I, Jessica Connor, hereby submit this original work as part of the requirements for the degree of Master of Science in Genetic Counseling.

It is entitled:
Chromosomal abnormalities identified in infants with congenital heart disease

Student's name: Jessica Connor

This work and its defense approved by:

Committee chair: Stephanie Ware, MD, PhD
Committee member: Robert Hinton, MD
Committee member: Erin Miller, MS, CGC
Committee member: Jennifer Ruschman, MS
Committee member: Kristen Sund, PhD
Chromosomal abnormalities identified in infants with congenital heart disease

A thesis submitted to the
Graduate School
of the University of Cincinnati
in partial fulfillment of the
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Master of Science
in the Program of Genetic Counseling
of the College of Medicine

by

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Committee Chair: Stephanie Ware, MD, PhD
Abstract

Background
Congenital heart disease (CHD) can occur as part of a genetic syndrome or as an isolated defect and genetic factors contribute to a majority of cases. Early diagnosis of syndromic CHD improves outcome but can be clinically challenging in the first year of life. Chromosome microarray analysis can identify causes of both syndromic and isolated CHD. The objectives of this study were to determine the diagnostic yield for chromosome microarray analysis and compare genetic testing practices among infants with CHD.

Methods and Results
A retrospective chart review was performed for infants with CHD identified by echocardiogram. CHD was classified using the National Birth Defects Prevention Study system, which takes into account complexity, CHD type, and extracardiac phenotype. Of 1087 infants with CHD, 277 (25%) had karyotype, FISH and/or chromosome microarray analysis. Of the 121 patients (11%) who had chromosome microarray analysis, genetic abnormalities were identified in 35 (29%) infants, including 16 isolated CHD and 19 non isolated CHD. Striking was the number of infants that received no genetic testing, and the inconsistent genetic testing practices. Infants with CHD do not receive consistent genetic testing, even though abnormalities were identified in infants with a variety of phenotypes.

Conclusions
The majority of infants with CHD do not undergo genetic testing, and only a small proportion receives chromosome microarray analysis. The frequency of abnormal chromosome microarray analysis results did not differ by CHD complexity or the presence of extracardiac malformations,
suggesting chromosome microarray analysis is warranted for first-line testing for infants with CHD. Chromosome microarray abnormalities of unknown significance present opportunities to identify novel causes of CHD and define disease etiology. Given the likelihood of an uncertain result, expertise is required for clinical interpretation and genetic counseling.
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List of Abbreviations

1. Congenital Heart Disease (CHD)
2. Fluorescent *in-situ* Hybridization (FISH)
3. Cincinnati Children’s Hospital Medical Center (CCHMC)
4. Comparative Genomic Hybridization (CGH)
5. Single Nucleotide Polymorphism (SNP)
6. Atrioventricular septal defect (AVSD)
7. Anomalous pulmonary venous return (APVR)
8. Left ventricular outflow tract obstruction (LVOTO)
9. Right ventricular outflow tract obstruction (RVOTO)
Introduction

Congenital heart disease (CHD) is the most common type of birth defect, affecting approximately 1 in 100 live births. CHD refers to any structural malformation of the heart that is present at birth. Importantly, the recurrence risk for isolated CHD ranges from 1-10 percent. Approximately 24% of CHD is thought to be caused by known chromosomal or single gene defects, and about 90% of the remainder of CHD is thought to be multifactorial with major genetic factors contributing to the phenotype. There is increasing evidence that genetic factors influence the development of most, if not all, CHD, but the genetic basis of nonsyndromic CHD is poorly understood.

Despite significant advances in the diagnosis and treatment of CHD, our understanding of the etiology of CHD remains incomplete. A recent scientific statement from the American Heart Association and American Academy of Pediatrics outlines several important reasons to determine an identifiable genetic cause for CHD: 1) to identify other organ systems involved; 2) to determine prognosis; 3) to inform families about recurrence risk; and 4) to identify at risk family members and provide necessary screening, including genetic testing. Elucidation of cause will help determine a developmental basis of CHD, and help to define disease risk as well as new treatments and interventions.

The two conventional methods for identifying large scale chromosomal abnormalities are G-banded karyotyping, which has a resolution of 3-5 Mb, and fluorescent in situ hybridization (FISH), which detects specific single gene deletions or duplications with a resolution of 150-200kb. In many patients with CHD, a genetic cause is not found after clinical examination, karyotyping, and FISH testing for known genetic disorders associated with
CHD. Chromosome microarray analysis can detect submicroscopic deletions or duplications are unable to be identified by karyotype or FISH.

Chromosome microarray analysis has been extensively studied and is routinely used in clinical settings to identify unbalanced chromosomal abnormalities associated with mental retardation. Chromosome microarray analysis has also been used to study unbalanced chromosomal abnormalities in patients with developmental delay, autism, dysmorphic features, and multiple congenital anomalies. A small number of studies have investigated the use of chromosome microarray analysis specifically in patients with CHD. Three studies have identified chromosome imbalances using chromosome microarray analysis in 17-25% of children with CHD and additional birth defects of unknown cause. One study identified chromosomal imbalances using chromosome microarray analysis in 17% of patients with isolated CHD that were not previously reported as common DNA copy number variants.

The purpose of this study was to assess current genetic testing practices in infants with CHD, and to more specifically determine the proportion of infants with CHD who have clinically significant cytogenetic abnormalities detected by chromosome microarray analysis. This study is the first to compare diagnostic yield based on CHD complexity or presence of extracardiac anomalies. Surprisingly, we determined that the frequency of abnormal chromosome microarray analysis results did not differ by CHD complexity or by the presence of extracardiac malformations, suggesting wide applicability for patients with cardiovascular malformations. Large multi-institutional studies are necessary to further refine testing recommendations and practice guidelines.
Methods

Study population

This is a single institution retrospective study. Infants under one year of age with CHD were included. Participants were identified by a local echocardiography database at the Cincinnati Children’s Hospital Medical Center (CCHMC) Heart Institute between January 1, 2008 and October 1, 2010. This study was approved by the CCHMC Institutional Review Board.

Genetic Testing

Cytogenetic tests completed during the study period were extracted for each infant with CHD. Cytogenetic testing included chromosome analysis by karyotype, fluorescence in-situ hybridization (FISH) analysis, and/or chromosome microarray analysis, including comparative genomic hybridization (CGH) microarray or single nucleotide polymorphism (SNP) microarray. Additional information was collected for infants who had both CHD and chromosome microarray analysis testing including indication for testing, subspecialty of ordering physician, platform type, reported results, type, size and locus of genetic aberration, and genes overlapping with the genetic aberration.

The significance of microarray results was determined by the CCHMC Clinical Cytogenetics Laboratory guidelines for reporting, which takes into account the size of the abnormality and the presence of genes in the abnormal region. Results are interpreted as clinically significant, unknown clinical significance, or no significant findings. In general, clinically significant deletions and duplications contained genes that are known to be associated with a genetic syndrome. Deletions and duplications greater than 500 kb and 200 kb, respectively that lacked identifiable genes were considered of unknown clinical significance.
Deletions and duplications that were less than 500 kb and 200 kb, respectively, were reported as no significant findings if they 1) did not contain genes known to be associated with a genetic syndrome, 2) contained 2 or fewer genes not known to be associated with a genetic condition, or 3) overlapped with multiple reported benign genetic changes.

**Classification of Congenital Heart Disease.**

CHD was defined using the classification system established by the National Birth Defects Prevention Study [18]. Eligible CHD included conotruncal defects, atrioventricular septal defects, anomalous pulmonary venous return, heterotaxy, left ventricular outflow obstructions, septal defects, and right ventricular outflow tract obstructions. Patients were excluded from the study if they had mild CHD (patent ductus arteriosus, patent foramen ovale), CHD that is poorly defined in infancy (bicuspid aortic valve, aortic dilation), or CHD that is primarily associated with the vascular system (right aortic arch, vascular rings). Rhythm abnormalities, cardiomyopathies and coronary abnormalities were also excluded.

Infants with CHD who had a chromosome microarray analysis were categorized by CHD complexity. Simple CHD was defined as one anatomically discrete heart defect or a well-recognized single defect (e.g. Tetralogy of Fallot), CHD Association was defined as common, uncomplicated combinations of heart defects (e.g. Tetralogy of Fallot and atrioventricular septal defect), and Complex CHD was defined as three or more heart defects other than septal defects, L-transposition of the great arteries, or a rudimentary single ventricle.

**Classification of Extracardiac Malformations**

Phenotypic data was obtained through retrospective chart review for each infant in the study who had chromosome microarray analysis testing performed. Subjects were classified as
having isolated CHD (without congenital malformations in other organ systems) or non-isolated CHD (with one or more congenital malformations in other organ systems). Physician-generated problem lists in patient electronic medical records, Human Genetics evaluations when present, and clinic notes from other subspecialties were reviewed to identify the presence of other anomalies. Anomalies were categorized into groups according to the organ system affected. Neither dysmorphic features nor failure to thrive were included in this study because of the age of patients and the lack of standard methods of reporting these features in medical records.

Statistical Analysis

Data analysis determined the proportion of patients with CHD who had various forms of cytogenetic testing, and focused on the significance of chromosome microarray results. Chi squared analysis was used to compare chromosome microarray results from the sub-cohort with CHD diagnosed in 2009 to all patients with a chromosome microarray analysis test in 2009, so that a full calendar year of testing data could be compared. Fisher Exact Testing was applied to identify differences in chromosome microarray analysis testing results based on heart defect complexity and also to compare patients with isolated heart defects to patients with multiple anomalies.
Results

Study population

CHD was identified in 1087 infants during the study period, and 277 (25%) had cytogenetic testing. Karyotype was performed on 212 (20%) patients, FISH testing on 136 (13%), and chromosome microarray analysis on 121 (11%). Patients frequently had more than one genetic test (Figure 1). Among infants with CHD who had chromosome microarray analysis testing, 39% also had karyotype, 13% also had FISH, and 24% had both karyotype and FISH studies in addition to chromosome microarray analysis. The frequency of chromosome microarray analysis during the study period for infants with CHD increased during each consecutive test year (Table 1). However, overall genetic testing decreased from 34% in the first year of the study to 20% in the last year.

Cytogenetic abnormalities

Clinically significant cytogenetic abnormalities were identified in 14% of patients undergoing genetic testing (40/277). Aneuploidy detected by karyotype accounted for 63% (25/40) of the abnormalities detected. Known microdeletion syndromes identified by FISH accounted for 25% (10/40) of the abnormalities. FISH abnormalities included 8 patients with 22q11 Deletion Syndrome (VCFS/DiGeorge Syndrome) and 2 with 7q11 deletion syndrome (Williams syndrome). In infants without aneuploidy or known deletion syndromes who had a karyotype, the yield for abnormal results was 0.5% (1/184) and the yield for abnormal chromosome microarray results was 3.4% (4/117) and accounted for 10% of all genetic abnormalities in patients undergoing genetic testing (4/40). Importantly, chromosome microarray analysis identified all aneuploidy and microdeletion syndromes (n=4) that were
identified through either karyotyping or FISH. Interestingly, 2.7% (5/184) of infants without aneuploidy had results of uncertain significance identified by karyotype, and those patients went on to have those results further characterized by chromosome microarray analysis. For example, one patient had a duplication on chromosome 17 of unknown clinical significance identified by karyotype and chromosome microarray analysis was performed to determine the breakpoints. The chromosome microarray revealed two abnormalities, a deletion at 17q24.2-q25.3 of clinical significance and a duplication of 22q13.32-q13.33 of unknown clinical significance, supporting chromosome microarray analysis as a tool to obtain more detailed information on duplications and deletions of various sizes.

No significant findings were identified in 71% (86/121) patients who had chromosome microarray analysis performed. Genetic abnormalities of uncertain clinical significance were identified in 22% (27/121) of patients who had chromosome microarray analysis performed. There were no statistically significant differences in the proportion of the three chromosome microarray significance groups when comparing infants with CHD to infants without CHD who had a chromosome microarray analysis in 2009 (X-squared =0.53, df=2, p-value=0.77). This finding suggests that there are no differences in chromosome microarray yield between patients with CHD and all other patients who have a chromosome microarray analysis CHD as valid an indication for chromosome microarray analysis as any other indication (intellectual disability, multiple congenital anomalies, and developmental delay).

CHD complexity

The majority of infants with CHD who had chromosome microarray analysis had simple CHD (Figure 2). The most common types of CHD were conotruncal defects and septal defects
Of patients with simple CHD, clinically significant microarray anomalies were identified in 7.5% (7/94) patients. One of these patients had 22q11 deletion syndrome, one had 7q11 deletion syndrome, one had Trisomy 21, and one had Trisomy 18. Results of unknown clinical significance were identified in 23.4% (22/94) of patients with simple CHD. Of patients with CHD associations, clinically significant microarray anomalies were identified in 4.8% (1/21). Results of unknown clinical significance were identified in 14.3% (3/21) of patients with CHD associations. Of patients with complex CHD, no clinically significant microarray anomalies were identified and results of unknown clinical significance were identified in 33.3% (2/6) of patients. A Fisher exact test showed that there was no statistical difference in the three chromosome microarray significance groups between the three CHD complexity groups (p=0.8117). There were no differences in the likelihood of abnormal results based on complexity of CHD.

Extracardiac phenotype

Isolated CHD occurred in 46% of the study cohort (N=55) while 56% (N=66) had CHD with additional congenital birth defects (non-isolated CHD). In infants with isolated CHD, chromosome microarray analysis found clinically significant chromosome aberrations in 3.6% (2/55) (one with Trisomy 21 and one with Williams Syndrome) and results of unknown clinical significance were identified in 26% (14/55). Clinically significant microarray abnormalities were identified by chromosome microarray analysis in three times as many infants with CHD and other congenital birth defects (9%, 6/66) compared to infants with isolated CHD, and aberrations of unknown clinical significance were identified in 20% (13/66). However, a Fisher exact test showed that there were no statistical differences in the frequency of the three
groups of chromosome microarray significance between infants with isolated CHD and infants with CHD and additional congenital birth defects (p=0.86).

Specialists who ordered chromosome microarray

In this study, 43% (52/121) of chromosome microarrays were ordered by cardiologists, 21% (25/121) were ordered by geneticists, and 17% (21/121) were ordered by neonatologists. Other subspecialties ordered the remaining 19.0% (23/121) of chromosome microarrays. Overall, geneticists had a higher number of patients with clinically significant abnormal results, but these were not statistically significant differences (Fisher exact test, p=0.2445). Cardiology was the only specialty who ordered chromosome microarray analysis for patients with complex CHD. The ordering frequencies for simple CHD and CHD association were similar among cardiologists, geneticists, and other specialists. Cardiologists tended to order chromosome microarray analysis more frequently for infants with isolated CHD while geneticists and other specialists ordered chromosome microarray analysis more frequently for patients with extracardiac birth defects in addition to CHD, however, these results were also not statistically significant (Fisher exact test, p=0.06682).

Description of variants of unknown clinical significance

Cytogenetic duplications and deletions of uncertain significance varied greatly in size. The largest abnormality of uncertain significance was a duplication of 25.88 Mb and the smallest abnormality was a 35.06 Kb deletion (Figure 4). At least one RefSeq gene, OMIM gene, or Decipher region of interest was detected within the deleted or duplicated region in 37% (10/27) of the cytogenetic abnormalities of uncertain clinical significance. Of those with follow up parental testing, 21% (3/14), cytogenetic abnormalities of unknown significance were de
novo mutations, 79% (11/14) were inherited from a parent. Parental studies were not completed in 48% (13/27) of patients. For the three infants where the imbalance was known to be de novo, two infants had loci that contained genes of interest (one had a RefSeq gene and one had three Decipher syndrome regions) and both infants had extracardiac malformations.
Discussion

In this study, we found that most infants with CHD are not having routine genetic testing, and only a small subset is having chromosome microarray analysis. The frequency of chromosome microarray analysis for infants with CHD increased over time, suggesting an increasing awareness in its utility. Chromosome microarray was able to identify all aneuploidies and microdeletion syndromes (22q11 Deletion Syndrome and Williams syndrome) detected using other genetic tests, further supporting the use of chromosome microarray analysis for infants with CHD as a first study when a specific genetic syndrome is not strongly suspected. Importantly, a large subset of infants with CHD had no significant chromosome microarray findings, ruling out genomic imbalances in these patients.

Of the infants with CHD who had genetic testing, 40 had clinically significant abnormal results. Microarray was the test used to detect 33 of these abnormal results; however, all 40 results could have been detected using microarray. There were 8 genetic abnormalities that would not have been identified by another genetic test. The 7 abnormalities detected using genetic testing other than microarray were defects associated with recognizable syndromes that were strongly suspected based on the patient’s phenotype.

This was the first study to our knowledge that assessed the use of chromosome microarray analysis in infants with CHD using the National Birth Defects Prevention Study classification system. The majority of infants with CHD who had chromosome microarray analysis did not have cytogenetic abnormalities identified, however, 7% infants had clinically significant results and 22% had results of uncertain clinical significance. This data is consistent with the detection rate for patients with intellectual disability, which ranges from 10-24% \(^6,8,11\).
Surprisingly there were no statistical differences in the yield of chromosome microarray analysis between infants with isolated CHD and CHD associated with extracardiac malformations, based on the spectrum of anomalies seen in this study. In addition, there was no difference in microarray yield among infants with CHD based on CHD complexity. Taken together, this suggests that chromosome microarray analysis testing for infants with CHD should not vary based on CHD complexity or the presence of extracardiac malformations. These results support a strategy to screen patients with isolated CHD for chromosomal imbalances using chromosome microarray analysis as proposed by Erdogan et al.\(^\text{17}\).

We found chromosome microarray analysis in infants with CHD identified a substantial number of infants with results of unknown clinical significance. The interpretation of significance of microarray results varies by lab in terms of how conservative they are with size cutoffs and presence of genes in the region. The identification of cytogenetic abnormalities of unknown clinical significance in those with both isolated and non-isolated CHD could allow insight into a possible genetic etiology in CHD. For example, two patients had deletions on 9p24.1 that contained the GLDC gene, which has not previously been implicated in CHD but could potentially play a role in heterotaxy. The potential importance of CHD candidate genes in regions of cytogenetic abnormalities of unknown clinical significance identified by chromosome microarray analysis may not be dependent on the size or number of genes in the aberration. This type of testing could be informative to researchers investigating genetic discovery (new causes) or complex inheritance (genetic modifiers) of CHD. Determining whether these regions contribute to the etiology of CHD would provide important information to convey to families during genetic counseling, including risk for other family members and recurrence risk in future
pregnancies. Providing more concrete information on the meaning of test results would strengthen genetic counseling for patients with chromosomal imbalances in these regions.

Cardiologists were the only specialists who ordered chromosome microarray analysis for patients with complex CHD. Geneticists and other specialists ordered chromosome microarray analysis more frequently for patients with non-isolated CHD. This data likely represents the biases regarding which specialties are the first to evaluate the infant (i.e. infants with CHD will be seen first by a cardiologist and infants with multiple congenital anomalies will be seen by geneticists), but it also may represent differences in ideas about which patients should have chromosome microarray analysis. Our study suggests that likelihood of finding abnormal results is similar regardless of type of CHD or additional abnormalities, therefore chromosome microarray analysis could be a screening test for all types of CHD.

Limitations of this study include the small sample size, especially for specific types of CHD and the presence of specific extracardiac anomalies. A larger sample size would allow future studies to perform statistical analysis using phenotype stratification. In addition, this was a single institution study, and the findings reported may not apply to all infants with CHD; however, the proportion of CHD types is consistent with epidemiology findings suggesting that our cohort is representative. Phenotypic data beyond CHD for infants that did not have genetic testing was not collected, so there may be differences between the infants with CHD that received genetic testing as compared to the group that did not receive testing. This does limit the applicability of the conclusions of this study to all infants with CHD identified through echocardiogram. In addition, some important extracardiac phenotypes could not be consistently obtained. For example, because the study population was restricted to infants,
information regarding developmental delay or dysmorphic features was limited, thus, the number of infants with non-isolated CHD may be underreported. The proportion of infants with CHD who had genetic testing may be underreported because these infants may have genetic testing in the future. This is especially true for the infants with CHD born at later dates in the study. The clinical significance of cytogenetic tests is continually being updated as new research uncovers novel disease causing genes, therefore, some of the variants of unknown significance may be reclassified as clinically significant after the time period of this retrospective chart review. Chromosome microarray analysis cannot detect all cytogenetic abnormalities. One type of chromosome anomaly that cannot be identified by microarray is a balanced translocation, though very few are disease causing, and a karyotype would need to be performed to rule these out. Point mutations in CHD genes would also not be detected using chromosome microarray analysis.

We propose that larger, multi-institutional studies be performed to examine utility of chromosome microarray analysis testing in complex or syndromic cases as well as simple CHD and isolated CHD. Chromosome microarray analysis in simple CHD and isolated CHD populations in particular could provide genetic targets for further studies related to cardiac development. Studies in older patients would be helpful in ensuring that the extracardiac phenotype of patients with CHD is properly characterized and that patients have sufficient time to have genetic testing. Future studies may also want to take into account genes identified in this study that are potentially associated with the genetic cause or a genetic predisposition to CHD. Understanding variants of uncertain significance is important to determine genetic cause of CHD and to provide information for genetic counseling for CHD. Cytogenetic abnormalities
of uncertain clinical significance in infants with CHD may contain potentially clinically significant
genes, and further research on these abnormalities may help to understand the importance of
these regions of uncertain significance. In addition, cost analysis regarding the use of
chromosome microarray as a first line genetic test would be important to determine whether
this testing strategy would be a good value for the cost.

In summary, most infants with CHD in this study do not have chromosome microarray
analysis, yet approximately 15% of identifiable clinically significant abnormalities will be missed
if this technology is not employed. In this study, chromosome microarray analysis detected all
abnormalities identified by chromosome analysis or FISH testing, indicating that it is the most
comprehensive approach. These data do not support limiting testing to specific CHD
populations based on complexity or extracardiac manifestations, and larger studies are needed
to refine yield in population subsets. Chromosome microarray analysis testing should be
considered as a first-line genetic test in those infants where a specific genetic diagnosis is not
strongly suspected based on phenotype.
Bibliography


### Tables and Figures

**Table 1:** Type of chromosome microarray analysis ordered by test year for patients who had CHD identified on echocardiogram between January 1, 2008 and October 1, 2010

<table>
<thead>
<tr>
<th>Microarray Type</th>
<th>All Years</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAC (constitutional)</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>BAC (expanded)</td>
<td>38</td>
<td>3</td>
<td>17</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>SNP</td>
<td>80</td>
<td>0</td>
<td>1</td>
<td>20</td>
<td>59</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>121</strong></td>
<td><strong>5</strong></td>
<td><strong>18</strong></td>
<td><strong>39</strong></td>
<td><strong>59</strong></td>
</tr>
</tbody>
</table>

**Table 2:** Frequency of types of CHD identified by echocardiogram between January 1, 2008 and October 1, 2010 in patients who also had chromosome microarray analysis

<table>
<thead>
<tr>
<th>Simple</th>
<th>Frequency</th>
<th>Percent</th>
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<tr>
<td>Conotruncal</td>
<td>23</td>
<td>19%</td>
</tr>
<tr>
<td>Attrioventricular septal defect (AVSD)</td>
<td>4</td>
<td>3.3%</td>
</tr>
<tr>
<td>Anomalous pulmonary venous return (APVR)</td>
<td>1</td>
<td>0.8%</td>
</tr>
<tr>
<td>Heterotaxy</td>
<td>17</td>
<td>14%</td>
</tr>
<tr>
<td>Left ventricular outflow tract obstruction (LVOTO)</td>
<td>15</td>
<td>12%</td>
</tr>
<tr>
<td>Septal</td>
<td>20</td>
<td>17%</td>
</tr>
<tr>
<td>Right ventricular outflow tract obstruction (RVOTO)</td>
<td>14</td>
<td>12%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>94</strong></td>
<td><strong>78%</strong></td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Association</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conotruncal, AVSD</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>APVR, AVSD</td>
<td>1</td>
<td>0.8%</td>
</tr>
<tr>
<td>Septal, LVOTO</td>
<td>2</td>
<td>1.7%</td>
</tr>
<tr>
<td>Septal, RVOTO</td>
<td>4</td>
<td>3.3%</td>
</tr>
<tr>
<td>Other Association</td>
<td>14</td>
<td>12%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>21</strong></td>
<td><strong>17%</strong></td>
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<table>
<thead>
<tr>
<th>Complex</th>
<th>Frequency</th>
<th>Percent</th>
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<tbody>
<tr>
<td>Left transposition of the great arteries</td>
<td>1</td>
<td>0.8%</td>
</tr>
<tr>
<td>Single Ventricle</td>
<td>4</td>
<td>3.3%</td>
</tr>
<tr>
<td>Multiple heart defects</td>
<td>1</td>
<td>0.8%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>6</strong></td>
<td><strong>5%</strong></td>
</tr>
</tbody>
</table>

| Grand Total                                 | **121**   | **100.00%** |
Table 3: Frequency of extracardiac malformations in study cohort

<table>
<thead>
<tr>
<th>Extracardiac Malformations</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal anomaly</td>
<td>19</td>
<td>16%</td>
</tr>
<tr>
<td>Eye anomaly</td>
<td>18</td>
<td>15%</td>
</tr>
<tr>
<td>Ear anomaly</td>
<td>14</td>
<td>12%</td>
</tr>
<tr>
<td>Hypotonia</td>
<td>13</td>
<td>11%</td>
</tr>
<tr>
<td>Cleft Lip/Palate</td>
<td>12</td>
<td>10%</td>
</tr>
<tr>
<td>Brain anomaly</td>
<td>11</td>
<td>9%</td>
</tr>
<tr>
<td>Genitourinary</td>
<td>10</td>
<td>8%</td>
</tr>
<tr>
<td>Skeletal</td>
<td>10</td>
<td>8%</td>
</tr>
<tr>
<td>Limb anomaly</td>
<td>8</td>
<td>7%</td>
</tr>
<tr>
<td>Seizures</td>
<td>4</td>
<td>3%</td>
</tr>
</tbody>
</table>

Table 4: Chromosome microarray findings by CHD complexity and presence of extracardiac features

<table>
<thead>
<tr>
<th></th>
<th>No significant Findings</th>
<th>Clinically Significant Findings</th>
<th>Unknown Clinical Significance</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td>Percent</td>
<td>Frequency</td>
<td>Percent</td>
</tr>
<tr>
<td>Simple</td>
<td>65</td>
<td>69%</td>
<td>7</td>
<td>7%</td>
</tr>
<tr>
<td>Isolated</td>
<td>29</td>
<td>69%</td>
<td>2</td>
<td>5%</td>
</tr>
<tr>
<td>Multiple Anomalies</td>
<td>36</td>
<td>69%</td>
<td>5</td>
<td>10%</td>
</tr>
<tr>
<td>Association</td>
<td>17</td>
<td>81%</td>
<td>1</td>
<td>5%</td>
</tr>
<tr>
<td>Isolated</td>
<td>8</td>
<td>89%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Multiple Anomalies</td>
<td>9</td>
<td>75%</td>
<td>1</td>
<td>8%</td>
</tr>
<tr>
<td>Complex</td>
<td>4</td>
<td>67%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Isolated</td>
<td>2</td>
<td>50%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Multiple Anomalies</td>
<td>2</td>
<td>100%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>86</strong></td>
<td><strong>71%</strong></td>
<td><strong>8</strong></td>
<td><strong>7%</strong></td>
</tr>
</tbody>
</table>
**Table 5:** Description of patients with clinically significant cytogenetic abnormalities

<table>
<thead>
<tr>
<th>Patient#</th>
<th>Final Result</th>
<th>Chromosome Locus</th>
<th>Size (Kb)</th>
<th>Inheritance</th>
<th>Select Genes</th>
<th>CHD Type</th>
<th>Other Anomalies</th>
</tr>
</thead>
<tbody>
<tr>
<td>027</td>
<td>Deletion</td>
<td>22q11.2</td>
<td>2732</td>
<td>Not Requested</td>
<td>Tbx1</td>
<td>VSD+ASD</td>
<td>hypotonia, Wilm’s tumor, speech apraxia</td>
</tr>
<tr>
<td>035</td>
<td>Deletion</td>
<td>2q32-2q34</td>
<td>23000</td>
<td>Not Requested</td>
<td></td>
<td>VSD-perimembranous</td>
<td>Pierre Robin Sequence, cleft palate</td>
</tr>
<tr>
<td>040</td>
<td>Trisomy</td>
<td>21</td>
<td>32482</td>
<td>Not Requested</td>
<td></td>
<td>AVSD</td>
<td>Down Syndrome</td>
</tr>
<tr>
<td>058</td>
<td>Deletion</td>
<td>7q11.23</td>
<td>1394</td>
<td>Not Requested</td>
<td></td>
<td></td>
<td>pulmonary valve stenosis, William’s syndrome</td>
</tr>
<tr>
<td>064</td>
<td>Tetrasomy</td>
<td>15q25.2-qter</td>
<td>17693</td>
<td>Not Requested</td>
<td>hapln, adams17, igf1r, mef2a</td>
<td>Pulmonary valve stenosis, ASD</td>
<td>cleft lip/palate</td>
</tr>
<tr>
<td>025</td>
<td>Deletion</td>
<td>17q24.2-q25.3</td>
<td>1474</td>
<td>de novo maternally inherited</td>
<td>Foxj1, DNAI2, Sox9</td>
<td>ASD-secundum</td>
<td>undescended testes, bilateral sensorineural hearing loss, craniosynostosis, astigmatism, dysphagia, platelet dysfunction</td>
</tr>
<tr>
<td></td>
<td>Duplication</td>
<td>22q13.32-q13.33</td>
<td>580</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>093</td>
<td>Deletion</td>
<td>6q23.3-24.3</td>
<td>11392</td>
<td>unknown paternally inherited</td>
<td>Coarctation of the Aorta</td>
<td></td>
<td>tracheomalacia, respiratory distress, sepsis syndrome</td>
</tr>
<tr>
<td></td>
<td>Duplication</td>
<td>2p21</td>
<td>1312</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>114</td>
<td>Trisomy</td>
<td>18</td>
<td>76116</td>
<td>Not Requested</td>
<td></td>
<td>VSD-perimembranous</td>
<td>imperforate anus, duplicated left kidney</td>
</tr>
</tbody>
</table>
**Table 6:** Description of patients with cytogenetic abnormalities of uncertain clinical significance.

<table>
<thead>
<tr>
<th>Patient#</th>
<th>Final Result</th>
<th>Chromosome Locus</th>
<th>Size (Kb)</th>
<th>Inheritance</th>
<th># of Genes</th>
<th>Select Genes</th>
<th>CHD Complexity</th>
<th>CHD Type</th>
<th>Other Anomalies</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>Duplication</td>
<td>2p13.1</td>
<td>645</td>
<td>maternally inherited</td>
<td>2</td>
<td>Simple</td>
<td>TOF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>85</td>
<td>Deletion</td>
<td>3q26.1</td>
<td>570</td>
<td>maternally inherited</td>
<td>2</td>
<td>Simple</td>
<td>TOF</td>
<td>agenesis of the corpus callosum, hypospadias, cryptorchidism, bilateral hearing loss, subglottic stenosis, micrognathia</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>Deletion</td>
<td>4q28.3</td>
<td>273</td>
<td>maternally inherited</td>
<td>8</td>
<td>Simple</td>
<td>HLHS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>Duplication</td>
<td>4q34.3-35.1</td>
<td>3167</td>
<td>de novo</td>
<td>14</td>
<td>Association</td>
<td>pulmonary valve stenosis, secundum ASD, dysplastic ears</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Duplication</td>
<td>5q14.1-q21.1</td>
<td>25880</td>
<td>de novo</td>
<td>11</td>
<td>MEF2C</td>
<td>Simple</td>
<td>pulmonary valve stenosis, dysplastic ears</td>
<td></td>
</tr>
<tr>
<td>79</td>
<td>Duplication</td>
<td>6q14.2,q14.3</td>
<td>690</td>
<td>paternally inherited</td>
<td>5</td>
<td>Riplly2</td>
<td>Simple</td>
<td>pulmonary atresia, plagiocephaly, strabismus, subglottic stenosis, tracheitis</td>
<td></td>
</tr>
<tr>
<td>98</td>
<td>Deletion</td>
<td>7p21.3</td>
<td>242</td>
<td>paternally inherited</td>
<td>7</td>
<td>THSD7A</td>
<td>Complex</td>
<td>Single Ventricle</td>
<td></td>
</tr>
<tr>
<td>54</td>
<td>Deletion</td>
<td>7q35</td>
<td>354</td>
<td>paternally inherited</td>
<td>1</td>
<td>Simple</td>
<td>TOF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>59</td>
<td>Duplication</td>
<td>8q24.22</td>
<td>700</td>
<td>Not Completed</td>
<td>1</td>
<td>Simple</td>
<td>VSD-perimembranous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>103</td>
<td>Deletion</td>
<td>9p24.1</td>
<td>65</td>
<td>maternally inherited</td>
<td>1</td>
<td>GLDC</td>
<td>Simple</td>
<td>HLHS</td>
<td></td>
</tr>
<tr>
<td>108</td>
<td>Deletion</td>
<td>9p24.1</td>
<td>35</td>
<td>Not Requested</td>
<td>1</td>
<td>GLDC</td>
<td>Simple</td>
<td>heterotaxy</td>
<td></td>
</tr>
<tr>
<td>68</td>
<td>Deletion</td>
<td>10q22.3</td>
<td>362</td>
<td>Not Completed</td>
<td>2</td>
<td>Association</td>
<td>Coarctation, AVSD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>Duplication</td>
<td>11q25</td>
<td>336</td>
<td>Not Completed</td>
<td>0</td>
<td>Simple</td>
<td>Coarctation of the Aorta, left eye ptosis, optic coloboma, hiatal hernia, hemifacial microsomia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>Deletion</td>
<td>15q13.1-13.2</td>
<td>1394</td>
<td>paternally inherited</td>
<td>6</td>
<td>Simple</td>
<td>HLHS</td>
<td>renal insufficiency, respiratory insufficiency</td>
<td></td>
</tr>
<tr>
<td>#</td>
<td>Type</td>
<td>Chromosome</td>
<td>Patient ID</td>
<td>Status</td>
<td>Gene Rich</td>
<td>Simple Disorder</td>
<td>Common Anomalies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----</td>
<td>----------</td>
<td>------------</td>
<td>------------</td>
<td>-------------------</td>
<td>-----------</td>
<td>----------------</td>
<td>--------------------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>82</td>
<td>Deletion</td>
<td>15q13.3</td>
<td>224</td>
<td>Not Completed</td>
<td></td>
<td>Simple</td>
<td>pulmonary valve stenosis, laryngotraceoesophageal cleft, bronchial atresia, pneumothorax, hemivertebrae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>Duplication</td>
<td>15q13.3</td>
<td>477</td>
<td>maternally inherited</td>
<td></td>
<td>Complex</td>
<td>I-TGA</td>
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<tr>
<td>39</td>
<td>Deletion</td>
<td>16p11.2</td>
<td>530</td>
<td>Not Completed</td>
<td>24</td>
<td>Gene Rich</td>
<td>DORV-other</td>
<td></td>
<td></td>
</tr>
<tr>
<td>73</td>
<td>Duplication</td>
<td>16p13.1</td>
<td>252</td>
<td>paternally inherited</td>
<td></td>
<td>Simple</td>
<td>ASD-secundum, renal agenesis, bilateral hearing loss, cleft palate, subglottic stenosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>Deletion</td>
<td>17q25.3</td>
<td>684</td>
<td>de novo</td>
<td>3</td>
<td>ID:2220</td>
<td>multiple VSD, coarctation, cryptorchidism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>53</td>
<td>Duplication</td>
<td>18q23</td>
<td>520</td>
<td>Not Completed</td>
<td>2</td>
<td>ZNF516</td>
<td>AVSD, hydronephrosis, astigmatism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>Deletion</td>
<td>20q13.33</td>
<td>60</td>
<td>paternally inherited</td>
<td></td>
<td>CHD4</td>
<td>VSD+ASD, focal cortical dysplasia, encephalopathy, pseudostrabismus, hyperopic astigmatism, otitis media</td>
<td></td>
<td></td>
</tr>
<tr>
<td>66</td>
<td>Deletion</td>
<td>22q11.21</td>
<td>402</td>
<td>Not Completed</td>
<td>2</td>
<td>MAPK</td>
<td>d-TGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>Deletion</td>
<td>22q11.21, q11.22</td>
<td>295</td>
<td>Not Completed</td>
<td>6</td>
<td>Simple</td>
<td>TOF, multiple anomalies noted, but the details are not documented.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Duplication</td>
<td>X22.2</td>
<td>227</td>
<td>Not Completed</td>
<td>0</td>
<td>Simple</td>
<td>pulmonary valve stenosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>119</td>
<td>Duplication</td>
<td>Xp22.31</td>
<td>1650</td>
<td>Not Completed</td>
<td>1</td>
<td>STS, ACSL4</td>
<td>pulmonary valve stenosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>Deletion</td>
<td>Xq28</td>
<td>880</td>
<td>Not Completed</td>
<td>3</td>
<td>Simple</td>
<td>heterotaxy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Deletion</td>
<td>Yq12qter</td>
<td>451</td>
<td>Not Completed</td>
<td>2</td>
<td>Simple</td>
<td>HLHS, duplication of left renal collecting system, anterior eye anomaly</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1:

Figure 2:
Figure 4:
Data Extraction Form

Demographics

Gender: male female Date of birth: __/__/____ MRN: _______

Ethnicity: White African American Native American Hispanic Asian Other: ___________________________ Unknown

Abnormal Echocardiograms

Date of echo: __/__/____

Person Requesting Test: ______________

Abnormal Obstetric/Fetal Diagnosis? no yes Explain _______________________

Was the infant cyanotic? yes no

Heart Defect Classification (CHECK ONE PER TEIR)

☐ Simple Cardiac Malformation
  ☐ Conotruncal
  ☐ Truncus Arteriosus
  ☐ Interrupted Aortic Arch
  ☐ Ventricular septal defect reported as conoventricular, malalignment type, or subaortic (not otherwise specified)
  ☐ D-transposition of the great arteries (d-TGA)
  ☐ Tetralogy of Fallot
  ☐ Double outlet right ventricle – other
  ☐ Double outlet right ventricle – TGA type
  ☐ Atrioventricular Septal Defect (AVSD)
    ☐ AVSD
  ☐ Anomalous Pulmonary Venous Return
    ☐ Anomalous Pulmonary Venous Return
  ☐ Heterotaxy
    ☐ Heterotaxy
  ☐ Left Ventricular Outflow Tract Obstruction (LVOTO)
    ☐ Hypoplastic Left Heart Syndrome (HLHS)
    ☐ IAA, A
    ☐ AS
    ☐ Coarctation
  ☐ Septal
    ☐ Ventricular septal defect, perimembranous
    ☐ Ventricular septal defect, muscular
    ☐ Ventricular septal defect, os/nos
    ☐ Atrial Septal Defect, secundum
    ☐ Atrial Septal Defect, os/nos
- VSD, multiple combination of perimembranous, muscular, or not otherwise specified types of ventricular septal defects
- VSD+ASD
- Right Ventricular Outflow Tract Obstruction
  - Pulmonary Atresia
  - Ebstein Anomaly
  - Tricuspid Atresia
  - Pulmonary Valve Stenosis

- **Cardiac association**
  - Conotruncal 1 AVSD
  - APVR 1 AVSD
  - Septal 1 LVOTO
  - Septal 1 RVOTO
  - Other Associations Two (occasionally three) major defects not specified elsewhere

- **Complex cardiac**
  - L-TGA
  - Single ventricle/complex
    - Multiple, complex heart anomaly (three or more defects in addition to simple ASD, VSD)
    - Single ventricle

Was surgical intervention required?
- Neonatal (0-1 month)
- Childhood
- Never

Seen by genetics?
- Was Seen
- Will be seen
- Never Seen

**Chromosome Testing**
Type: High Resolution Routine Other: 
Result: Normal Abnormal
List result: 

**FISH Testing**
Type: 
List result: 
SNP Microarray Testing

Date of microarray testing: Month:   Year:

Referring Physician: _________________

Physician Specialty: _________________

Indication for microarray testing: _______________

Platform used:
- constitutional
- expanded
- SNP

Significance
- Clinically Significant
- Unknown Clinical Significance

Final Result:
- No significant findings
- Signif: copy neutral LOH
- Previously known abnormality, no imbalance found
- Trisomy
- Duplication
- Multiple abnormalities
- Mosaic
- Previously known abnormality, imbalance found
- Tetrasomy
- Equivocal
- Deletion
- Sex chromosome abnormality
- Failure

Chromosome locus: _________________

Size: ____________

Genes involved: _________________

Parental chromosomes requested: yes no
- Completed
- Partially completed
- Not completed
Other Anomalies (only include if significant)

- Hypotonia
- Seizures
- Cleft lip/ palate
- Genitourinary: ________________
- Ear anomaly: ________________
- Renal anomaly: ________________
- Brain anomaly: ________________
- Limb anomaly: ________________
- Eye anomaly: ________________
- Seizures
- Dysmorphic features
- Developmental delay
- Failure to thrive
- Multiple congenital anomalies
  - List anomalies:
    - ___________________________________________________________________
    - ___________________________________________________________________
    - ___________________________________________________________________
  - Other: ________________

Diagnosed Syndrome: ________________

Other specialists seen:

- Aerodigestive
- Audiology
- DDBP
- ENT (Otolaryngology)
- Gastro
- Nephrology
- Neurology
- Neurosurgery
- Ophthalmology
- Pulmonary Medicine
- Other: ________________