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Copy Number Variants of Undetermined Significance are not Associated with Worse Clinical Outcomes in Hypoplastic Left Heart Syndrome

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Abstract

Objective: To determine the prevalence, spectrum, and prognostic significance of copy number variants of undetermined significance (cnVUS) seen on chromosomal microarray (CMA) in neonates with hypoplastic left heart syndrome (HLHS).

Study design: Neonates with HLHS who presented between June 2008 and December 2016 to Texas Children's Hospital were identified. CMA results were abstracted and compared against copy number variations (CNVs) in ostensibly healthy individuals, gathered from the literature. Findings were classified as normal, consistent with a known genetic disorder, or cnVUS. Survival was then compared using Kaplan Meier analysis. Secondary outcomes included tracheostomy, feeding tube at discharge, cardiac arrest, and extracorporeal membrane oxygenation (ECMO).

Results: Our study included 105 neonates with HLHS; 70 (66.7%) had normal CMA results, 9 (8.6%) had findings consistent with known genetic disorders, and 26 (24.7%) had a cnVUS. Six of the 26 (23.0%) cnVUS patients had a variant that localized to a specific region of the genome seen among the healthy control population. One-year survival in patients with a cnVUS, normal CMA, or known genetic disorders was 84.0%, 68.3% and 33.3%, respectively ($P=0.003$). There was no significant difference in secondary outcomes, although notably ECMO was utilized in 15.7% of patients with normal CMA and was not used in patients with cnVUS and abnormal results ($P=0.038$).

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The authors declare no conflicts of interest.

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Conclusions: Among children with HLHS, cnVUSs detected on CMA are common. The cnVUSs did not localize to specific regions of the genome and were not associated with worse outcomes compared with children with a normal CMA.

Keywords

copy number variation; genetic test; mutation variant of undetermined significance

Despite surgical palliation and medical management evolving tremendously in the past 40 years, HLHS still carries significant morbidity and mortality.^{3, 5, 6} The backbone of palliation is the 3-stage surgical intervention comprised of the Norwood, Glenn, and Fontan procedures, respectively. Even with major centers achieving survival rates to discharge of up to 90% following Norwood, these neonates remain at a high risk of death prior to Glenn.⁷ This so-called interstage mortality can be as high as 24% of children with HLHS.⁸ At Texas Children's Hospital (TCH), interstage mortality was 12% from 2002 to 2007, before the single ventricle program (SVP) was established and 8% from 2007 to 2010, with a 12.5% overall hospital discharge mortality following Norwood (2013–2016).^{9, 10} Further, several clinical factors have been found to be associated with increased mortality, including prematurity, low birth weight, pulmonary venous obstruction, restrictive or intact atrial septum, or children above 1 month of age undergoing the Norwood procedure, and genetic syndrome-associated copy number variation (CNV).^{11–15}

Given the prognostic relevance and high prevalence of CNVs, genetic testing by chromosomal microarray (CMA) has become routine and standard of care.^{4, 16} CMA is a genome-wide screening technique, utilized to detect chromosomal imbalances.⁴ As CMA testing in neonates with HLHS has increased, so has the detection of copy number variants of unknown/undetermined significance (cnVUS).⁴ The uncertainty inherent in these findings impairs the ability to adequately predict the potential for morbidity and mortality with consequences for provider management strategies and appropriate counseling of families. To this end, we sought to determine the spectrum, prevalence, and diagnostic relevance of cnVUSs in HLHS.

In this study, we evaluate the association between CMA results and morbidity and mortality in patients with HLHS in order to determine the potential prognostic implications of cnVUS results.

METHODS

In this Institutional Review Board-approved study, we conducted a retrospective review of all patients diagnosed with HLHS at TCH (Houston, Texas, United States) between June 2008 and December 2016. Inclusion criteria were neonates with HLHS who presented to TCH within thirty days of birth, underwent CMA testing through the Baylor Genetics Laboratories (Houston, Texas, United States; previously Baylor College of Medicine Medical Genetics Laboratories). Exclusion criteria were children who received palliative surgical procedures at other centers prior to transfer, and single ventricle lesions not clearly defined as HLHS, including unbalanced atrioventricular septal defects and double outlet right ventricle. Data collected included patient sex, race/ethnicity, age at presentation,

gestational age at birth, birthweight, atrial level restriction, echocardiographic characteristics, and CMA testing results. Echocardiograms were analyzed to determine the presence of mitral stenosis (MS)/aortic stenosis (AS), MS/aortic atresia (AA), mitral atresia (MA)/AA, left-sided superior vena cava (LSVC), total anomalous pulmonary venous return/anomalous pulmonary venous return, degree of tricuspid regurgitation, diameter of ascending aorta, and the degree of RV dysfunction.

The patients were studied by V7, V8, V9, V10, or V11 arrays designed by Baylor Medical Genetics Laboratories and manufactured by Agilent Technology (Santa Clara, CA, USA). The V7 array included approximately 105,000 interrogating oligonucleotides, selected from Agilent's online library (eArray; <https://earray.chem.agilent.com/earray/>), with backbone coverage of about 30 kb.^{17, 18} The V8 array included about 180,000 oligonucleotides targeting ~1,714 genes plus 101,644 probes used for SNP analysis for the detection of uniparental disomy (UPD) and absence of heterozygosity (AOH).¹⁸ The V9-V11 arrays targeted over 4,800 genes with oligonucleotides including at the exon level and had an average of >4.2 probes per exon for SNP analysis.¹⁹ Further details are available at <https://www.bcm.edu/geneticlabs/>.

We defined structural variation in the genome as variants involving more than 50 base pairs. Our patient cohort was compared with CNV data from a stringent database of CNVs that was later compiled as the Database for Genomic Variants (DGV) genome browser – a database of structural variants of the human genome in healthy patient cohorts²⁰. This database included 72 studies to create a reference cohort including 2,057,386 variants among 2647 subjects across diverse ethnicities with identified structural variations in the genome²⁰. Each CNV locus labeled as a cnVUS was compared with the healthy reference cohort to discern the frequency of healthy CNV seen at each cnVUS locus. The stringent map variants were utilized to determine control cohort frequency.

The primary outcome studied was overall survival. Secondary outcomes included tracheostomy, nasogastric or gastrostomy tube placement at discharge from initial hospitalization, cardiopulmonary resuscitation (CPR), or extracorporeal membrane oxygenation (ECMO).

Statistical Analyses

Continuous variables were presented as means with standard deviation and medians with interquartile ranges (IQRs). One way ANOVA or Kruskal-Wallis Test were applied to compare the difference among the three groups for data with normal or non-normal distribution, respectively. Categorical variables were expressed as counts with percentages and proportions were compared using the Fisher exact test. Survival was compared using Kaplan-Meier analysis, with comparison by log rank, with birth as time 0, and censoring at death or last follow-up. All statistics were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA).

RESULTS

Given the broad use of CMAs as a diagnostic tool in children with a variety of underlying diseases, we first sought to determine the overall prevalence of abnormal and cnVUS findings in pediatric patients seen at TCH regardless of diagnosis. We identified a total of 6410 CMAs performed on individual subjects. Among these, 4089 (63.2%) individuals were interpreted as “normal”, 1692 (26.2%) as cnVUSs, and 689 (10.6%) as abnormal genetic test results associated with a known genetic syndrome (Figure 1; available at www.jpeds.com). This suggests that a considerable proportion of CMA results, which were cnVUS, did not provide clinicians any clear diagnostic utility.

In total, 105 infants met our inclusion and exclusion criteria. Sixty (57%) were male, and the median age of presentation in all groups was the first day of life. These findings, along with birth weight, gestational age, and fronto-occipital circumference are summarized in Table 1 (available at www.jpeds.com). CMAs performed on these children demonstrated no CNV abnormalities in 70 (66.7%), and 26 (24.7%) had cnVUSs, and 9 (8.6%) hosted CNV interpreted as syndromic/pathologic (Table 2; available at www.jpeds.com). The pathologic CNVs identified in patients with HLHS were associated with several medically relevant phenotypes. These include syndromes such as Turner syndrome ($N = 4$, 67% of abnormal females) and Pallister-Killian syndrome ($N = 1$). In addition, pathologic CNV was associated with gastrointestinal dysmotility and renal and craniofacial abnormalities. Although loci affected were diverse, four chromosomal rearrangements occurred on the X chromosome. The CNVs were due to copy number losses (50%) and copy number gains (50%). These findings are summarized in Table 3 (available at www.jpeds.com). In comparison, CNV interpreted as cnVUSs were due to copy number gain (59%), loss (37%), and absence of heterozygosity (4%) and were highly variable in their location. These findings are summarized in Table 4 (available at www.jpeds.com).

To determine whether HLHS-associated cnVUSs were associated with variation seen in ostensibly healthy individuals, we cross-referenced them with a stringent meta-database of CNV seen in healthy individuals, published in *Nature Reviews Genetics*.²⁰ This database compiled information on CNVs regarded as healthy “background” genetic variation. Six of the 26 (23.0%) cnVUS probands with HLHS had a variant that localized to a specific region of the genome identified among the healthy cohort CNV. The remaining 20 (77.0%) had CNV not included in the healthy reference population. In addition, only two of our six patients had loci involved in more than 1% of the healthy patient cohort including the 9p21.3 gain cnVUS which was seen in 12.9% of the cohort and 15q11.2 loss which was seen in 4.16%. These results are summarized in Table 5. Overall, this suggests that cnVUSs do not clearly represent healthy “background” genetic variation and may be a distinct genetic subset with children with HLHS.

To evaluate the possibility that the remaining cnVUSs not seen in otherwise healthy individuals may involve clinically-relevant genes, we mapped each against a comprehensive list of 935 clinical-relevant genes that are understood to involve CNVs.²⁰ Only a small minority of cnVUS-positive subjects were found to host genes associated with genetic diseases (Table 6). Of the remaining 20 that did not have CNV in the healthy population,

only 5 cnVUSs involved loci containing genes in which single gene mutations or CNV have been associated with predisposition to disease. Overall, these genes were associated with developmental delay, autism spectrum disorders, craniofacial abnormalities, macrocephaly and epilepsy. A single cvVUS was associated with congenital heart disease and arrhythmias. The cnVUS with a gain in 1q21.1 contains *GJA5*, which encodes a gap junction protein, alpha 5 (connexin40) and has been associated with the development of atrial fibrillation, atrial standstill, and cardiac malformations.

The one-year Kaplan-Meier survival in patients with a normal CMA was 68.3%, 84.0% in patients with cnVUS, and 33.3% in those with pathologic CNV ($P=0.003$ via log rank test). Survival by KM analysis is illustrated in Figure 2. We noted a significantly higher prevalence of extracardiac abnormalities among HLHS infants with abnormal CMAs (55.6%) compared with the much lower prevalence seen in infants with normal (5.7%) and cnVUS (8%) CMA results ($P<0.001$).

RV dysfunction was also more common in our abnormal group (22.2%) as compared with the cnVUS (7.7%) and normal (5.7%) population ($P=0.016$). There were no significant differences in the HLHS morphologic variation based on CMA class result including prevalence of MS/AS, MS/AA, MA/AA, presence of a LSVC, degree of tricuspid regurgitation, levocardinal vein, abnormal coronary arteries, ascending aorta diameter, and anomalous pulmonary venous return (Table 7).

Given the differences in survival between CMA group, we next evaluated a number of secondary endpoints by CMA class. We identified no significant difference between CMA class and tracheostomy, feeding tube, or CPR ($P=1.000$, $P=1.000$, $P=0.139$ respectively, Table 8). Of note, ECMO was not used among probands hosting either cnVUS or pathologic CMA findings, and probands with normal CMAs required ECMO in 11 (15.7%) individuals ($P=0.038$).

DISCUSSION

CNVs, or unbalanced chromosomal rearrangements, have a wide array of clinical associations. Although there is a broad spectrum of syndromic CNVs, HLHS probands hosting this class of CMA are associated with a worse prognosis when compared with their normal counterparts. Multiple studies have found chromosomal abnormalities and genetic syndromes negatively impact inter-stage mortality. One study found one and ten year survival was half for HLHS patients with pathologic genetic syndromes compared with patients with normal genetics.^{6, 21–23} Amongst other known syndromes, Turner syndrome, trisomy 18, trisomy 13, and Down syndrome are associated with higher early mortality in HLHS.^{24, 25} One study demonstrated 50% 1-month survival in patients with HLHS with Turner syndrome, as opposed to 85% survival in non-syndromic patients with HLHS²⁶, and another exhibited significantly increased cumulative mortality compared with females without Turner Syndrome, even with multivariate analysis controlling for low birth weight.²⁷ Another study demonstrated five-year survival of 61% in patients with Down syndrome as opposed to 85% in non-syndromic patients.²⁸ Therefore, an abnormal result detected on CMA is widely considered a predictor of increased mortality in CHD. However,

with the rapidly expanded use of clinical CMAs, which are becoming the gold-standard for neonates with HLHS, there is increasing awareness of cnVUS. With 26.2% of all children at our institution referred for CMA testing and 20% of HLHS patients having a cnVUS result, this is a common issue that will likely only increase as genetic testing use increases.

The general consensus amongst most studies is that chromosomal anomaly prevalence in CHD is higher when extracardiac anomalies are also present.²⁹ Hence, both genetic syndromes and extracardiac anomalies, as individual and combined factors, are associated with heightened mortality.^{30, 31} However, patients with cnVUS results did not exhibit the same characteristics as their abnormal counterpart, achieving significantly higher survival rates and a significantly lower prevalence of extra-cardiac abnormalities.

To determine the genetic significance of cnVUS results, we compared results with loci identified in healthy individuals and determined if there was any overlap with loci of clinical-actionable genes. Studies exploring incidentally identified VUSs associated with cardiomyopathies and channelopathies have suggested that the majority of these variants are likely background genetic noise.³² When comparing our cohort to cnVUS results among ostensibly healthy individuals, fewer than a quarter of patients in our cohort had identical loci identified in healthy individuals. As such, we cannot assume that cnVUS results in HLHS patients are comparable with cnVUS results in healthy individuals and seem genetically distinct. Of the remaining CNVs not included in the cross-referenced database, 5 had clinical-actionable genes involved. Among these, *GJA5* has been associated with cardiac disease. Importantly, polymorphisms of *GJA5* detected in families with cases of atrial standstill were only clinically manifesting if co-inherited with mutations in the *SCN5A* gene.^{33, 34} Further, clinical manifestations of atrial fibrillation were associated with rare, rare, novel missense and nonsense mutations.^{35–38} Mouse models with limited or absence of expression of *GJA5* have demonstrated a higher prevalence of cardiac anomalies usually of conotruncal origin, with one third of the hearts exhibiting tetralogy of Fallot or double outlet right ventricle.³⁹ Although most of these genes have little immediate clinical impact on HLHS patient survival, they may have an impact on patient long-term morbidity that is difficult to determine. Aside from this single cnVUS, associated phenotypes with the other clinical-actionable genes include mainly long-term neurobehavioral/psychiatric conditions. These conditions often cause significant functional limitations, in regards to executive planning, visual-motor integration, and thought-processing.⁴⁰ Nonetheless, although genetics play a role, neurobehavioral limitations are usually due to a variety of factors, including parental IQ, cardiopulmonary bypass conduct, hemodynamic instability, intraoperative procedures, and perioperative neuroprotection.^{40–42} Ultimately, cnVUS results were not commonly found among ostensibly healthy individuals nor did loci contain clinically significant genes, suggesting that cnVUSs represent a distinct genetic subclass within HLHS and that there does not appear to be a genetic explanation for the survival seen in individuals hosting cnVUSs

Our finding that patients with HLHS hosting cnVUSs have superior survival to syndromic counterparts suggests the worse prognosis associated with abnormal CMA is restricted to known genetic syndromes. Although we identified a statistically significant greater number of neonates with normal CMAs who received ECMO support compared with patients with

cnVUS and abnormal CMA findings, from our study method we cannot definitively conclude the genetic test results altered patient management. The decision to initiate ECMO support is a multifaceted process. Previous studies have noted the inclusion of known pathologic mutations as part of end of life care discussions with the families of patients, and clinical management has the capacity to be influenced by the presence of abnormal CMA^{43, 44}.

We recognize the limitations of our smaller sample size and the fact that CMA testing of infants with HLHS is not universal. Hence, estimates of CNVs could be amplified in our patient cohort. A better understanding of the effects of cnVUS results on physician/patient family decision-making is needed.

We find that cnVUS are common among children who receive CMAs and among children with HLHS. Although CNV associated with known genetic syndromes carries a negative prognostic association, we find cnVUS-associated survival to be equal, and perhaps superior to, normal CMA class.

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ABBREVIATIONS

CHD	congenital heart disease
CMA	chromosomal microarray
CNV	copy number variation
cnVUS	copy number variant of undetermined/unknown significance
CPR	cardiopulmonary resuscitation
DGV	Database of Genomic Variation
ECMO	extracorporeal membrane oxygenation
HLHS	hypoplastic left heart syndrome
LSVC	left superior vena cava
MA/AA	mitral atresia and aortic atresia
MS/AA	mitral stenosis and aortic atresia
MS/AS	mitral stenosis/aortic stenosis

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Figure 1;online only:

Pie chart of CMA results at Texas Children Hospital's from June 2008 through December 2016 for any test indication. Test results are divided into those interpreted as abnormal (black fill), cnVUS (gray), and normal (white).

Figure 2: Kaplan-Meier analysis of transplant-free survival among neonates with HLHS by CMA result with normal (gray), cnVUS (light blue), and abnormal (dark blue). Censored observations are indicated as plus signs (+).

Table 1; online only.

Summary of clinically relevant findings in subjects with HLHS who underwent CMA testing

	Normal N=70		cnVUS N=26		Abnormal N=9		
	Mean±SD	Median (IQR)	Mean±SD	Median (IQR)	Mean±SD	Median (IQR)	P-value
Age at Presentation (d)	0.71 ± 2.30	0 (0,0)	1.04 ± 5.09	0 (0,0)	1.00 ± 2.00	0 (0,1)	0.197
Gestational Age	38.32 ± 1.59	38.93 (37.29, 39.43)	38.92 ± 0.91	39.00 (38.71, 39.43)	37.78 ± 2.00	38.29 (36.00, 39.00)	0.145
Birth Weight (kg)	3.03 ± 0.55	3.08 (2.70, 3.46)	3.20 ± 0.46	3.21 (2.90, 3.46)	2.56 ± 0.56	2.74 (2.23, 2.90)	0.008
FOC (cm)	33.28 ± 1.64	33.00 (32.50, 34.50)	33.83 ± 1.95	33.70 (32.00, 35.00)	32.00 ± 1.77	32.00 (30.50, 33.50)	0.103

AA, ascending aorta; *cm*, centimeter; *d*, days; *FOC*, fronto-occipital circumference; *kg*, kilogram.

Table 2; online only.

Summary of clinical demographics for subjects with HLHS who underwent CMA testing

HLHS Cohort	N (%)
Total Cases	105
Male	60 (57.0%)
Female	45 (43.0%)
CMA cohort	
Normal	70 (66.7%)
cnVUS	26 (24.7%)
Abnormal	9 (8.6%)
Race/Ethnicity	
Caucasian	54 (51.0%)
Hispanic	49 (47.0%)
African-American	2 (2%)

HLHS, hypoplastic left heart syndrome; *CMA*, chromosomal microarray; *cnVUS*, copy number variant of unknown significance; *d*, days.

Table 3; online only:

Summary of copy number variation for subjects with HLHS hosting abnormal/pathogenic CNVs

CMA Pathologic Variants			
Subject	CNV		Clinical Presentation or Syndrome
1	Xp22.33q28	Loss	Turner syndrome, renal pyelectasis, cystic kidneys bilaterally
2	1p36.33p36.32	Gain	Gastroparesis and esophageal dysmotility
3	Xp22.33q28	Loss	Turner syndrome
4	15q11.2,	Loss	Turner Syndrome
	Xp22.33q28,	Loss	
	Yp11.31q11.1	Gain	
5	X,	Loss	Turner Syndrome
	Yp11.31p11.2	Gain	
6	12p13.33p11.1	Gain	Pallister-Killian syndrome
7	18q23	Gain	Partial Trisomy 18q and dysmorphic features
8	13q13.1	Gain	BRCA2 Duplication
9	22q11.21	Loss	Cleft lip/palate (features of DiGeorge Syndrome)

Table 4; online only:

Summary of copy number variation for subjects with HLHS hosting copy number variants of unknown significance

Subject	CNV	Genes
1	Xp22.13p22.12	Gain, Gain <i>MTTP, PHKA2, GPR64, PDHA1, MAP3K15</i>
2	9p21.3	Gain none
3	4q25	Loss <i>PAPSS1</i>
4	8p23.1	Gain <i>GATA4, NEIL2, FDFT1, CTSB</i>
5	7q21.11	Gain <i>PHTF2, MAGI2, RPL13A17</i>
6	22q13.2	Gain <i>BIK, MCAT, TSPO, TTLL12, SCUBE1</i>
7	1p36.33	Gain <i>PRKCZ, C1orf86, LOC100128003, SKI</i>
8	Xq21.31	Loss <i>CPXCR1</i>
9	16q24.2	Gain <i>ZNF469, ZFPM1</i>
10	Xq28	Gain <i>TMLHE</i>
11	1q21.1q21.2	Gain <i>PRKAB2, PDIA3P, FMO5, CHD1L, LOC100289211, BCL9, ACP6, GIA5, GIA8, GPR89B, GPR89C, PDZK1P1, NBPF11, NBPF24</i>
12	15q13.3	Gain <i>CHRNA7</i>
13	20q12q13.11	Loss <i>PTPRT</i>
14	15q11.2	Loss <i>TUBGCP5, CYFIP1, NIPA2, NIPA1</i>
15	6p25.3	Gain <i>LOC285768</i>
16	2p21	Gain <i>CALM2</i>
17	1q21.1	Gain <i>SEC22B, NOTCH2NL, NBPF10, HFE2, TXNIP, POLR3GL, RBM8A, GNRHR2, PEX11B, ITGA10, ANKRD34A, LIX1L, ANKRD35, PIAS3, NUDT17, POLR3C, RNF115, CD160, PDZK1</i>
18	2p13.1p12	Gain <i>SEMA4F, HK2, POLE4, TACR1</i>
19	17q23.2	Loss <i>PPM1D</i>
20	6q23.3	Loss <i>AH11</i>
21	7q21.13	Gain <i>ZNF804B, C7orf62, DPY19L2P4,</i>
22	5p13.2	Loss <i>IL7R</i>
23	9p24.3	Loss <i>DOCK8</i>
24	6q25.1	Loss <i>PLEKHG1</i>
25	16q22.1	Loss <i>PDXDC2, PDPR</i>
26	6q24.2q25.3	Absence of Heterozygosity <i>EPM2A, LOC100507557, FBXO30, SHPRH, GRM1, RAB32, C6orf103, LOC729176, LOC729178, STXBP5, SAMD5, SASH1, UST, LOC100128176, TAB2, SUMO4, ZC3H12D, PPIL4, C6orf72, KATNA1, LATS1, NUP43, PCMT1, LRP11, RAET1E, RAET1G, ULBP2, ULBP1, RAET1K, RAET1L, ULBP3, PPP1R14C, IYD, PLEKHG1, MTHFD1L, AKAP12, ZBTB2,</i>

Subject	CNV	Genes
		<i>RMND1, C6orf211, C6orf97, ESR1, SYNE1, MYCT1, VIP, FBXO5, MTRF1L, RGS17, OPRM1, IPCEF1, CNKSR3.</i>

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Table 5; online only:

Frequency of HLHS-identified cnVUSs within the Stringent Database for Genomic Variants

Subject	Chromosome	CMA Change	Interval	Genome Map Frequency*
1	Xp22.13p22.12	Gain	100532453–100545071	-
			18936760–19507586	-
2	9p21.3	Gain	22453166–22808555	1513/11732 =12.9%
3	4q25	Loss	108456682–108806918	49/11732=0.42%
4	8p23.1	Gain	11598718–11843369	107/11732=0.912%
5	7q21.11	Gain	77530094–78018726	-
6	22q13.2	Gain	43506447–43722430	-
7	1p36.33	Gain	1978815–2201821	-
8	Xq21.31	Loss	87851064–88148650	-
9	16q24.2	Gain	88385967–88530955	-
10	Xq28	Gain	154741174–154785986	-
11	1q21.1q21.2	Gain	146618988–147825678	-
12	15q13.3	Gain	32218274–32445252	114/11732 = 0.97%
13	20q12q13.11	Loss	41617589–41737558	-
14	15q11.2	Loss	22842145–23086692	488/11732= 4.16%
15	6p25.3	Gain	908246 –1063595	-
16	2p21	Gain	47388806–47539278	-
17	1q21.1	Gain	145114722–145740657	-
18	2p13.1p12	Gain	74908297–75285980	-
19	17q23.2	Loss	58740319–58741680	-
20	6q23.3	Loss	135715759–135732797	-
21	7q21.13	Gain	88424763–89858498	-
22	5p13.2	Loss	35903054–35903339	-
23	9p24.3	Loss	272815–428641	-
24	6q25.1	Loss	151051089–151152761	70 /11732 = 0.60%
25	16q22.1	Loss	68567940 – 68753268	-
26	6q24.2q25.3	AOH	145377585–155757781	-

* No value indicates no CNV observed involving the locus

AOH, Absence of Heterozygosity.

Table 6:

CMA-identified cnVUSs in patients with HLHS associated with medically relevant genes/phenotypes.

Chromosome	CMA Change	Medically Relevant / Phenotype
Xp22.13p22.12	Gain, Gain	-
7q21.11	Gain	<i>MAGI2</i> : associated with bipolar affective disorder, schizophrenia, and infantile spasms.
22q13.2	Gain	-
1p36.33	Gain	<i>SKI</i> : monosomy 1p36, which is associated with facial clefting anomalies, generalized epilepsy with febrile seizures, cranial suture closure anomalies, and seizures.
Xq21.31	Loss	-
16q24.2	Gain	-
Xq28	Gain	-
1q21.1q21.2	Gain	<i>GJA5</i> : associated with learning disability, autism spectrum disorders, macrocephaly, behavioral features, atrial fibrillation, atrial standstill, and tetralogy of Fallot. <i>GJA8</i> : associated with cataracts.
20q12q13.11	Loss	-
6p25.3	Gain	-
2p21	Gain	-
1q21.1	Gain	<i>HFE2</i> : 1q21.1 duplication syndrome (no further info.)
2p13.1p12	Gain	-
17q23.2	Loss	-
6q23.3	Loss	<i>AHI1</i> : haploinsufficient 6q23.3
7q21.13	Gain	-
5p13.2	Loss	-
9p24.3	Loss	-
16q22.1	Loss	-
6q24.2q25.3	Absence of	-
	Heterozygosity	

Table 7:

Clinical characteristics of HLHS cohort by CMA class

Cardiac Parameters on Presentation					
		Normal	cnVUS	Abnormal	
		N=70	N=25	N=10	
Parameter		Mean (SD)	Mean (SD)	Mean (SD)	P-value
MS/AS, MS/AA, MA/AA	MA/AA	32 (46.4)	17 (65.4)	3 (33.3)	0.140
	MS/AA	24 (34.8)	6 (23.1)	6 (66.7)	.
	MS/AS	13 (18.8)	3 (11.5)	0	.
	Missing	1 (1.4)	0	0	.
LSVC	No	65 (92.9)	26 (100)	9 (100)	0.436
	Yes	5 (7.1)	0	0	.
PAPVR/TAPVR	No	68 (97.2)	24 (92.3)	9 (100.0)	0.510
	Yes	2 (2.8)	2 (7.7)	0	.
Degree of TR	Trivial/None	40 (57.1)	11 (42.3)	4 (44.4)	0.503
	Mild	23 (32.9)	12 (46.2)	3 (33.3)	.
	Moderate/Severe	7 (10.0)	3 (11.5)	2 (22.2)	.
Mod/Severe TR	No	64 (91.4)	23 (88.5)	7 (77.8)	0.333
	Yes	6 (8.6)	3 (11.5)	2 (22.2)	.
RV function, n (%)	Normal	62 (89.9)	20 (80.0)	5 (55.6)	0.008
	Mildly/Moderately	7 (10.1)	5 (20.0)	4 (44.4)	.
	Depressed				
RV moderate dysfunction, n (%)	No	69 (98.6)	24 (92.3)	7 (77.8)	0.016
	Yes	1 (1.4)	2 (7.7)	2 (22.2)	.
Extra cardiac abnormalities	No	66 (94.3)	24 (92.3)	4 (44.4)	<0.001
	Yes	4 (5.7)	2 (7.7)	5 (55.6)	.
Sano, BTS, or Hybrid	BTS	45 (64.3)	17 (65.4)	3 (33.3)	0.010
	Sano	16 (22.9)	9 (34.6)	1 (11.1)	.
	Hybrid	3 (4.3)	0	1 (11.1)	.
	None	6 (8.6)	0	4 (44.4)	
Restrictive ASD, n (%)	No	54 (77.1)	22 (84.6)	7 (77.8)	0.743
	Yes	16 (22.9)	4 (15.4)	2 (22.2)	.
Ascending aorta diameter (cm)		0.34 ± 0.28	0.27 ± 0.15	0.24 ± 0.07	0.204

AA, aortic atresia; AS, aortic stenosis; ASD, atrial septal defect; BTS, Blalock-Taussig shunt; LSVC, left superior vena cava; MA, mitral atresia; MS, mitral stenosis; RV, right ventricle; TR, tricuspid regurgitation; PAPVR/TAPVR, partial anomalous pulmonary venous return/total anomalous pulmonary venous return.

Table 8:

Secondary outcome variables divided by CMA class.

Outcome Variables by CMA Class					
		Normal	cnVUS	Abnormal	
Parameter		Mean (SD)	Mean (SD)	Mean (SD)	P-value
		n (%)	n (%)	n (%)	
Tracheostomy	No	67 (95.7)	25 (96.2)	9 (100)	1.000
	Yes	3 (4.3)	1 (3.8)	0	
Feeding tube	No	53 (75.7)	20 (76.9)	7 (77.8)	1.000
	Yes	17 (24.3)	6 (23.1)	2 (22.2)	
CPR	No	61 (87.1)	26 (100)	8 (88.9)	0.139
	Yes	9 (12.9)	0	1 (11.1)	
ECMO	No	59 (84.3)	26 (100)	9 (100)	0.038
	Yes	11 (15.7)	0	0	

CPR, cardiopulmonary resuscitation; *ECMO*, extracorporeal membrane oxygenation