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Cytogenetic and microarray analysis of prenatally detected congenital heart defects (CHD): Diagnostic findings and variation among CHD subtype

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Introduction

Congenital heart defects (CHD) are common abnormalities seen in approximately 9-10 of every 1,000 babies born. It comprises several different subtypes and can be classified from simple to complex. While most can be diagnosed prenatally, and some associations with cytogenetic abnormalities have been reported, most studies evaluating chromosome and microarray analysis for CHD lack sufficient numbers to make satisfactory correlations. During the past 12 years, over 13,000 prenatal patients referred because of prenatal ultrasound findings, indicating a concern for heart defects, were studied with a single nucleotide polymorphism (SNP) microarray and/or chromosome analysis. This large number of patients has provided a unique understanding of large visible chromosome abnormalities and smaller copy number variants in this group in general, and for many of the specific subclassifications of heart defects. This study not only indicates the increased diagnostic yield by microarray analysis but provides a better understanding of the genes involved in heart defects.

Methods

We report on the analysis of over 13,000 prenatal patients ascertained with heart defects utilizing both standard chromosome analysis and/or a SNP microarray. These specimens include at least 15 different subclassifications of different congenital heart defects **(Table 1)**. Chromosome studies were done using standard analysis. All microarray studies were done utilizing the Affymetrix® Cytoscan® HD array (Affymetrix® and CytoScan® HD are Registered Trademarks of ThermoFisher Scientific). This array contains approximately 2.695 million markers across the entire human genome. There are approximately 743,000 SNPs and 1,953,000 non-polymorphic probes (NPCNs).

Results

This study looks at the frequency of pathogenic abnormalities detected by both chromosomal and microarray analysis in prenatal patients with CHD. It has broad specific implications regarding the types of genes and aberrations involved in the different subclassifications of CHD.

- Over 10,000 patients with an isolated defect were studied and the frequency of chromosome aberrations was ~18.5%. There were additional array findings in 6.3% of patients **(Table 1)**. Both numbers were elevated if there were additional major extracardiac abnormalities, reaching as high as ~38% with chromosome abnormalities and 9.4% with additional pathogenic abnormalities detected by array analysis **(Table 2)**.
- There was a wide variation in the diagnostic yield detected by standard chromosome analysis in CHD with a structural defect (as an isolated defect) ranging from 4.3% in transposition of great arteries to 70.2% in double-outlet right ventricle (DORV) **(Table 1)**.
- The microarray studies showed an increase in detectable aberrations (not seen by chromosome analysis), in this group, as high as 15.9% in tetralogy of Fallot (TOF) and 19.2% in pregnancies with truncus arteriosus **(Table 1)**.
- An increased frequency of consanguinity was detected in several CHD subtypes (atrioventricular septal defect – 5.9%; aortic stenosis – 5.3%) and in cases of TOF with an additional extracardiac major abnormality (6.3%).
- In patients with an isolated simple heart defect, the frequency of chromosome aberrations was ~19.2% and there were additional array findings in 5.3% of patients. However, both numbers were elevated if there were additional major extracardiac abnormalities, reaching as high as ~31% of referrals **(Table 3)**.
- In patients with an isolated conotruncal heart defect, the frequency of chromosome aberrations was ~11.3% and there were additional array findings in 13.9% of patients. However, both numbers were elevated if there were additional major extracardiac abnormalities, reaching as high as almost half of referrals **(Table 4)**.
- While 22q deletions (including *TBX1*) were common in conotruncal defects, these only accounted for 32.4% of the abnormalities detected by microarray analysis in isolated heart defects and only 15.2% if there were additional major extracardiac abnormalities.
- In addition to the deletion of *TBX1* (seen in 172 patients with isolated heart defects), the analysis has revealed an additional 23 different genes in 92 patients as responsible for the heart defect. The most common genes involved outside of *TBX1* include *ETS1*, *GATA4* and *MSX1* **(Figure 1)**. The most common involved subtypes were DORV, hypoplastic left heart syndrome and ventricular septal defect.
- Variable microdeletions associated with neurodevelopmental disorders were identified in about 1-2% of these patients, which has significance for additional family members.
- Variants of uncertain significance were identified in 1.5% of patients with ~90% inherited from a parent.

Discussion

CHD are birth defects that affect the normal physiology of the heart and may change the normal flow of blood. Congenital heart defects are the most common type of birth defect, known to occur in about one percent of live births in the United States. This number could be much higher as it is a defect that is difficult at times to diagnose. Approximately 15% of families learn about these defects prenatally, but many cannot be detected prenatally. It is estimated that 25-75% of CHD are conotruncal defects.

This is the most comprehensive study to date utilizing chromosome and microarray studies in patients with prenatal heart defects and demonstrates the importance of cytogenetic abnormalities in the etiology of congenital heart defects. Overall, approximately 18.5% of the patients with an isolated CHD, studied prenatally, had an abnormality detected by standard chromosome analysis while another 6.3% had a pathogenic abnormality only detectable by microarray analysis. However, these frequencies varied depending on the type of abnormality **(Table 1)**. The occurrences also varied and were elevated if there was a concurrent extracardiac major abnormality (e.g., holoprosencephaly). In these more complex patients almost 50% of patients had a cytogenetic abnormality [~38% had a detectable chromosome abnormality and another 9.4% had a pathogenic copy number variation (CNV) detectable by microarray analysis **(Table 2)**].

Simple CHD include ventricular septal defects, atrial septal defects, pulmonary atresia, patent ductus arteriosus and aortic stenosis **(Table 3)**. These defects are associated with a longer survival, but ~19.2% of isolated simple heart defects have a chromosome abnormality and another 5.3% have a pathogenic CNV. In patients with a simple heart defect and a concurrent extracardiac defect ~22.6% of isolated simple heart defects have a chromosome abnormality and another 8.1% have a pathogenic CNV detected by microarray.

Conotruncal CHD include, double outlet right ventricle, transposition of great arteries, tetralogy of Fallot, truncus arteriosus and interrupted aortic arch **(Table 4)**. Approximately 11.3% of isolated conotruncal heart defects have a chromosome abnormality and another 13.9% have a pathogenic CNV detected by microarray. In patients with a conotruncal heart defect and a concurrent extracardiac defect, these numbers are elevated with ~30.2% having a chromosome abnormality and another 15.6% have a pathogenic CNV. Although most abnormalities (~71.9%) only detected by microarray analysis involved deletions of 22q associated with velo-cardio-facial syndrome (VCFS), almost 30% have pathogenic CNVs outside of the VCSF region demonstrating the need for microarray analysis on these patients and not just FISH for 22q deletions.

The increased diagnostic yield of microarray compared to FISH for 22q is even more evident for other isolated CHD not involving conotruncal defects, where 22q deletions only comprise one-third of the microarray detectable abnormalities and for CHD. In CHD patents with extracardiac abnormalities, ~85% of microarray CNVs detected do not involve 22q deletions. The importance of microarray analysis in these patients can be seen by the rate of detection of abnormalities not seen in chromosome studies. However, microarray analysis was not requested for 50% of the cases with normal chromosomal findings; ~35% of the patients with a simple heart defect were not studied and ~20% of the patients with conotruncal defects were not studied by microarray analysis.

As demonstrated in Figure 1, in addition to deletions of *TBX1*, 23 different deleted genes were identified that appear to be responsible for the CHD present in 92 patients. This number will likely grow as more is learned about the genetics of CHD.

This work has identified that approximately 1-2% of patients studied for CHD have a CNV associated with a neurodevelopmental disorder (NDD) with variable features. Most of these disorders appear unrelated to the heart defect; however, some (e.g., 1q21.1 deletions and duplications) have been shown to be associated with CHD. More work needs to be done to determine if other NDD regions we identified might also have implications for CHD. These disorders have broad implications when detected in the prenatal setting, because of the neurodevelopmental findings that need to be counseled on, but also the familial implications since ~70% of these disorders are inherited.

Although these studies provide new information and insights into the importance of cytogenetic abnormalities in CHD, there are some limitations. In some instances, chromosome analysis was not done concurrently with microarray analysis, and if a defect was detected by the array, judgment had to be used to determine if the abnormality would have been detected by standard chromosome analysis. Although all patients studied had some indication of CHD provided, in only around 50% of the patients was a definitive CHD subtype diagnosis provided, limiting the ability to correlate our findings with specific CHD subtypes in those.

Many of the CHD are not detected early in gestation and most patients were not referred for chromosome/microarray studies until after 20 weeks of gestation. This might present health care issues due to the timing of the referrals in some cases.

Conclusion

- An increased yield of abnormalities underlying CHD can be detected by microarray studies. In some subtypes, many patients are still examined only by chromosome studies rather than array analysis, resulting in a lower yield of abnormalities for those patients.
- The frequency of abnormalities seen by chromosome and microarray analysis varies widely between the different subtypes.
- Most of these patients were not referred to the laboratory until after 20 weeks gestation, which has important health care implications.
- Findings that may not be strictly correlated with heart defects are detected in 1-2% of all groups of patients. These findings are a challenge and may be problematic from a genetic counseling perspective, but are clinically important, and proper counseling is imperative given they could have implications for family members.

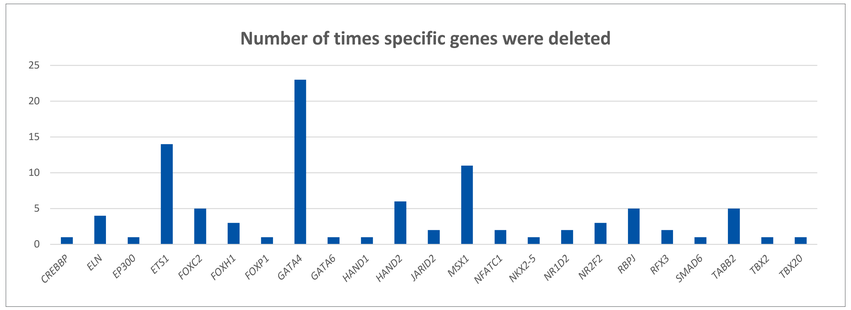

Figure 1. Frequency of deletions seen in CHD-associated genes in over 13,000 prenatal cases referred for cardiac abnormalities seen on ultrasound.

	Chromosome			Array		
Type	Total	Abnormal chromosomes	Percent	Total	Abnormal CNVs	Percent
Ventricular septal defect	1629	335	20.6%	759	38	5.0%
Truncus arteriosus	117	6	5.1%	78	15	19.2%
Hypoplastic left heart syndrome	871	72	8.3%	669	38	5.7%
Tetrology of Fallot	740	66	8.9%	547	87	15.9%
Aortic stenosis	32	2	6.3%	19	0	0.0%
Atrial septal defect	51	10	19.6%	24	3	12.5%
Atrioventricular canal	332	111	33.4%	160	6	3.8%
Atrioventricular septal defect	104	73	70.2%	17	0	0.0%
Pulmonary atresia	73	6	9.0%	32	4	12.5%
Double outlet right ventricle	188	65	34.6%	98	10	10.2%
Complex heart defects, nos	293	25	8.5%	205	16	7.8%
Coarctation of aorta	67	4	6.0%	45	2	4.4%
Conotruncal, nos	16	0	0.0%	14	2	14.3%
Interrupted aortic arch	42	4	9.5%	31	8	25.8%
Transposition of great arteries	232	10	4.3%	161	7	4.3%
Dextrocardia	38	0	0.0%	25	0	0.0%
Displaced heart	10	2	20.0%	0	0	0.0%
Heterotaxy	130	4	3.1%	96	4	4.2%
Situs inversus	50	0	0.0%	30	1	3.3%
Epstein anomaly	50	3	6.0%	39	2	5.1%
Endocardial cushion defect	35	15	42.9%	15	1	6.7%
Double aortic arch	23	0	0.0%	17	0	0.0%
Heart defect, nos	4955	1101	22.2%	2788	130	4.7%
Pentology of Cantrell	15	1	6.7%	11	0	0.0%
Rhabdomyoma	25	0	0.0%	12	0	0.0%
Right aortic arch	171	4	2.3%	142	11	7.7%
Single ventricle	32	8	25.0%	13	1	7.7%
Tricuspid valves	90	13	14.4%	37	2	5.4%
Enlarged heart	78	16	20.5%	32	2	6.3%
Hypoplastic right heart abnormality	122	4	3.3%	83	1	1.2%
TOTAL	**10611**	**1960**	**18.5%**	**6199**	**391**	**6.3%**

Table 1. Frequency of cytogenetic abnormalities in patients with isolated heart defects.

	Chromosome			Array		
Type	Total	Abnormal chromosomes	Percent	Total	Abnormal CNVs	Percent
Ventricular septal defect	741	170	22.9%	461	33	7.2%
Truncus arteriosus	22	6	27.3%	15	2	13.3%
Hypoplastic left heart syndrome	157	40	25.5%	91	11	12.1%
Tetrology of Fallot	115	22	19.1%	80	14	17.5%
Aortic stenosis	13	2	15.4%	11	2	18.2%
Atrial septal defect	39	7	17.9%	29	5	17.2%
Atrioventricular canal	91	31	34.1%	52	2	3.8%
Atrioventricular septal defect	30	16	53.3%	10	1	10.0%
Pulmonary atresia	8	2	25.0%	3	1	33.3%
Double outlet right ventricle	61	42	68.9%	18	3	16.7%
Complex heart defects, nos	103	30	29.1%	68	7	10.3%
Coarctation of aorta	9	2	22.2%	5	0	0.0%
Conotruncal, nos	2	0	0.0%	2	0	0.0%
Interrupted aortic arch	10	1	10.0%	6	2	33.3%
Transposition of great arteries	25	0	0.0%	20	1	5.0%
Dextrocardia	29	1	3.4%	20	3	15.0%
Displaced heart	18	3	16.7%	14	0	0.0%
Heterotaxy	20	1	5.0%	16	0	0.0%
Situs inversus	11	0	0.0%	11	1	9.1%
Epstein anomaly	10	0	0.0%	9	2	22.2%
Endocardial cushion defect	4	1	25.0%	3	0	0.0%
Double aortic arch	0	0	0.0%	0	0	0.0%
Heart defect, nos	1469	462	31.4%	817	76	9.3%
Pentology of Cantrell	15	1	6.7%	12	0	0.0%
Rhabdomyoma	1	0	0.0%	1	0	0.0%
Right aortic arch	0	0	0.0%	0	0	0.0%
Single ventricle	7	1	14.3%	6	0	0.0%
Tricuspid valves	6	1	16.7%	3	1	33.3%
Enlarged heart	60	12	20.0%	42	3	7.1%
Hypoplastic right heart abnormality	25	5	20.0%	18	4	22.2%
TOTAL	**3101**	**859**	**27.7%**	**1843**	**174**	**9.4%**

Table 2. Frequency of cytogenetics abnormalities in patients with heart defects that also had additional extracardiac defects.

Isolated heart defect	Total	Abnormal chromosomes	Percent	Total	Abnormal CNVs	Percent
Ventricular septal defect	1629	335	20.6%	759	38	5.0%
Atrial septal defect	51	10	19.6%	24	3	12.5%
Pulmonary atresia	73	6	9.0%	32	4	12.5%
Aortic stenosis	32	2	6.3%	19	0	0.0%
Coarctation of aorta	67	4	6.0%	45	2	4.4%
TOTAL	**1852**	**357**	**19.2%**	**879**	**47**	**5.3%**
Heart defect with extracardiac abnormality	**Total**	**Abnormal chromosomes**	**Percent**	**Total**	**Abnormal CNVs**	**Percent**
Ventricular septal defect	741	170	22.9%	461	33	7.2%
Atrial septal defect	39	7	17.9%	29	5	17.2%
Pulmonary atresia	8	2	25.0%	3	1	33.3%
Aortic stenosis	13	2	15.4%	11	2	18.2%
Coarctation of aorta	9	2	22.2%	5	0	0.0%
TOTAL	**810**	**183**	**22.6%**	**509**	**41**	**8.1%**

Table 3. Abnormalities associated with simple heart defects.

Isolated heart defect	Total	Abnormal chromosomes	Percent	Total	Abnormal CNVs	Percent
Double outlet right ventricle	188	65	34.6%	98	10	10.2%
Interrupted aortic arch	42	4	9.5%	31	8	25.8%
Tetralogy of Fallot	740	66	8.9%	547	87	15.9%
Conotruncal, nos	16	0	0.0%	14	2	14.3%
Transposition of great arteries	232	10	4.3%	161	7	4.3%
Truncus arteriosus	117	6	5.1%	78	15	19.2%
TOTAL	**1335**	**151**	**11.3%**	**929**	**129**	**13.9%**
Heart defect with extracardiac abnormality	**Total**	**Abnormal chromosomes**	**Percent**	**Total**	**Abnormal CNVs**	**Percent**
Double outlet right ventricle	61	42	68.9%	18	3	16.7%
Interrupted aortic arch	10	1	10.0%	6	2	33.3%
Tetralogy of Fallot	115	22	19.1%	80	14	17.5%
Conotruncal, nos	2	0	0.0%	2	0	0.0%
Transposition of great arteries	25	0	0.0%	20	1	5.0%
Truncus arteriosus	22	6	27.3%	15	2	13.3%
TOTAL	**235**	**71**	**30.2%**	**141**	**22**	**15.6%**

Table 4. Abnormalities associated with conotruncal heart defects.

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