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

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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 to Texas Children's Hospital between June 2008 and December 2016 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 cohort comprised 105 neonates with HLHS, including 70 (66.7%) with normal CMA results, 9 (8.6%) with findings consistent with a known genetic disorder, and 26 (24.7%) with a cnVUS. Six of the 26 (23.0%) neonates with a cnVUS had a variant that localized to a specific region of the genome seen in the healthy control population. One-year survival was 84.0% in patients with a cnVUS, 68.3% in those with normal CMA results, and 33.3% in those with a known genetic disorder ($P = .003$). There were no significant differences in secondary outcomes among the groups, although notably ECMO was used in 15.7% of patients with normal CMA and was not used in those with cnVUS and abnormal results ($P = .038$).

Conclusions Among children with HLHS, cnVUSs detected on CMA are common. The cnVUSs do not localize to specific regions of the genome, and are not associated with worse outcomes compared with normal CMA results. *(J Pediatr 2018;202:206-11).*

Despite the significant advances in surgical palliation and medical management over the past 4 decades, hypoplastic left heart syndrome (HLHS) still carries significant morbidity and mortality.[1-3] The backbone of palliation is the 3-stage surgical intervention comprising the Norwood, Glenn, and Fontan procedures. Even with major centers achieving survival rates to discharge of up to 90% following the Norwood procedure, these neonates remain at a high risk of death before the Glenn procedure.[4] This so-called "interstage mortality" can be as high as 24% in children with HLHS.[5] At Texas Children's Hospital (TCH), interstage mortality was 12% from 2002 to 2007, before the single-ventricle program was established, and 8% from 2007 to 2010, with a 12.5% overall hospital discharge mortality following the Norwood procedure in 2013 to 2016.[6,7]

Several clinical factors have been associated with increased mortality, including prematurity, low birth weight, pulmonary venous obstruction, restrictive or intact atrial septum, performance of the Norwood procedure in children aged >1 month, and genetic syndrome-associated copy number variation (CNV).[8-12]

Given the prognostic relevance and high prevalence of CNVs, genetic testing by chromosomal microarray (CMA) has become routine and a standard of care.[13,14] CMA is a genome-wide screening technique used to detect chromosomal imbalances.[13] As CMA testing in neonates with HLHS has increased, so has the detection of copy number variants of unknown/

AA	Aortic atresia
AS	Aortic stenosis
CHD	Congenital heart disease
CMA	Chromosomal microarray
CNV	Copy number variation
cnVUS	Copy number variant of undetermined/unknown significance
ECMO	Extracorporeal membrane oxygenation
HLHS	Hypoplastic left heart syndrome
MA	Mitral atresia
MS	Mitral stenosis
TCH	Texas Children's Hospital

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undetermined significance (cnVUS).[13] 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 neonates with HLHS.

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

Methods

In this Institutional Review Board-approved study, we conducted a retrospective review of all neonates diagnosed with HLHS at TCH between June 2008 and December 2016. Inclusion criteria were neonates with HLHS who presented to TCH within 30 days of birth and underwent CMA testing through the Baylor Genetics Laboratories (previously Baylor College of Medicine Medical Genetics Laboratories). Exclusion criteria were receipt of a palliative surgical procedure at another center before 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, birth weight, 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 superior vena cava, total anomalous pulmonary venous return/anomalous pulmonary venous return, degree of tricuspid regurgitation, diameter of the ascending aorta, and degree of right ventricular dysfunction.

The patients were studied using V7, V8, V9, V10, and V11 arrays designed by Baylor Medical Genetics Laboratories and manufactured by Agilent Technologies (Santa Clara, California). 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 ~30 kb.[15,16] The V8 array included ~180 000 oligonucleotides targeting ~1714 genes plus 101 644 probes used for single nucleotide polymorphism analysis for the detection of uniparental disomy and absence of heterozygosity.[16] The V9, V10, and V11 arrays targeted more than 4800 genes with oligonucleotides including those at the exon level and had an average of >4.2 probes per exon for single nucleotide polymorphism analysis.[17] 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 genome browser, a database of structural variants of the human genome in healthy patient cohorts.[18] 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 genome.[18] 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 used 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, and extracorporeal membrane oxygenation (ECMO).

Statistical Analyses

Continuous variables are presented as mean with SD and median with IQR. One-way ANOVA or the Kruskal-Wallis test was applied to compare differences among the 3 groups for data with a normal or nonnormal distribution, respectively. Categorical variables are expressed as counts with percentages, and proportions were compared using the Fisher exact test. Survival was compared using Kaplan-Meier analysis, with comparisons by the log-rank test, with birth as time 0 and censoring at death or the last follow-up. All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, North Carolina).

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 in individual subjects. Among these CMAs, 4089 (63.2%) 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 any clear diagnostic utility for clinicians.

A total of 105 infants, including 60 males (57%) and 45 females (43%), met our inclusion criteria. The median age of presentation in all groups was the first day of life. These findings, along with birth weight, gestational age, and frontooccipital circumference, are summarized in **Table I** (available at www.jpeds.com). CMAs performed on these children demonstrated no CNV abnormality in 70 (66.7%), a cnVUS in 26 (24.7%), and a CNV interpreted as syndromic/pathological in 9 (8.6%) (**Table II**; available at www.jpeds.com). The pathological CNVs identified in patients with HLHS were associated with several medically relevant phenotypes, including Turner syndrome (n = 4; 67% of abnormal females) and Pallister-Killian syndrome (n = 1). In addition, pathological CNV was associated with gastrointestinal dysmotility and renal and craniofacial abnormalities. Although diverse loci were affected, 4 chromosomal rearrangements occurred on the X chromosome. The CNVs were due to both copy number losses (50%) and copy number gains (50%). These findings are summarized in **Table III** (available at www.jpeds.com). In

comparison, CNVs interpreted as cnVUSs were due to copy number gain (59%), copy number loss (37%), and absence of heterozygosity (4%) and were highly variable in location. These findings are summarized in **Table IV** (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*.[18] This database compiled information on CNVs considered healthy "background" genetic variations. 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 a CNV not included in the healthy reference population. In addition, only 2 of these 6 patients with HLHS had loci involved in >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 V** (available at www.jpeds.com). Overall, these findings suggest that cnVUSs do not clearly represent healthy "background" genetic variation and may be a distinct genetic subset of 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 clinically relevant genes known to involve CNVs.[18] Only a small minority of cnVUS-positive subjects were found to host genes associated with genetic diseases (**Table VI**). Of the remaining 20 subjects who did not have CNV in the healthy population, only 5 cnVUSs involved loci containing genes in which single gene mutations or CNV are 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 (CHD) and arrhythmias. The cnVUS with a gain in 1q21.1 contains *GJA5*, which encodes a gap junction protein, alpha 5 (connexin 40), and has been associated with the development of atrial fibrillation, atrial standstill, and cardiac malformations.

The 1-year Kaplan-Meier survival was 68.3% in patients with a normal CMA, 84.0% in those with cnVUS, and 33.3% in those with pathological CNV ($P = .003$, log-rank test). Survival by Kaplan-Meier analysis is illustrated in **Figure 2**. We noted a significantly higher prevalence of extracardiac abnormalities in infants with HLHS with abnormal CMAs (55.6%) compared with infants with normal (5.7%) and cnVUS (8%) CMA results ($P < .001$).

Right ventricular dysfunction was also more common in the abnormal CMA group (22.2%) compared with the cnVUS (7.7%) and normal (5.7%) groups ($P = .016$). There were no significant differences in the HLHS morphological variation based on CMA class result, including prevalence of MS/AS, MS/AA, or MA/AA; presence of a left superior vena cava; degree of tricuspid regurgitation; levocardinal vein; abnormal coronary arteries; ascending aorta diameter; and anomalous pulmonary venous return (**Table VII**).

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

Chromosome	CMA change	Medically relevant gene/phenotype
Xp22.13p22.12	Gain, gain	—
7q21.11	Gain	*MAGI2*, associated with bipolar affective disorder, schizophrenia, and infantile spasms
22q13.2	Gain	—
1p36.33	Gain	*SKI*, monosomy 1p36, 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	*GJA5*, associated with learning disability, autism spectrum disorders, macrocephaly, behavioral features, atrial fibrillation, atrial standstill, and tetralogy of Fallot *GJA8*, associated with cataracts
20q12q13.11	Loss	—
6p25.3	Gain	—
2p21	Gain	—
1q21.1	Gain	*HFE2*, 1q21.1 duplication syndrome (no further information)
2p13.1p12	Gain	—
17q23.2	Loss	—
6q23.3	Loss	*AHI1*, haploinsufficient 6q23.3
7q21.13	Gain	—
5p13.2	Loss	—
9p24.3	Loss	—
16q22.1	Loss	—
6q24.2q25.3	Absence of heterozygosity	—

Given the differences in survival based on CMA groups, we next evaluated a number of secondary endpoints by CMA class. We identified no significant differences in the use of tracheostomy, feeding tube, or cardiopulmonary resuscitation based on CMA class ($P = 1.00$, 1.00, and .139, respectively; **Table VIII**). Of note, ECMO was not used in probands with either cnVUS or pathological CMA findings, but 11 probands with normal CMAs required ECMO (15.7%) ($P = .038$).

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 have a worse prognosis compared with their normal counterparts. Multiple studies have found that chromosomal abnormalities and genetic syndromes negatively impact interstage mortality. One study found that the 1-year and 10-year survival in patients with HLHS and a pathological genetic syndrome was one-half that in those with a normal genetic profile.[3,12,19,20] Among other known syndromes, Turner syndrome, trisomy 18, trisomy 13, and Down syndrome are associated with higher early mortality in HLHS.[21,22] One study reported 50% 1-month survival in patients with HLHS with

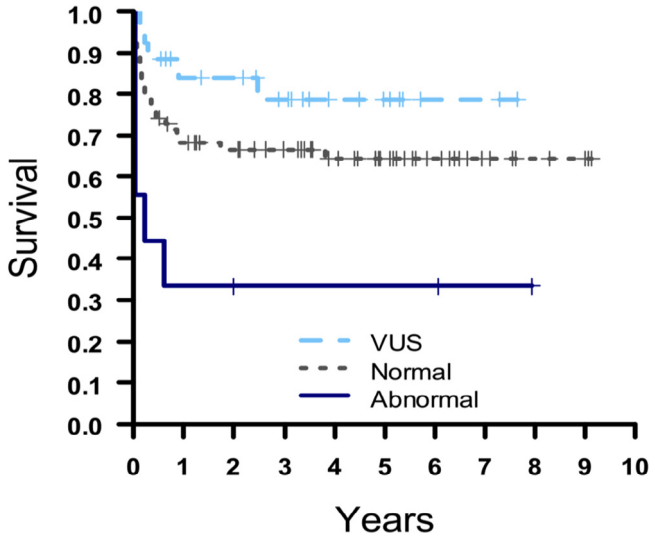

Figure 2. Kaplan-Meier analysis of transplantation-free survival among neonates with HLHS by CMA results, with normal (*gray*), cnVUS (*light blue*), and abnormal (*dark blue*). Censored observations are indicated as plus signs (+).

Table VII. Clinical characteristics of HLHS cohort by CMA class

Characteristics	Normal (n = 70)	cnVUS (n = 25)	Abnormal (n = 10)	*P* value
MS/AS, MS/AA, MA/AA, n (%)				
MA/AA	32 (46.4)	17 (65.4)	3 (33.3)	.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, n (%)				
No	65 (92.9)	26 (100)	9 (100)	.436
Yes	5 (7.1)	0	0	
PAPVR/TAPVR, n (%)				
No	68 (97.2)	24 (92.3)	9 (100.0)	.510
Yes	2 (2.8)	2 (7.7)	0	
Degree of TR, n (%)				
Trivial/none	40 (57.1)	11 (42.3)	4 (44.4)	.503
Mild	23 (32.9)	12 (46.2)	3 (33.3)	
Moderate/severe	7 (10.0)	3 (11.5)	2 (22.2)	
Moderate/severe TR, n (%)				
No	64 (91.4)	23 (88.5)	7 (77.8)	.333
Yes	6 (8.6)	3 (11.5)	2 (22.2)	
RV function, n (%)				
Normal	62 (89.9)	20 (80.0)	5 (55.6)	.008
Mildly/moderately depressed	7 (10.1)	5 (20.0)	4 (44.4)	
Moderate RV dysfunction, n (%)				
No	69 (98.6)	24 (92.3)	7 (77.8)	.016
Yes	1 (1.4)	2 (7.7)	2 (22.2)	
Extracardiac abnormalities, n (%)				
No	66 (94.3)	24 (92.3)	4 (44.4)	<.001
Yes	4 (5.7)	2 (7.7)	5 (55.6)	
Sano, BTS, or hybrid, n (%)				
BTS	45 (64.3)	17 (65.4)	3 (33.3)	.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)	.743
Yes	16 (22.9)	4 (15.4)	2 (22.2)	
Ascending aorta diameter, cm, mean ± SD	0.34 ± 0.28	0.27 ± 0.15	0.24 ± 0.07	.204

ASD, atrial septal defect; *BTS*, Blalock-Taussig shunt; *LSVC*, left superior vena cava; *RV*, right ventricular; *TR*, tricuspid regurgitation; *PAPVR/TAPVR*, partial anomalous pulmonary venous return/total anomalous pulmonary venous return.

Turner syndrome, as opposed to 85% in nonsyndromic patients with HLHS,[23] and another study found significantly increased cumulative mortality compared with females without Turner syndrome, even with multivariate analysis controlling for low birth weight.[24] Another study demonstrated a 5-year survival of 61% in patients with Down syndrome, compared with 85% in nonsyndromic patients.[25] Therefore, an abnormal result detected on CMA is widely considered a predictor of increased mortality in CHD. However, with the rapidly expanding use of clinical CMA, which is 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 patients with HLHS having a cnVUS result, this is a common issue that likely will increase in importance with the expanding use of genetic testing.

The general consensus among most studies is that the prevalence of chromosomal anomalies in CHD is higher when extracardiac anomalies are also present.[26] Thus, both genetic syndromes and extracardiac anomalies, as individual and combined factors, are associated with heightened mortality.[20,27] However, our patients with cnVUS results did not exhibit the same characteristics as their abnormal counterparts, achieving significantly higher survival rates with a significantly lower prevalence of extracardiac abnormalities.

To evaluate the genetic significance of observed cnVUS results, we compared results with loci identified in healthy individuals and determined whether there was any overlap with loci of clinically 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.[28] When comparing the cnVUS cohort with ostensibly healthy individuals, fewer than one-quarter of our patients had the same loci identified in the healthy individuals. As such, we cannot assume that cnVUS results are comparable between patients with HLHS and healthy individuals; rather, they appear to be genetically distinct. Of the remaining CNVs not included in the cross-referenced database, 5 had clinically 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 clinically manifested only if coinherited with mutations in the *SCN5A* gene.[29,30] Furthermore, clinical manifestations of atrial fibrillation were associated with rare, novel missense and nonsense mutations.[31-34] Mouse models with limited or absent expression of *GJA5* have demonstrated a higher prevalence of cardiac anomalies, usually of conotruncal origin, with one-third of hearts exhibiting

Table VIII. Secondary outcome variables by CMA class

Variables	Normal	cnVUS	Abnormal	*P* value
Tracheostomy, n (%)				
No	67 (95.7)	25 (96.2)	9 (100)	1.000
Yes	3 (4.3)	1 (3.8)	0	
Feeding tube, n (%)				
No	53 (75.7)	20 (76.9)	7 (77.8)	1.000
Yes	17 (24.3)	6 (23.1)	2 (22.2)	
CPR, n (%)				
No	61 (87.1)	26 (100)	8 (88.9)	.139
Yes	9 (12.9)	0	1 (11.1)	
ECMO, n (%)				
No	59 (84.3)	26 (100)	9 (100)	.038
Yes	11 (15.7)	0	0	

CPR, cardiopulmonary resuscitation.

tetralogy of Fallot or double-outlet right ventricle.[35] Although most of these genes have little immediate clinical impact on survival in patients with HLHS, 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 clinically actionable genes include mainly long-term neurobehavioral/psychiatric conditions. These conditions are often associated with significant functional limitations in executive planning, visual-motor integration, and thought processing.[36] 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.[36-38] Ultimately, cnVUS results were not commonly found in ostensibly healthy individuals, and loci did not commonly 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 with cnVUSs.

Our finding that patients with HLHS with cnVUSs have superior survival to their syndromic counterparts suggests that 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, given our study methodology, we cannot definitively conclude that 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 pathological mutations as part of end-of-life care discussions with the families of patients, and clinical management has the potential to be influenced by the presence of abnormal CMA.[39,40]

We recognize the limitations of our small sample size and the fact that CMA testing of infants with HLHS is not universal. Thus, 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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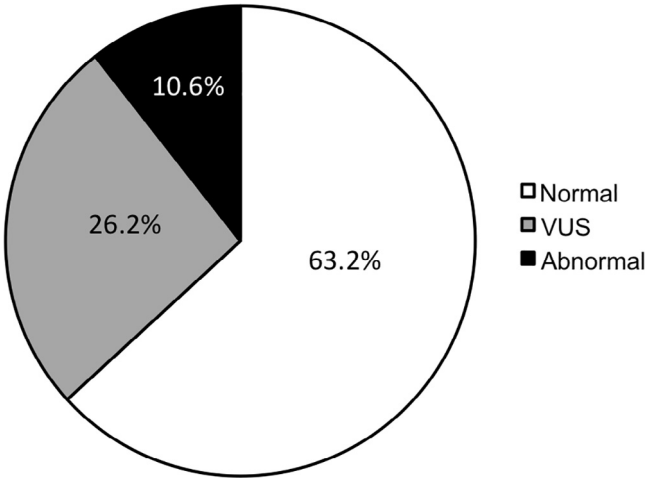

Figure 1. Pie chart of CMA results for any test indication at TCH between June 2008 and December 2016. Test results are divided into those interpreted as abnormal (*black*), cnVUS (*gray*), and normal (*white*).

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

Characteristics	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)	.197
Gestational age, wk	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)	.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)	.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)	.103

FOC, fronto-occipital circumference.

Table II. Summary of clinical demographic data for neonates with HLHS who underwent CMA testing

Characteristics	Value
Total cases, n	105
Sex, n (%)	
Male	60 (57.0)
Female	45 (43.0)
CMA cohort, n (%)	
Normal	70 (66.7)
cnVUS	26 (24.7)
Abnormal	9 (8.6)
Race/ethnicity, n (%)	
White	54 (51.0)
Hispanic	49 (47.0)
African American	2 (2)

Table III. Summary of CNV for subjects with HLHS hosting abnormal/pathogenic CNVs

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, Xp22.33q28	Loss	Turner syndrome
	Yp11.31q11.1	Loss	
		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 IV. Summary of CNV for subjects with HLHS hosting CNVs of unknown significance

Subject	CNV		Genes
1	Xp22.13p22.12	Gain, Gain	*MTTP, PHKA2, GPR64, PDHA1, MAP3K15*
2	9p21.3	Gain	None
3	4q25	Loss	*PAPSS1*
4	8p23.1	Gain	*GATA4, NEIL2, FDFT1, CTSB*
5	7q21.11	Gain	*PHTF2, MAGI2, RPL13AP17*
6	22q13.2	Gain	*BIK, MCAT, TSPO, TTLL12, SCUBE1*
7	1p36.33	Gain	*PRKCZ, C1orf86, LOC100128003, SKI*
8	Xq21.31	Loss	*CPXCR1*
9	16q24.2	Gain	*ZNF469, ZFPM1*
10	Xq28	Gain	*TMLHE*
11	1q21.1q21.2	Gain	*PRKAB2, PDIA3P, FMO5, CHD1L, LOC100289211, BCL9, ACP6, GJA5, GJA8, GPR89B, GPR89C, PDZK1P1, NBPF11, NBPF24*
12	15q13.3	Gain	*CHRNA7*
13	20q12q13.11	Loss	*PTPRT*
14	15q11.2	Loss	*TUBGCP5, CYFIP1, NIPA2, NIPA1*
15	6p25.3	Gain	*LOC285768*
16	2p21	Gain	*CALM2*
17	1q21.1	Gain	*SEC22B, NOTCH2NL, NBPF10, HFE2, TXNIP, POLR3GL, RBM8A, GNRHR2, PEX11B, ITGA10, ANKRD34A, LIX1L, ANKRD35, PIAS3, NUDT17, POLR3C, RNF115, CD160, PDZK1*
18	2p13.1p12	Gain	*SEMA4F, HK2, POLE4, TACR1*
19	17q23.2	Loss	*PPM1D*
20	6q23.3	Loss	*AHI1*
21	7q21.13	Gain	*ZNF804B, C7orf62, DPY19L2P4, STEAP1, STEAP2*
22	5p13.2	Loss	*IL7R*
23	9p24.3	Loss	*DOCK8*
24	6q25.1	Loss	*PLEKHG1*
25	16q22.1	Loss	*PDXDC2, PDPR*
26	6q24.2q25.3	Absence of heterozygosity	*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, RMND1, C6orf211, C6orf97, ESR1, SYNE1, MYCT1, VIP, FBXO5, MTRF1L, RGS17, OPRM1, IPCEF1, CNKSR3.*

Table V. 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	1513/11 732 = 12.9%
3	4q25	Loss	108456682-108806918	49/11 732 = 0.42%
4	8p23.1	Gain	11598718-11843369	107/11 732 = 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	114/11 732 = 0.97%
13	20q12q13.11	Loss	41617589-41737558	—
14	15q11.2	Loss	22842145-23086692	488/11 732 = 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 /11 732 = 0.60%
25	16q22.1	Loss	68567940-68753268	—
26	6q24.2q25.3	Absence of heterozygosity	145377585-155757781	—

*No value indicates no CNV observed involving the locus.