other than iCHD or MCA, including cardiomyopathy, aortopathy, and arrhythmia, were noted for volume accounting but were excluded from the remainder of the study. All patients meeting the above inclusion criteria were included in the full retrospective chart review.

Data Collection

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 (24). Data collected included demographics, echocardiography and other imaging results, clinical notes, family history, prenatal history, genetic testing results and geneticists' evaluation (including dysmorphology exam). 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.

Classification of Cardiac Disease

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 (25). Level III categories of Aortopathy, Arteriopathy, Coronary anomaly, and Cardiomyopathy were also added, as previously described (reference: https://www.mdpi.com/2308–3425/2/2/76). 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 (HLHS). Patients were allowed to have more than one level I diagnosis recorded. Level I diagnoses which were the combinations of two 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 would also have been recorded and specified (e.g. perimembranous VSD). Level I diagnoses that may have been excluded in the Botto system were also recorded (e.g. an atrial septal defect in a patient with tetralogy of Fallot) in order 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 one level III

diagnosis recorded. In addition to this non-hierarchical phenotyping, the level I diagnoses were utilized 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

Genetic testing included in the study cohort included chromosome analysis, fluorescence in situ hybridization (FISH) for 22q11.2, single nucleotide polymorphism (SNP) microarray (CMA), and any molecular testing that may have included disease-specific gene panels or single gene testing. While 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) CMA 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.

Statistical Analysis

The associations between categorical clinical/phenotype variables and abnormal genetic testing were tested using 2×2 cross tables. Pearson's chi-square testing was utilized when all values in the cross table were 5 or greater. When at least one value was less than 5, the Fisher's exact 2-tail test was utilized. 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 <0.05 for statistical significance. Statistical analyses were performed using JMP statistical software package (SAS Institute, Cary, North Carolina).

Results

Description of Cohort

The CICU at CCHMC admitted 2,391 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), MCA including CHD (95), cardiomyopathy (32), arrhythmia (15), aortopathy/concern for connective tissue disorder (2), and other (10) (Figure 2, online). All infants < 1 month of age at the time of consultation with iCHD or MCA who had a genetics and/or genetic counseling consultation were included for study (n=293; Table 1, online). Among these, 204 (70%) patients had prenatal diagnosis of CHD and 21 (7%) patients had family history of CHD.

Results of Genetic Testing

Table 2 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 MCA (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 higher in patients with MCA than iCHD (p=0.001) (odds ratio (OR) 2.7 and 95% confidence interval (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 higher in MCA than iCHD groups (p=0.0007) with an OR 3.3 (CI 1.6–6.6). While testing yields were lower in iCDH, the 12% testing yield in iCHD is clinically significant.

Genetic testing included chromosome analysis, CMA, 22q11.2 FISH, and molecular analysis. Figure 3 (online) summarizes the testing strategies and results. Of the 245 patients who had genetic testing, 155 (63%) had one type of genetic testing, 76 (31%) had two types, 11 (4%) had three types and 3 (1%) had all four types. Two types of genetic testing were ordered together as the initial testing for 49 patients (20%). CMA was the most common initial test (n=182). Second, third, and fourth line testing primarily consisted of CMA (n=21) or molecular testing (n=22). First line testing had a yield of 21%, 2nd tier testing had a yield of 26%, none of the 3rd line testing was positive, and both 4th 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 one test completed.

Table 3 (online) summarizes yields for each type of genetic testing. Chromosome analysis was abnormal in 13 patients, including aneuploidies (9), large deletions (2), and translocations (2). CMA was abnormal in 30 patients. Five of these CMA abnormalities helped to define abnormal chromosome analysis findings (three were sent together with chromosome analysis and two were sent as follow up testing). Syndromic diagnoses identified by CMA included 22q11.2 deletion (3) and Turner syndrome (1). Two patients had regions of homozygosity (ROH) identified on CMA that led to further molecular testing that identified pathogenic sequence variants (*DNAH11* and *CFC2*) within the ROH. The 19 other CMA abnormalities included 5 pathogenic 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 above, molecular analysis in concert with CMA 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 4 (online).

Cardiac phenotype and genetic testing yields

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). AVSD lesions had the highest yield of abnormal genetic testing (13/31, 42%) (Table 5). 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 CTDs for 22q11.2 deletion using CMA or FISH. Also, patients with one of these aneuploidy syndromes are often diagnosed prenatally or soon after birth based on external features and CHD phenotypes. Therefore, in order to study the impact of genetic evaluations in CHD patients beyond these relatively common and well-characterized syndromes, further analyses excluded these 23 patients. Interestingly, in this analysis right ventricular obstructive defect (RVOTO) was significantly associated with abnormal genetic testing (OR 3.4, CI 1.7–7.0; p=0.0005) (Table 6). The association was statistically significant with Bonferroni correction for multiple comparisons consisting of 11 separate tests (corrected p=0.0055).

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 ASD was present in 63 (28%) and pulmonary valve stenosis/hypoplasia in 34 (15%) patients (Table 7, online). There were nominally significant associations between abnormal genetic testing and pulmonary valve stenosis/hypoplasia (p=0.02) or specified pulmonary valve malformation (e.g. dysplastic) (p=0.03). However, Bonferroni correction (15 CHD lesions were separately tested) determined that these associations were not statistically significant. Nonetheless, these associations likely contributed to the significant association for the overall CHD type RVOTO and abnormal genetic testing. Also of note, only one of 23 (4%) genetically tested patients with HLHS and intact ventricular septum was found to have a genetic abnormality.

Finally, each patient's set of CHD lesions was classified into a single CHD type using a hierarchical classification method from the prior 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 8, online).

Non-cardiac phenotypes and genetic testing yields.

Recognizing that the overall rates of genetic testing were similar between iCHD and MCA groups but yields were higher in patients with MCA (Table 2), 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 9, online). 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=0.003) and brain abnormalities (OR 31.9, CI 3.7–273.8; p=0.0001) were significantly associated with abnormal genetic testing after Bonferroni correction for 9 tests.

A possible association between renal anomalies and abnormal genetic testing was suggested based on unadjusted p value (p=0.048). In addition, a diagnosis of intrauterine growth restriction (IUGR) or small for gestational age (SGA) was present in 13 (6%) patients and was significantly associated with abnormal genetic testing (OR 4.5, CI 1.4–14.1; p=0.0061). Thus, these results indicate that compared with other congenital abnormalities, patients with brain and ENT anomalies may have an increased likelihood for abnormal genetic testing.

Impact of clinical genetics evaluation on genetic testing.

Among the whole cohort, 162 (55%) patients had a physical exam by a geneticist. A geneticist examined all 88 patients in the cohort who had MCA. Among the total 162 with genetics exam, 88 (54%) were documented by the geneticist to have dysmorphic features. Genetic testing was completed in 144 (89%) of 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 MCA when not considering the presence of dysmorphic features. Of the 14 without MCA, 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 MCA or dysmorphic features had 22q11.2 deletion.

A physical exam 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 LVOTO were less frequently examined (29%) (complete list in Table 10, online). Forty-seven (39%) had one genetic test, 60 (50%) had two 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%) of patients examined by a geneticist. Four patients had abnormal chromosomes and CMA defining the chromosome abnormality, and two had CMA with ROH and positive molecular testing with a heterotaxy panel. Otherwise, 12 had CMA abnormality and 15 had abnormal molecular testing. In contrast, genetic testing results were abnormal in only 7 of the 101 patients (7%) that had genetic testing sent without ever being examined by a geneticist. Ninety (89%) had one test and 11 (11%) had two 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<0.0001). 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 two were given a diagnosis of diabetic embryopathy. Overall, 38 (31%) of 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 MCA. In the iCHD group, 30 patients were noted to have dysmorphic features, of which 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. While the frequency of abnormal genetic testing was higher in the dysmorphic group with iCHD, it did not reach statistical significance (p=0.12). In the MCA 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=0.0053) with OR 4.6 [1.5–13.8]. Considering patients with dysmorphic features in both iCHD and MCA 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=0.0033).

Discussion

The present study provides important data regarding results of a comprehensive approach to incorporate cardiovascular genetics services into the care of infants with critical CHD with an effort to identify iCHD vs MCA executed by a dedicated team with expertise in cardiovascular genetics. Consideration of genetic testing yield associated with CHD subtype, presence/absence of extracardiac features, growth, and dysmorphology is important for risk stratification and further delineation of infants that require additional evaluation.

Within our overall cohort, 26% of infants with CHD had genetic testing that was abnormal. Infants with MCA had a higher yield (39%) than infants with iCHD (20%). Other centers have reported similar yields (25–36%) among their CHD cohorts utilizing a similar approach (21, 22). However, our study is the first to assess genetic testing yield in iCHD versus MCA exclusively among infants under the age of one month. Abnormal testing yield differed for iCHD and MCA across most testing modalities. Chromosome testing had the highest abnormal yield within both the iCHD and MCA groups (32%). The proportion of infants tested by chromosome analyses was approximately 20% of those tested using the more sensitive CMA modality, likely reflecting the fact that chromosome analysis was primarily ordered in infants in whom there was a high suspicion of aneuploidy. Molecular testing had the second highest yield in the MCA group (31%) compared to the iCHD group in which 22q11.2 FISH (25%) had the second highest yield. These results suggest that infants with MCA 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 (22). In contrast, ENT anomalies and brain anomalies were found to be associated with abnormal genetic testing in our cohort. The use of screening head and renal ultrasounds is an easy and accurate method to assess extracardiac features that may not be apparent on physical exam. In previous studies renal abnormalities were reported in 28% of infants with CHD and head abnormalities were seen in 22% using ultrasound (23). This is higher 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 odds ratio of 31.9. More than 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. While 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.

Similar to the study by Ahrens-Nicklas et al (22) we observed frequent abnormal genetic testing for CTDs and AVSDs when including all patients. Additionally, our study was able to glean new insight by analyzing genetic testing and evaluation rates with and without common syndromes (trisomies, Turner syndrome and 22q11.2 deletion). This is necessary to begin to address the important question of approach to patients with CHD who do not have a commonly recognized syndrome.

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 (22). 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 (i.e. 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 (e.g. dysplastic, bicuspid, redundant) and abnormal genetic testing, which likely contributed to the larger RVOTO association. Fundamentally, there is still a need to develop cardiac phenotyping/grouping systems that are more predictive of genetic etiology which can only be accomplished through a larger multi-center study.

We restricted our analyses of dysmorphic features to those patients who were evaluated by a geneticist in order to better standardize the phenotyping. Dysmorphic features were identified both in infants with iCHD as well as MCA and infants with dysmorphic features, regardless of cohort, were more likely to have a positive genetic testing result than those classified as nondysmorphic. In addition, 5 patients were given etiologic diagnoses based on clinical evaluation despite normal genetic testing. We suggest that 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. Future studies are needed to directly address this. Interestingly, the genetic testing yield in nondysmorphic infants was relatively similar between the MCA group (18%) and the iCHD group (12.5%) suggesting some baseline rate of syndromic diagnoses in infants with CHD regardless of presentation. This finding

also highlights that even though numbers are small, nondysmorphic infants with isolated CHD have identifiable genetic diagnoses.

This study illustrates the experience of a single pediatric medical center with a comprehensive cardiovascular genetics approach in which all infants with critical CHD are evaluated. As such, it does not reflect the current practice at all pediatric institutions. 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 22q whereas in 2015 only 6% had a FISH for 22q. This is likely due to the fact that FISH was being replaced by microarray technology. Additionally, molecular genetic testing can now reliably identify CNVs while it could not at the time of this study. Exome sequencing (ES) and genome sequencing (GS) were not standardly used for clinical care during the course of this study, however both tests are now being incorporated into clinic care at some institutions. Prior studies have demonstrated that the likelihood of identifying a pathogenic or likely pathogenic variant for CHD through ES/GS ranges from 10–43% (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 ES in 1,213 CHD parent-offspring trios which identified de novo mutations in 20% of patients with CHD, extracardiac features and neurodevelopmental disabilities compared to 2% of patients with iCHD (31). While the variant interpretation process utilized 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. To date no studies have assessed the clinical utility and cost effectiveness of ES/GS in patients with CHD and no guidelines have been published to establish best practices or algorithms for incorporation into the care of patients with CHD. Our study and others suggest that involvement of a geneticist improves diagnosed yields among patients with CHD, however genetics providers are not always an available resource. Many institutions lack the infrastructure required for ES/GS including the consent process, complex results and possibility of secondary findings. When available, a geneticist or genetic counselor should be utilized to guide ES/GS use. When not available, standardized incorporation of ES/GS 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).

In conclusion, using a comprehensive cardiovascular genetics approach for infants with critical CHD, we found that 26% of infants had an abnormal genetic test. When including infants with a clinical diagnosis assigned by a geneticist, 28% were given an etiologic diagnosis. Once common aneuploidies and 22q11.2 deletion syndrome were excluded, patient features associated with increased yield of genetic testing included the presence of ENT or brain anomalies, history of IUGR/SGA, presence of dysmorphic features identified by a geneticist (especially within the MCA group), and RVOTO lesion when allowing for multiple CHD types. Head and renal ultrasounds should be considered among infants with CHD given the frequencies of abnormalities identified and the association with positive genetic testing results. A geneticist evaluation to identify dysmorphic features in infants without MCA appears to identify a group at highest risk for abnormal genetic testing.

The present study provides important evidence to support a comprehensive approach to cardiovascular genetic service and testing in infants with critical CHD.

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Abbreviations and Acronyms:

APVR	anomalous pulmonary venous return
AVSD	atrioventricular septal defect
CHD	congenital heart disease
CCHMC	Cincinnati Children’s Hospital Medical Center
CICU	cardiac intensive care unit
CMA	chromosome microarray
CNV	copy number variant
CTD	conotruncal defect
ES	exome sequencing
FISH	florescent in situ hybridization
GS	genome sequencing
IUGR	intrauterine growth retardation
LVOTO	left ventricular outflow tract obstruction
MCA	multiple congenital anomalies
ROH	regions of homozygosity
SGA	small for gestational age
VUS	variant of uncertain significance
RVOTO	right ventricular outflow tract obstruction

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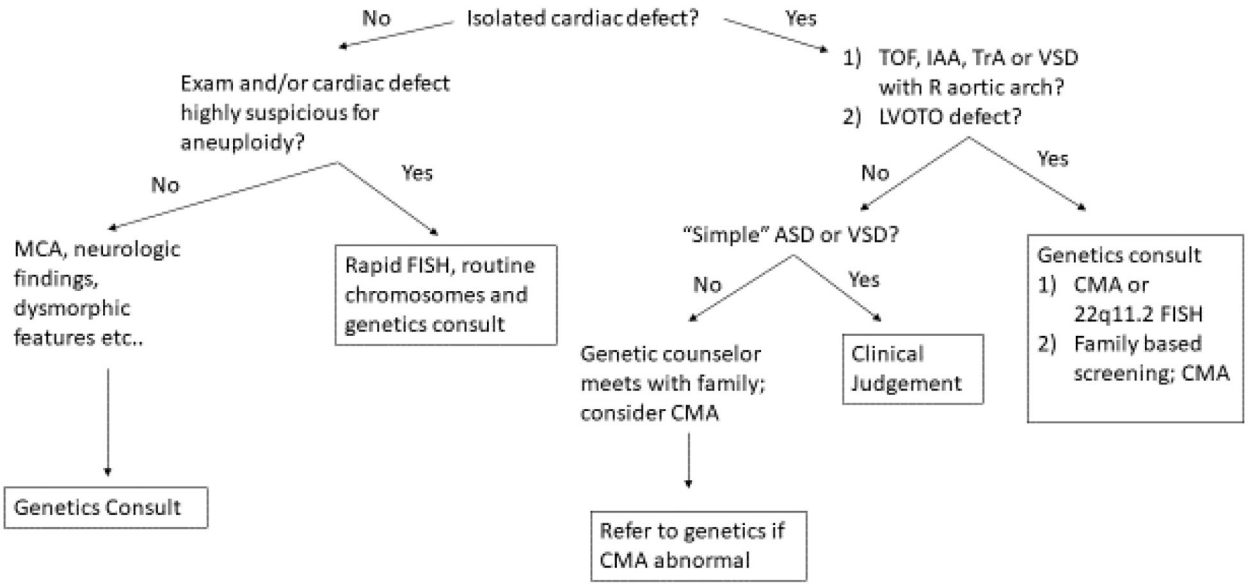

Figure 1:

Genetic Evaluation and Testing Algorithm for Infants with Critical Congenital Heart Disease

ASD: atrial septal defect, CMA: chromosome microarray, IAA: interrupted aortic arch, MCA: multiple congenital anomalies, LVOTO: left ventricular outflow tract obstruction, TOF: tetralogy of fallow, TrA: truncus arteriousus, VSD: ventricular septal defect

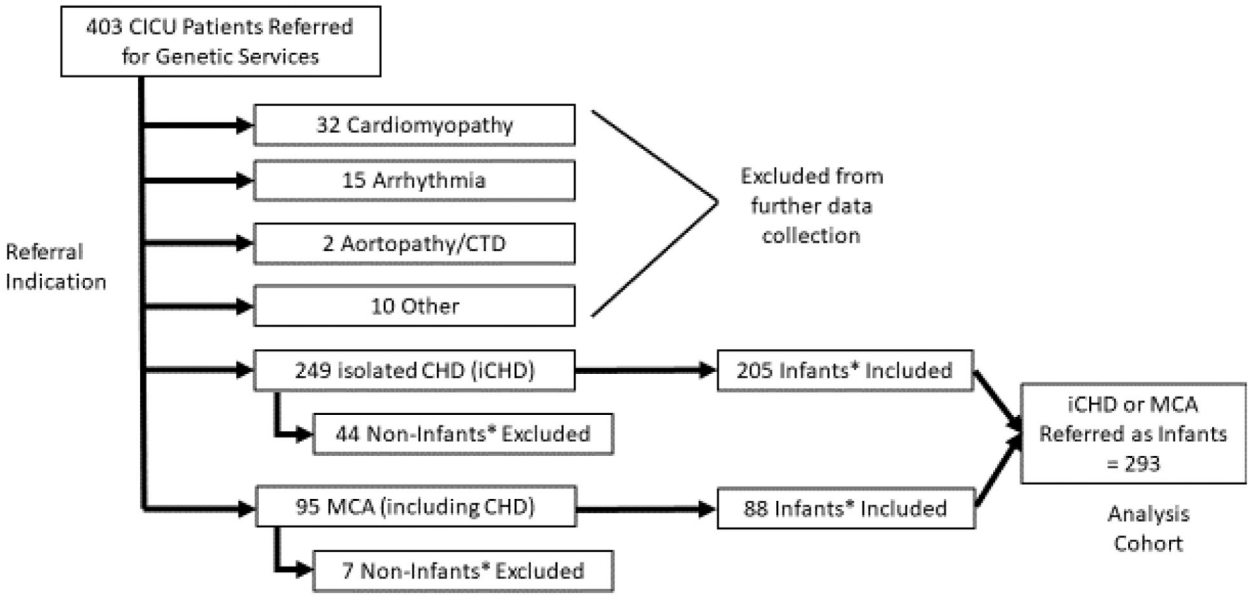

Figure 2 –.

Indications for Cardiovascular Genetics Consultation among Patients Admitted to the Cardiac Intensive Care Unit

*Infants defined as less than one month of age at the time of consultation

CHD: congenital heart disease, CTD: connective tissue disorder, MCA: multiple congenital anomalies

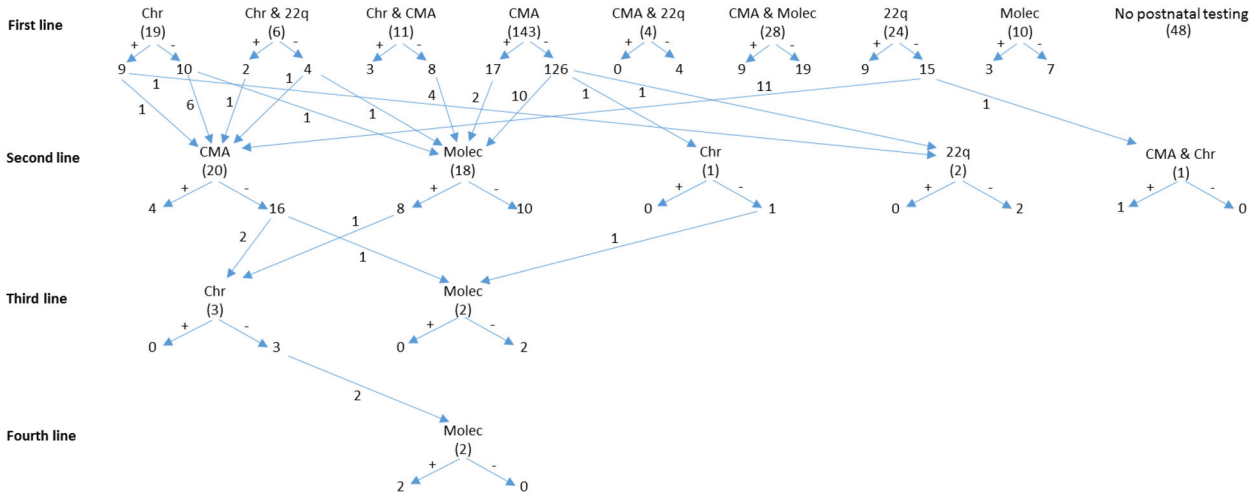

Figure 3 –.

Completed Genetic Testing in CHD and MCA Cohort

CHD: congenital heart disease, Chr: chromosomes, CMA: chromosome microarray, MCA: multiple congenital anomalies, Molec: molecular, 22q: deletion 22q11.2 FISH

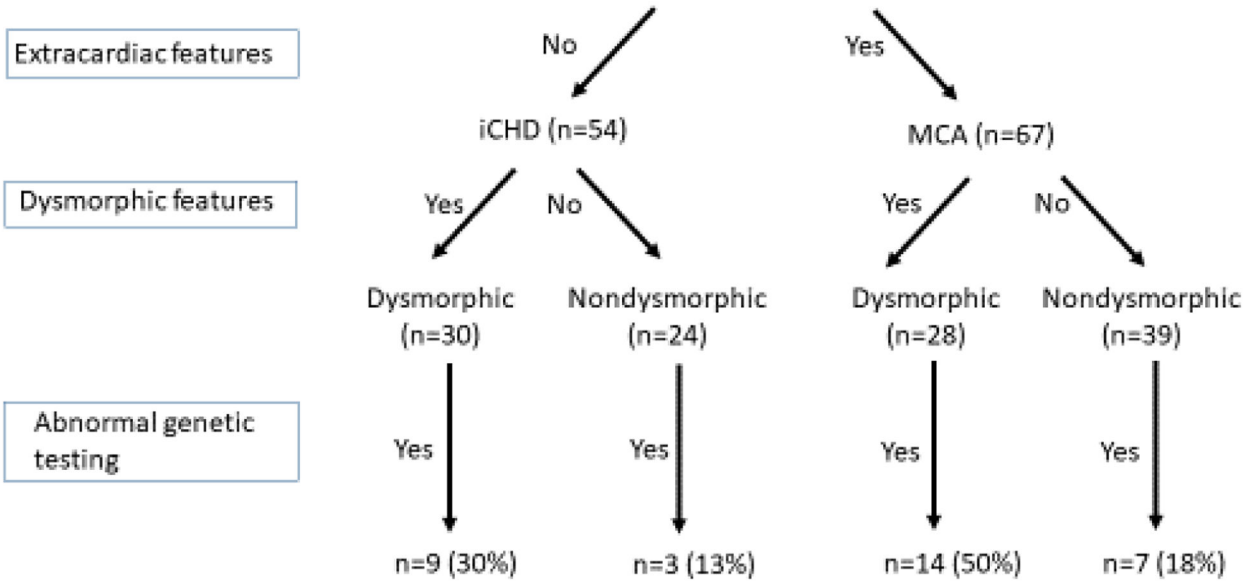

Figure 4:
Geneticist evaluation of patients without aneuploidy or 22q11.2 deletion (n=121)

Table 1.

Characteristics of infants with CHD and age < 1 month (N=293)

Characteristics	N (%)
Sex	
Male	193 (66)
Female	100 (34)
Race	
Caucasian	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 2.

Rates and yields of genetic testing

Group	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%
MCA (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%
MCA (N=79)	67 (85%)	21 (27%)	21/67 = 31%

iCHD: isolated congenital heart disease, MCA: multiple congenital anomalies, T21: Trisomy 21, T13: Trisomy 13, TS: Turner syndrome, 22q11: 22q11.2 deletion syndrome

Table 3.

Yields for different genetic testing types.

Group	All			MCA			iCHD		
	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%
CMA	210	30	14%	60	13	22%	150	17	11%
Molecular	62	17	27%	39	12	31%	23	5	22%

Note: 8 patients had two abnormal test results where one test result clarified the other. These tests are counted in both categories. CMA: chromosomal microarray, iCHD: isolated congenital heart disease, MCA: multiple congenital anomalies

Table 4.

Abnormal Genetic Testing Results

Study ID	iCHD/ MCA	Cardiac phenotype	Result	Interpretation
Chromosome analysis				
47*	MCA	HLHS+VSD ASD nos Possible aberrant RSCA	46,XX,der(1)t(1;4)(p36.3;q25)	Pathogenic
279*	MCA	CoA-VSD PM VSD AV thickened Mitral valve thick and redundant Pulmonary valve thickened Tricuspid valve thick and redundant Arch hypoplasia	46,XX,del(2)(q36.3q37.1)	Pathogenic
148*	MCA	Dilated AscAo Balanced CAVC PA-VSD (non-TOF) LSVC Sec ASD	46,XX,der(8)t(5;8)(p15.2;p23.1) pat	Pathogenic
384*	MCA	Inlet VSD R arch PS	47,XY,+8[8]/46,XY[12]	Pathogenic
331	MCA	Balanced CAVC PA-VSD (TOF anatomy) LSVC	47,XX,+13	Pathogenic (trisomy 13)
58	iCHD	Balanced complete AVSD CoA-VSD Sec ASD Distal transverse arch hypoplastic	47,XX,+21	Pathogenic (trisomy 21)
114	iCHD	Root dilation TOF Probably discontinuous Pas vs. severe proximal LPA stenosis	47,XY,+21	Pathogenic (trisomy 21)
248*	iCHD	Balanced CAVC CoA-VSD Hypoplastic arch	47,XY,+21	Pathogenic (trisomy 21)
277	MCA	LVDCAVC LSVC RV hypoplasia No RSVC Dysplastic AV valve leaflets	47,XY,+21	Pathogenic (trisomy 21)
295	iCHD	Root dilation TOF TV thickened with redundant chordae	47,XX,+21	Pathogenic (trisomy 21)
359	MCA	CAVC (LV dominant) RV hypoplasia Dysplastic pulmonary valve	46,XX,+21,der(21;21)(q10;q10)	Pathogenic (trisomy 21)
15*	iCHD	Type B IAA Aberrant SCA Conoventricular VSD AS BAV Sec ASD Sub AS	46,XYdel(22)(q11.2q11.2)	Pathogenic (22q11.2 deletion syndrome)
321	iCHD	BAV CoA-IVS LSVC	45,X	Pathogenic (Turner syndrome)

Study ID	iCHD/ MCA	Cardiac phenotype	Result	Interpretation
Chromosomal microarray analysis				
34	iCHD	CoA-VSD AS BAV PM VSD	arr[GRCh37] 22q11.21(18891398_21463730)x3	Pathogenic (22q11.2 deletion syndrome)
48	iCHD	PA-VSD (TOF) Discont PAs LV hypoplasia AP collaterals Midline abdominal aorta	arr[GRCh37] 22q11.21(17269490_19796715)x1	Pathogenic (22q11.2 deletion syndrome)
294	iCHD	Truncus Sec ASD Mildly thickened trileaflet truncal valve	arr[GRCh37] 22q11.21(18640300_21608479)x1	Pathogenic (22q11.2 deletion syndrome)
150	iCHD	CoA-VSD ASD RVDCAVC LV hypoplasia Sec ASD BAV Dysplastic AV LSVC	arr[GRCh36] Xp22.33q28(262_154899943)x1	Pathogenic (Turner syndrome)
248 *	iCHD	Complete balanced AVCD CoA-VSD Hypoplastic arch	arr[GRCh37] 21p11.2q22.3(10824040_48090629)x3	Pathogenic
47 *	MCA	HLHS+VSD ASD, nos Possible aberrant RSCA	arr[GRCh37] 4q25q35.2(109970465_190915650)x3	Pathogenic
279 *	MCA	CoA-VSD PM VSD Thickened AV Thick and redundant MV Thickened PV Thick and redundant TV Arch hypoplasia	arr[GRCh37] 2q36.3q37.1(229119155_234050398)x1	Pathogenic
384 *	MCA	Inlet VSD R arch PS	arr[GRCh37] 8p23.3q24.3(213–146,264,218)x2–3	Pathogenic
148 *	MCA	Complete balanced AVCD PA-VSD (non-TOF) LSCV Sec ASD	arr[GRCh36] 5p15.33p15.2(66648_8920419)x3, 8p23.3p23.1(213_11,898,254)x1	Pathogenic
144 *	MCA	TAPVR Sec ASD Mesocardia 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 *	MCA	Atrial isomerism Dextrocardia RVDCAVC LV hypoplasia	arr[GRCh37] 7p21.1p15.1(16974692_30970344)x2 hmz	VUS

Study ID	iCHD/ MCA	Cardiac phenotype	Result	Interpretation
		L-looped ventricle DORV (side by side with aorta leftward) SubPS TAPVR+RVOTO LSVC Common atrium		
137	iCHD	CoA-VSD BAV Parachute MV Musc VSD ASD, nos LSVC	arr[GRCh37] 20q13.33(59497040_62431738)x3	Pathogenic
61	MCA	Atrial isomerism L-looped ventricle PA-VSD (nonTOF) LSVC TAPVR+RVOTO LVDCAVC RV hypoplasia DORV (aorta left and anterior to PA) LV trabeculations	arr[GRCh37] 17p12(14101029_15449627)x1	Pathogenic (unrelated to cardiac phenotype)
401	iCHD	Root dilation DORV (TOF type) Musc VSD Dysplastic and redundant TV LV hypoplasia LSVC Sec ASD	arr[GRCh36] 5p15.33p15.31(66648_7175604)x1, 8p23.3p21.2(213_26130535)x3	Pathogenic
78	MCA	Tricuspid valve stenosis/hypoplasia RV hypoplasia d-TGA+RVOTO ASD, nos Musc VSD AS CoA-VSD Severe arch hypoplasia	arr[GRCh37] 5q23.2q34(123730483_167621784)x3	Pathogenic
393	MCA	PA-VSD (TOF) BAV R arch Sec ASD	arr[GRCh36] Xp22.33p22.11(262_22215611)x1, Xp22.11q28(22217004_154894859)x2, Y,22q11.1q11.21(14430822_18692668)x1	Pathogenic
30	iCHD	PS Dysplastic PV TS Thickened/dysplastic TV	arr[GRCh37] 5p13.1(38777383_39021044)x1	VUS
36	iCHD	LV hypoplasia AS CoA-IVS MS ASCA SubAS Hypoplastic arch	arr[GRCh37] 19p13.3(374160_1380367)x3	VUS
182	iCHD	AS Thickened AV leaflets CoA-IVS Arch hypoplasia	arr[GRCh37] 15q11.2(22652330_23272733)x1	VUS
207	iCHD	Conoventricular VSD AS CoA-VSD Sec ASD Arch hypoplasia	arr[GRCh37] 1q21.1q21.2 (146501348_147843733)x1	VUS

Study ID	iCHD/ MCA	Cardiac phenotype	Result	Interpretation
227	iCHD	DILV-L-malposition Sec ASD MA SubPS	arr[GRCh37] 16p11.2(29647342_30200975)x1	VUS
239	iCHD	Truncus	arr[GRCh37] 1q21.1q21.2(146089254_147826789)x1	VUS
278	iCHD	RV hypoplasia Common atrium TAPVR	arr[GRCh37] 1p36.32(2449711–4473263)x3	VUS
307	iCHD	CoA-IVS	arr[GRCh37] 15q13.3(29806023_30303141)x1	VUS
317	iCHD	CoA-IVS BAV AS Closely spaced mitral papillary muscles MS	arr[GRCh37] 7p15.3(21294396_23528927)x3	VUS
325	iCHD	Single ventricle, OS (no identifiable LV) RVDAVC DORV (side-by-side with aorta rightward) PS PV bicuspid and thickened Sec ASD R arch	arr[GRCh37] 17q21.31(44211338–44326245)x1–2	VUS
328	iCHD	HLHS ASD nos	arr[GRCh37] 6p22.1p21.33(27623511_30649134)x2 hmz,6p21.31p21.2(33864998_39723709)x2 hmz	VUS
346	iCHD	DILV, nos PS SubPS PV dysplastic	arr[GRCh37] 13q12.3(27886795_28398922)x3	VUS
64	MCA	MA LV hypoplasia AS CoA-VSD PV slightly thickened, mildly dysplastic TAPVR+LVOTO DORV (NRGV) Hypoplastic arch No discernible LV cavity	arr[GRCh37] 15q23(68815034_70018990)x1	VUS
230	MCA	LVDAVCD RV hypoplasia L-looped ventricle PA-VSD (nonTOF) R arch LSVC Common atrium Abdominal situs inversus with levocardia Anterior and leftward aorta Pulmonary venous return to confluence before entering common atrium	arr[GRCh37] 2q14.3q22.1(123225623_138447427)x2 hmz,8p21.3p12(19989194_32119175)x2 hmz,19p13.12q12(14893513_30050668)x2 hmz	VUS
FISH 22q11				
2	iCHD	Type B IAA Aberrant SCA Conoventricular VSD AS SubAS BAV ASD vs. PFO	ish del(22)(q11.2q11.2)(TUPLE1-)	Pathogenic (22q11.2 deletion syndrome)

Study ID	iCHD/ MCA	Cardiac phenotype	Result	Interpretation
15 *	iCHD	Type B IAA Aberrant SCA Conoventricular VSD AS BAV Sec ASD SubAS	ish del(22)(q11.2q11.2)(HIRA-)	Pathogenic (22q11.2 deletion syndrome)
256	iCHD	TOF Root dilation AscAo dilation STJ dilation Right arch Aberrant SCA AP collaterals	ish del(22)(q11.2q11.2)(TUPLE1-)	Pathogenic (22q11.2 deletion syndrome)
300	MCA	Root dilation STJ dilation TOF-APV Redundant TV Right arch	ish del(22)(q11.2q11.2)(TUPLE1-)	Pathogenic (22q11.2 deletion syndrome)
315	iCHD	Type B IAA Aberrant SCA BAV Conoventricular VSD SubAS AS	ish del(22)(q11.2q11.2)(TUPLE1-)	Pathogenic (22q11.2 deletion syndrome)
350	iCHD	Truncus Bicuspid truncal valve with thickened cusps	ish del(22)(q11.2q11.2)(TUPLE1-)	Pathogenic (22q11.2 deletion syndrome)
355	MCA	TOF	ish del(22)(q11.2q11.2)(TUPLE1-)	Pathogenic (22q11.2 deletion syndrome)
370	iCHD	Type B IAA Conoventricular VSD AS BAV PV thickened	ish del(22)(q11.2q11.2)(TUPLE1-)	Pathogenic (22q11.2 deletion syndrome)
374	iCHD	DORV (doubly committed) PS Right arch ASD nos	ish del(22)(q11.2q11.2)(TUPLE1-)	Pathogenic (22q11.2 deletion syndrome)
390	iCHD	TOF-APV R arch	ish del(22)(q11.2q11.2)(TUPLE1-)	Pathogenic (22q11.2 deletion syndrome)
Molecular analysis				
31	MCA	d-TGA-IVS+LVOTO PV bicuspid and dysplastic and prolapsing PS Sec ASD LSVC	*CHD7* sequencing	*CHD7* Pathogenic
139	MCA	Root dilation STJ dilation DORV (TOF-type) PS PV thickened, bicuspid SubPS Pfo vs. asd Likely aberrant RSCA	*CHD7* sequencing	*CHD7* Pathogenic
157	MCA	DORV-TGA type MS LV hypoplasia PS	*CHD7* sequencing	*CHD7* Pathogenic

Study ID	iCHD/ MCA	Cardiac phenotype	Result	Interpretation
		SubPS Sec ASD R arch Side-by-side great arteries		
163	MCA	PS D-TGA-VSD PM VSD PV bicuspid Sec ASD MS Ddeficient mitral anterioalateral papillary muscle and posterior leaflet) LSVC R arch Aberrant SCA TV mildly redundant	*CHD7* sequencing	*CHD7* Pathogenic
402	MCA	Type B IAA Conoventricular VSD SubAS AS Aberrant SCA Sec ASD Deficient mitral posteromedial papillary TV septal leaflet shortened/tethered	*CHD7* sequencing	*CHD7* Pathogenic
309	MCA	Dextrocardia TS RV hypoplasia d-TGA-VSD+RVOTO VSD nos LV trabeculations TAPVR+RVOTO LSVC Coronary anomaly (LAD off RCA off anterior facing sinus) ASD nos Arch hypoplasia	*CHD7* sequencing	*CHD7* VUS
90	iCHD	AS BAV AV dysplastic PS PV dysplastic AscAo dilation	Noonan panel	*PTPN11* Pathogenic
162	MCA	PS PV dysplastic SubAS AV dysplastic Musc VSD Outlet VSD	Noonan panel	*PTPN11* Pathogenic
106	MCA	AS Asymmetric septal hypertrophy Musc VSD ASD nos LSVC	Noonan panel	*KRAS* Pathogenic
284	iCHD	Balanced CAVC Parachute “mitral” valve variant DORV-TOF type	Noonan panel	*PTPN11* Pathogenic
403	MCA	Tri atresia-IVS PA-IVS ASD nos AV thickened	Noonan panel	*PTPN11* Likely-Pathogenic
386	iCHD	PS ASD NOS PV dysplastic, bicuspid	Noonan panel	*KRAS* VUS

Study ID	iCHD/ MCA	Cardiac phenotype	Result	Interpretation
357	iCHD	PA-IVS ASD nos TS RV hypoplasia AP collaterals	*JAG1* sequencing	*JAG1* Pathogenic
358	MCA	CoA-IVS BAV MS TV dysplastic Anterior mitral leaflet moves abnormally and hinges at its midpoint and papillary muscles closely spaced	*CREBBP* sequencing	*CREBBP* Pathogenic
144*	MCA	TAPVR Sec ASD Mesocardia RPA moderately hypoplastic	Heterotaxy panel	*CFC1* Pathogenic
379*	MCA	Atrial isomerism Dextrocardia RVDCAVC LV hypoplasia L-looped ventricle DORV (side by side with aorta leftward) SubPS TAPVR+RVOTO LSVC Common atrium	*DNAH11* sequencing	*DNAH11* Pathogenic
146	iCHD	CoA-VSD Conoventricular VSD AS SubAS BAV Sec ASD	Branchio-oto-renal panel	*EYA1* Pathogenic

* Multiple abnormal genetic tests.

AP: aortopulmonary, AS: aortic stenosis, AscAo: ascending aorta, AV: aortic valve, ASD: atrial septal defect, BAV: bicuspid aortic valve, CAVC: complete AV 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, LSVC: left superior vena cava, LV: left ventricular, LVDAVCD: left ventricular dominant complete AV canal defect, LVOTO: left ventricular outflow tract obstruction, MA: mitral atresia, MS: mitral stenosis, Musc: muscular, nos: not otherwise specified, os: otherwise specified, PA: pulmonary atresia, PM: primary muscular, PFO: patent foramen ovale, PS: pulmonary stenosis, RPA: right pulmonary artery , RV: right ventricular, RVOTO: right ventricular outflow tract obstruction; RVDCAVC: right ventricular dominate complete AV canal defect, SCA: subclavian artery, Sec ASD: secundom atrial septal defect, STJ; sino tubular junction; SubAS: subaortic stenosis, SVC: superior vena cava, TAPVR: total anomalous pulmonary venous return, TGA: transposition of the great arteries, TOF: tetralogy of fallot, TS: tricuspid stenosis, TV: tricuspid valve, VSD: ventricular septal defect

Table 5.

Frequency of abnormal genetic testing for different CHD types.

CHD type	No.	Patients with any abnormal genetic test (%)	No. of abnormalities by genetic test			
			Chromosome analysis	22q11 FISH	CMA	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
CTD	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, AVSD: atrioventricular septal defect, CHD: congenital heart disease, CMA: chromosome microarray CTD: conotruncal defect, LVOTO: left ventricular outflow tract obstruction, RVOTO: right ventricular outflow tract obstruction

Table 6.

Genetic testing yields for different CHD types.

CHD type	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]	0.6827
LVOTO	129	25 (19)	1.25 [0.62–2.53]	0.5341
CTD	88	16 (18)	1.02 [0.51–2.05]	0.9590
RVOTO	90	26 (29)	**3.42 [1.67–7.02]**	**0.0005**
Laterality	59	12 (20)	1.23 [0.58–2.61]	0.5883
Arteriopathy	33	8 (24)	1.57 [0.65–3.79]	0.3467
AVSD	25	7 (28)	1.93 [0.75–5.00]	0.1680
Aortopathy	20	4 (20)	1.15 [0.36–3.65]	0.7644 *
APVR	17	6 (35)	2.74 [0.95–7.92]	0.0538
Coronary	12	1 (8)	0.40 [0.05–3.18]	0.6985 *
Single ventricle	10	3 (30)	2.03 [0.50–8.21]	0.3917 *

* Fisher's exact test

Data excludes patients with 22q11.2 deletion (13), Down syndrome (7), trisomy 13 (1), or Turner syndrome (2).

APVR: anomalous pulmonary venous return, AVSD: atrioventricular septal defect, CHD: congenital heart disease, CI: confidence interval, CTD: conotruncal defect, LVOTO: left ventricular outflow tract obstruction, OR: odds ratio, RVOTO: right ventricular outflow tract obstruction

Table 7.

Genetic testing yields for the most frequent CHD lesions.

CHD lesion	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]	0.8917
ASD, nos	48	9 (19)	1.06 [0.47–2.42]	0.8815
Left SVC	45	10 (22)	1.40 [0.63–3.13]	0.4112
Aortic valve stenosis/hypoplasia	43	11 (26)	1.78 [0.81–3.93]	0.1752
CoA with VSD	41	7 (17)	0.92 [0.38–2.26]	0.8217
Pulmonary valve malformation, os	36	11 (31)	2.38 [1.06–5.37]	0.0325
Pulmonary valve stenosis/hypoplasia	34	11 (32)	2.62 [1.15–5.96]	0.0181
Muscular VSD	28	5 (18)	0.99 [0.35–2.78]	0.9811
CoA with IVS	27	5 (19)	1.04 [0.37–2.93]	0.9424
Mitral valve malformation, os	27	7 (26)	1.72 [0.67–4.39]	0.2540
RV hypoplasia	27	6 (22)	1.35 [0.51–3.60]	0.5442
Right aortic arch	26	5 (19)	1.09 [0.39–3.10]	0.8640
HLHS with IVS	23	1 (4)	0.19 [0.02–1.42]	0.0868 *
BAV	23	6 (26)	1.71 [0.63–4.66]	0.2876
LV hypoplasia	23	5 (22)	1.30 [0.45–3.74]	0.6611

* Fisher's exact test.

ASD: atrial septal defect, BAV: bicuspid aortic valve, CI: confidence interval, CoA: coarctation of the aorta, HLHS: hypoplastic left heart syndrome, IVS: intact ventricular septum, LV: left ventricular, nos: not otherwise specified, OR: odds ratio, os: otherwise specified, RV: right ventricular, SVC: superior vena cava, VSD: ventricular septal defect

Table 8.

Genetic testing yields for CHD types defined using a hierarchical classification method of CHD

CHD class	No. with genetic testing (N=222)	No. with abnormal genetic testing (%)	P value
CTD	66	11 (17)	0.7333
LVOTO	56	8 (14)	0.4007
RVOTO	27	5 (19)	0.9424
Laterality	24	3 (13)	0.5822 *
LVOTO + septal defect	18	4 (22)	0.7480 *
CTD + AVSD	9	4 (44)	0.0578 *
APVR	6	2 (33)	0.2955 *
AVSD	6	1 (17)	1 *
Other	3	0	1 *
SV	4	1 (25)	0.5510 *
RVOTO + septal defect	2	1 (50)	0.3286 *
Septal defect	1	0	1 *

* Fisher's exact

Data excludes patients who did not undergo genetic testing.

APVR: anomalous pulmonary venous return, AVSD: atrioventricular septal defect, CHD: congenital heart disease, CTD: conotruncal defect, LVOTO: left ventricular outflow tract obstruction, RVOTO: right ventricular outflow tract obstruction, SV: single ventricle

Table 9.

Genetic testing yields among patients with non-cardiac abnormalities.

Organ system	Total no. (%) (N=222)	No. with abnormal genetic testing (%)	OR [95% CI]	P value
All MCA	67 (30)	21 (31)	3.26 [1.61–6.61]	0.0007
Gastrointestinal	15 (7)	4 (27)	1.73 [0.52–5.73]	0.4829 *
Ribs/vertebrae	15 (7)	4 (27)	1.73 [0.52–5.73]	0.4829 *
Renal	13 (6)	5 (38)	3.11 [0.96–10.06]	0.0481
Hepatobiliary	13 (6)	3 (23)	1.39 [0.37–5.32]	0.7082 *
Spleen	13 (6)	5 (38)	3.11 [0.96–10.06]	0.0481
ENT	12 (5)	6 (50)	**5.18 [1.57–17.00]**	**0.0030**
Genitourinary	8 (4)	3 (38)	2.87 [0.66–12.54]	0.1577 *
Limb	8 (4)	3 (38)	2.87 [0.66–12.54]	0.1577 *
Brain	7 (3)	6 (86)	**31.9 [3.73–273.79]**	**0.0001** *
IUGR/SGA	13 (6)	6 (46)	**4.47 [1.41–14.14]**	**0.0061**

* Fisher's exact test

Data excludes patients with 22q11.2 deletion (13), Down syndrome (7), trisomy 13 (1), or Turner syndrome (2).

CI: confidence interval, ENT: ears, nose and throat, IUGR: intrauterine growth retardation, MCA: multiple congenital anomalies, OR: odds ratio, SGA: small for gestational age

Table 10.

Frequency of CHD types among patients who had geneticist examination.

CHD type	No. with geneticist examination (%)
All (N=222)	121 (55)
CTD (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)
CTD + 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)

APVR: anomalous pulmonary venous return, AVSD: atrioventricular septal defect, CHD: congenital heart disease, CTD: conotruncal defect, LVOTO: left ventricular outflow tract obstruction, RVOTO: right ventricular outflow tract obstruction, SV: single ventricle