Yield of Genetic Testing in Congenital Heart Disease

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Abstract:

Congenital heart disease(CHD) is the most common birth defect in the United States. In 25-40% of cases CHD is associated with a known genetic syndrome, and among children with chromosomal abnormalities approximately 30% will have CHD. As genetic testing options continue to evolve and become more available, reassessing the frequency of clinically relevant results is important to optimize genetic care for individuals with complex CHD as well as to identify targets for further research.

409 charts of patients with CHD who underwent surgical intervention prior to one year of age at Advocate Hope Children's Hospital in Oak Lawn, IL from January 2010 to May 2013 were retrospectively reviewed for specific CHD lesion, genetic testing, and results of genetic testing. There were 28 unique CHD lesions identified. Of 257 G-Banded Karyotypes 27(10.5%) were abnormal. Of 241 fluorescence in situ hybridization (FISH) probes for 22q11.2 deletion 17(7.1%) were positive. Of 103 SNP microarrays 34(33%) were abnormal.

The most commonly encountered genetic condition within the sample was Down syndrome (51, 12.5%) followed by DiGeorge syndrome (19, 4.6%). DiGeorge Syndrome was most commonly identified in patients with Interrupted Aortic Arch, Truncus Arteriosus and Pulmonary Atresia with Ventricular Septal Defect. None of the 43 patients with Tetralogy of Fallot in the sample had DiGeorge syndrome, which was found to be statistically significant when compared to the rate of positive FISH for 22q11.2 deletion in other conotruncal lesions with a p value of 0.0176.

Atrioventicular septal defects not associated with Down Syndrome were the lowest yield for abnormal microarray results. When compared to the rate of abnormal microarrays in other CHD lesions this was not found to be statistically significant, with a p value of 0.0501, but was a trend worth noting. Septation defects have multiple causal single gene defects identified, further reinforcing the utility of retrospective reviews of this kind to identify potential high-yield targets for further research for new single gene disorders. Even within this relatively small sample size, two distinct microarray abnormalities not currently known to be associated with CHD were noted multiple times, perhaps suggesting these two findings would be of value to review more in depth for potential association with CHD.

Introduction:

- In twenty-five to forty percent of cases congenital heart disease is associated with a known genetic syndrome. (1)
- Previous studies of abnormal microarray (also known as comparative genomic hybridization) frequency in congenital heart disease have found up to twenty two percent of patients have a significant chromosomal anomaly. (2)
- Multiple genetic conditions identified by microarray are known to have a high association with congenital heart disease. DiGeorge syndrome (also known as 22q11.2 Deletion Syndrome) has been found in up to eight percent of patients with congenital heart disease. (2)
- Previous reviews of frequency of positive FISH probe for 22q11.2 deletion in congenital heart disease have found a rate of five percent positive results. (3)
- Specific groups of congenital heart lesions, particularly Left Ventricular Outflow Tract Obstructive (LVOTO) lesions, have been identified as a genetically related group based on identified evidence of genetic heterogeneity with multiple potential loci. (4) In that study LVOTO lesions were classified as bicuspid aortic valve, aortic valve stenosis, coarctation of the aorta, and hypoplastic left heart. (4)
- For atrioventricular septal defect multiple single gene causes have been identified, for example, CRELD1, GJA1, GATA4, and ALK2. (1,4) Identification of single gene defects allows better understanding of the formation of the heart, which allow for identification of biological pathways critical to cardiac formation. (4)
- Single gene mutations are being identified with success by using exome sequencing.(5) Exome sequencing, or sequencing of the entirety of the protein-coding portion of the genome, is becoming increasingly available.

Methods:

This study was reviewed by the Advocate Institutional Review Board and approved for informed consent waiver and exemption status. Charts of patients under one year of age who were admitted to the pediatric surgical heart unit at Advocate Hope Children's Hospital after January 1, 2010 for surgical cardiac intervention were retrospectively reviewed. Electronic medical record was reviewed for all recorded genetic testing with notation of G-Band Karyotype, FISH probe for 22q11.2 deletion, and SNP Microarray results. Initial operative reports were reviewed for operative diagnosis and classification of congenital heart lesion.

Descriptive statistics were used to identify the proportion of specific congenital heart lesions represented within the sample and the frequency of abnormal genetic test results in proportion to tests completed. As appropriate, Fisher's exact test analyses were utilized to compare the frequency of abnormal genetic test results between groups congenital heart disease. A significance level of $p<.05$ was used to evaluate all comparisons.

Characteristic	N	%
Total Patients	409	100%
G-Banded Karyotypes	257	62.8%
FISH for 22q11.2 Deletion	241	58.9%
SNP Microarray	103	25.2%
Patients where Microarray not Indicated	74	18%
Female (Including 45X, 47XXX)	178	43.5%
Male	231	56.5%

Table 1. Sample Characteristics

Congenital Heart Disease	n	Positive
Coarctation of the Aorta	15	1 (6.7%)
Double Outlet Right Ventricle	13	1 (7.7%)
Interrupted Aortic Arch	14	7 (50%)
Pulmonary Atresia with Ventricular Septal Defect	12	4 (33.3%)
Tetralogy of Fallot	20	0
Truncus Arteriosus	12	3 (25%)*
Ventricular Septal Defect	9	1 (11.1%)
Conotruncal Defects	86	15 (17.4%)
LVOTO Lesions	71	1 (1.4%)
Total	241	17 (7.1%)
*(+2 with 22q11.2 deletion on SNP microarray 5/14=36%)		

Table 3. CHD with positive FISH Probe for 22q11.2 deletion and percentage of positive results.

Congenital Heart Disease:	N	%			
Anomalous Pulmonary Artery from Ascending Aorta	1	0.2%	Hypoplastic Right Heart	1	0.2%
Aortic Stenosis	3	0.7%	Interrupted Aortic Arch	16	3.9%
Atrial Septal Defect	3	0.7%	Patent Ductus Arteriosus	2	0.5%
Atrioventricular Canal Spectrum Lesions	52	13%	Pericardial Teratoma	1	0.2%
Cardiomyopathy	1	0.2%	Pulmonary Atresia	11	2.7%
Coarctation of the Aorta	28	6.9%	Pulmonary Atresia with Ventricular Septal Defect	15	3.7%
Congenitally Corrected Transposition of the Great Vessels	4	0.9%	Pulmonary Stenosis	10	2.4%
Cor Triatriatum	1	0.2%	Shone's Complex	6	1.5%
Double Inlet Left Ventricle	7	1.7%	Supravalvar Aortic/Pulmonary Stenosis	3	0.7%
Double Outlet Right Ventricle	18	4.4%	Tetralogy of Fallot	43	10.5%
D-Transposition of the Great Vessels	13	3.2%	Total Anomalous Pulmonary Venous Return	16	3.9%
Ebstein Anomaly	2	0.5%	Tricuspid Atresia	19	4.6%
Heterotaxy Syndrome	10	2.4%	Truncus Arteriosus	15	3.7%
Hypoplastic Left Heart	62	15.2%	Ventricular Septal Defect	46	11.3%

Table 2. Congenital Heart Diseases observed in 409 patients who underwent cardiac surgical intervention at less than one year of age.

Results:

Review of 409 charts yielded records of 257 G-banded karyotypes, 241 FISH probes for 22q11.2 deletion, and 103 SNP microarrays. Please see **Table 1** for sample characteristics. In the sample 231 patients were male, 176 patients were female, one patient was 45X, and one patient was 47 XXX. Of the 409 patients there were twenty-eight unique congenital heart lesions identified as illustrated in **Table 2**. Two groups of lesions were evaluated in addition to specific lesions, a conotruncal defect group and a LVOTO lesion group. Lesions categorized within the conotruncal defect group were congenitally corrected transposition of the great vessels, double outlet right ventricle, D-transposition of the great vessels, interrupted aortic arch, pulmonary atresia with ventricular septal defect, tetralogy of fallot, and truncus arteriosus. Lesions placed within the LVOTO lesion group were aortic stenosis, coarctation of the aorta, and hypoplastic left heart.

Of the 257 G-banded karyotypes there were twenty-seven abnormal results. Of the twenty-seven abnormal results seventeen were trisomy 21.

Of the 241 FISH probes for 22q11.2 deletion there were seventeen abnormal results. Please see **Table 3** for the lesions with abnormal findings. There were a total of nineteen patients identified with DiGeorge syndrome with two additional patients identified based of SNP microarray results of 22q11.2 deletion.

Of the 103 SNP microarrays there were thirty-four abnormal results. Please see **Table 4** for the lesions with abnormal findings. Specific abnormal results are detailed in **Table 5**. Atrioventricular septal defects not associated with Down Syndrome were particularly low-yield for abnormal microarray results. While this was not statistically significant, with a p value of 0.0501, it was a trend worth noting.

Congenital Heart Disease	n	Abnormal
Aortic Stenosis	1	1 (100%)
Coarctation of the Aorta	5	1 (20%)
Double Outlet Right Ventricle	4	2 (50%)
D-Transposition of the Great Vessels	5	2 (40%)
Ebstein's Anomaly	2	2 (100%)
Heterotaxy Syndrome	4	1 (25%)
Hypoplastic Left Heart	19	4 (21.1%)
Interrupted Aortic Arch	7	2 (28.6%)
Pulmonary Atresia with Ventricular Septal Defect	8	6 (75%)
Pulmonary Stenosis	1	1 (100%)
Shone's Complex	1	1 (100%)
Tetralogy of Fallot	10	4 (40%)
Total Anomalous Pulmonary Venous Return	5	3 (60%)
Truncus Arteriosus	7	1 (14.3%)
Ventricular Septal Defect	5	3 (60%)
Conotuncal Defects	42	17 (40.4%)
LVOTO Lesions	25	6 (24%)
Total	103	34 (33%)

Table 4. CHD with abnormal SNP Microarrays and percentage of abnormal results.

References:

1. Richards AA, Garg V. Genetics of Congenital Heart Disease. Current Cardiology Reviews. 2010; 6:91-97.
2. Lu XY, Phung MT, Shaw CA, et al. Genomic Imbalances in Neonates with Birth Defects: High Detection Rates by Using Chromosomal Microarray Analysis. Pediatrics. 2008; 122(6):1310-1318.
3. Baker K, Sanchez-de-Toledo J, Munoz R, et al. Critical Congenital Heart Disease- Utility of Routine Screening for Chromosomal and other Extracardiac Malformations. Congenital Heart Disease. 2012; 7(2):145-150
4. Ware SM, Jefferies JL. New Genetic Insights into Congenital Heart Disease. Journal of Clinical & Experimental Cardiology. 2012;S8:003
5. Ng SB, Buckingham KJ, Lee C, et al. Exome Sequencing Identifies the Cause of a Mendelian Disorder. Nature Genetics. 2010; 42(1):30-35.

Results for Down Syndrome and 22q11.2 Deletion

Down Syndrome: Down syndrome was the most commonly encountered genetic abnormality in the sample group. Among the 409 patients 12.5%(51) had Down syndrome. In addition one patient with coarctation of the aorta was noted to be mosaic for trisomy 21. Among patients with Down syndrome, thirty-nine had atrioventricular septal defects, seven had ventricular septal defects, two had tetralogy of fallot, one had a patent ductus arteriosus, one had pulmonary atresia with ventricular septal defect, and one had truncus arteriosus.

DiGeorge Syndrome(22q11.2 Deletion Syndrome, Velocardiofacial Syndrome): DiGeorge Syndrome is a the most common microdeletion syndrome associated with congenital heart disease, and the second most common genetic anomaly identified in the patient sample. Seventy-five percent of patients with DiGeorge have congenital heart disease. (2) Among the 409 patients 4.6% (19) of the patients had identified DiGeorge syndrome. DiGeorge syndrome is classically associated with conotruncal congenital heart disease, specifically tetralogy of fallot, interrupted aortic arch, and truncus arteriousus.(2) As illustrated in **Table 3** our data had the highest yield in interrupted aortic arch, truncus arteriousus, and pulmonary atresia with ventricular septal defect. Of the twenty FISH probes for 22q11.2 deletion performed on patients with tetralogy of fallot there were no positive results. When comparing the twenty negative results in patients with tetralogy of fallot to the fifteen positive in the other lesions comprising the conotruncal lesion group this was found to be statistically significant with a p value of 0.0176. None of the forty-three patients with tetralogy of fallot within the study had DiGeorge syndrome by history or available testing results.

Abnormal Microarray Results*	Congenital Heart Disease
15q25.3 Dup (KCS)	Aortic Stenosis
Mosaic Trisomy 21(KCS,ACHD) , 8q24.21 Dup (UKS,PI)	Coarctation of the Aorta
Xq21.31 Del (UCS)	Double Outlet Right Ventricle
4q16.3 Del (KCS,ACHD), 17q25.1-q25.3 Dup (KCS)	Double Outlet Right Ventricle
2q23.1 Del (KCS)	D-Transposition of the Great Vessels
5q23.1 Del (UCS)	D-Transposition of the Great Vessels
22q11.21 Dup (KCS,ACHD)	Ebstein's Anomaly
Increased Homozygosity (KCS)	Ebstein's Anomaly
15q13.2-13.3 Dup (KCS)	Heterotaxy Syndrome
2q23.3 Dup (UCS,PI)	Hypoplastic Left Heart
2p16.3 Del (KCS,PI) 12q13.33 Dup (UCS,PI)	Hypoplastic Left Heart
5q23.1 Del (UCS), 8p23.1 Dup (KCS,ACHD)	Hypoplastic Left Heart
9p22.2 Del (UCS)	Hypoplastic Left Heart
Increased Homozygosity (KCS)	Hypoplastic Left Heart
Xp22.33-22.2 Dup (KCS), Yq11.222-11.23 Del (UCS), 7q33 Dup (UCS)	Interrupted Aortic Arch
22q11.21 Dup (KCS,ACHD)	Pulmonary Atresia with Ventricular Septal Defect
20p12.1-q11.21 Dup (KCS,ACHD)	Pulmonary Atresia with Ventricular Septal Defect
Increased Homozygosity (KCS)	Pulmonary Atresia with Ventricular Septal Defect
15q13.2-q13.3 Dup (KCS)	Pulmonary Stenosis
2q13 Del (CP), 5q15.33 Dup (UCS)	Shone's Complex
16q11.2 Del (KCS)	Tetralogy of Fallot
20q11.13-13.2 Del (KCS)	Tetralogy of Fallot
Mosaic Trisomy 15 (KCS,ACHD)	Tetralogy of Fallot
22q11.2 Dup (KCS,ACHD)	Tetralogy of Fallot
15q11.2 Del (KCS), 5p15.2 Dup (UCS,ACHD)	Total Anomalous Pulmonary Venous Return
10q11.21 Trip (UCS), 16q23.1-q24.3 UPD (KCS,ACHD)	Total Anomalous Pulmonary Venous Return
47 XXX (KCS)	Total Anomalous Pulmonary Venous Return
13q12.3 Dup (UCS,PI)	Truncus Arteriosus
16p13.11 Del (UCS)	Ventricular Septal Defect
7 Del/Dup, 3 Dup (Results from history, Microarray Report Unavailable)	Ventricular Septal Defect
CP=Common Polymorphism, UCS=Unknown Clinical Significance, KCS=Known Clinical Significance, PI=Parentally Inherited, ACHD=Associated with Congenital Heart Disease	*Four microarrays with findings of 22q11.2 deletion were omitted from this table

Table 5. Abnormal SNP Microarray results with notation of clinical significance as reported with the findings, and associated congenital heart disease. Four abnormal microarray results with the findings of 22q11.2 deletion were omitted from this table for simplicity. Please see **Table 3** for all lesions associated with a 22q11.2 deletion

Conclusions:

Further evaluation of microarray testing yield by specific congenital heart disease is necessary to identify lesions with highest rates of normal microarray, as these lesions may be best suited for the focus of exome sequencing studies for new single gene disorders.

Atrioventricular septal defects not associated with Down Syndrome were particularly low yield for abnormal genetic testing in this sample, suggesting these patients may be a suitable target for more in-depth study for single gene defects. As atrioventricular septal defects have multiple associated single gene defects identified, this further reinforces the utility of retrospective reviews of this kind to identify potential high-yield targets for further research.

Also, this review showed two microarray results (5q23.1 Deletion, 15q13.2-13.3 Duplication) not known to be associated with congenital heart disease multiple times within our sample. Given the small sample size no direct association can be drawn from these results, but it is an interesting observation.