# Microcephaly is associated with early adverse neurologic outcomes in hypoplastic left heart syndrome

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**BACKGROUND:** Hypoplastic left heart syndrome (HLHS) is associated with significant mortality and morbidity. Fetal head growth abnormalities have been identified in a subset of HLHS fetuses, but it is unclear whether specific patterns of maladaptive growth affect clinical outcomes. We hypothesized that poor fetal head growth is associated with an increased frequency of adverse clinical outcomes.

**METHODS:** We retrospectively examined a cohort of HLHS patients from midgestation to 1 y of age. Fetal and birth anthropometric measurements were analyzed using the Olsen standard, and clinical outcomes were obtained.

**RESULTS:** A total of 104 HLHS patients were identified over a 12-y period; fetal data were available in 38 cases. HLHS neonates demonstrated a high incidence of microcephaly (12%), small head size (27%), and poor head growth (32%). All-cause mortality was 31% at 30 d and 43% at 1 y. Neurologic outcomes were observed in 12% of patients and were significantly increased with microcephaly (43 vs. 4%; $P = 0.02$). The average length of hospital stay following stage I palliation was 33.4 ± 33 d, correcting for early death.

**CONCLUSION:** In term nonsyndromic HLHS, fetal and neonatal microcephaly are associated with early adverse neurologic outcomes but not mortality.

Hypoplastic left heart syndrome (HLHS) is a complex cardiovascular malformation (CVM) strictly defined as atresia or stenosis of the aortic and mitral valves and hypoplasia of the left ventricle and ascending aorta (1). HLHS is uniformly fatal without a series of palliative reconstructive surgeries in the first years of life. Despite dramatic improvements in treatment, HLHS continues to be associated with significant mortality and morbidities (2–4), including short- and long-term central nervous system (CNS) abnormalities (5–7). In addition, there is a rapidly growing population of adult survivors with chronic morbidities that are only now beginning to be understood (8). Although some surgical risk factors have been identified, such as the presence of a genetic syndrome, low birth weight (LBW, <2.5 kg), or a prohibitively small ascending aorta, our ability to predict adverse clinical outcomes remains limited (4,9,10). Understanding the noncardiac parameters of fetal HLHS may lead to the identification of new risk factors that may ultimately improve clinical management.

Impaired postnatal growth is commonly associated with many types of CVM, including HLHS, and has been attributed to disruption of normal feeding behavior in the neonatal period. Postoperative failure to thrive is common, often requiring gastrostomy (G)-tube placement (11). Poor growth has been associated with genetic and environmental factors, but the prevailing view identifies a hypermetabolic state and malabsorption as common causes (11,12). However, increasing evidence shows that growth abnormalities begin before birth (11–13), suggesting the contribution of other necessary genetic and environmental factors. The majority of HLHS cases are now diagnosed *in utero*, contributing to increased survival, and are not typically associated with fetal demise (9,14). Given the complexity of development and growth in general, it remains unclear why some HLHS fetuses and infants have growth abnormalities and others do not.

Late CNS outcomes have been observed in stage III surgical survivors at school age, including learning disabilities, attention-deficit hyperactivity disorder, fine and gross motor abnormalities, and speech and behavioral problems (2,15). The prevailing view is that chronic cyanosis and multiple cardiopulmonary bypass surgeries early in life lead to cognitive, motor, and behavioral deficits later in life (6,16). However, recent studies have implicated additional factors in the relationship between CNS abnormalities identified in neonates after birth but before surgery (17,18). Furthermore, early CNS abnormalities, including agenesis of the corpus callosum, as well as holoprosencephaly and microcephaly, have been identified in approximately one-quarter of HLHS neonates (5,15). Recently, we described poor fetal head growth and white matter injury despite normal brain weight in HLHS, identifying a spectrum of fetal CNS pathology (19). It is unknown whether these fetal head growth abnormalities are associated with adverse clinical outcomes.

In this study, we examined fetal head growth and clinical outcomes in HLHS. There is an emerging interest in fetal

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Received 28 June 2012; accepted 13 December 2012; advance online publication 15 May 2013. doi:10.1038/pr.2013.61

growth in the context of CVM, but its relationship to clinical course is largely unknown. We hypothesized that poor fetal head growth would be associated with an increased frequency of early adverse clinical outcomes. In term nonsyndromic HLHS, fetal and neonatal microcephaly is associated with early adverse neurologic outcomes but not with mortality or other clinical outcomes. A better understanding of fetal growth, especially fetal head growth, may improve long-term risk stratification and counseling for late outcomes in HLHS and other CVM patients.

**Table 1.** Fetal and neonatal anthropometrics by timing of diagnosis

| | All patients | Fetal | Neonatal |
|---|---|---|---|
| *N* | 104 | 42 | 62 |
| General characteristics | | | |
| Male sex (*n*, %) | 69 (66) | 26 (62) | 43 (69) |
| Newborn HLHS phenotype | | | |
| Aortic atresia/mitral atresia (*n*, %) | 51 (55) | 27 (64) | 24 (48) |
| Aortic atresia/mitral stenosis (*n*, %) | 21 (23) | 5 (12) | 16 (32) |
| Aortic stenosis/mitral stenosis (*n*, %) | 20 (22) | 10 (24) | 10 (20) |
| Fetal characteristics | | | |
| EGA (wk) | | 26.6 ± 5.0 | NA |
| Fetal weight (g) | | 1,211 ± 783 | NA |
| Fetal weight percentile | | 61 ± 29 | NA |
| SGA (weight ≤ 10th percentile) | | 3 (9%) | NA |
| Fetal HC (mm) | | 242 ± 47 | NA |
| Fetal HC percentile | | 46 ± 29 | NA |
| Fetal microcephaly (HC ≤ 3rd percentile) | | 3 (8%) | NA |
| Fetal small head (HC ≤ 10th percentile) | | 5 (13%) | NA |
| Birth characteristics | | | |
| EGA (wk) | 39.0 ± 1.2 | 38.7 ± 1.1 | 39.2 ± 1.2* |
| Birth weight (g) | 3,205 ± 485 | 3,060 ± 444 | 3,300 ± 490* |
| Birth weight percentile | 38 ± 24 | 33 ± 24 | 42 ± 24 |
| Low birth weight (weight < 2,500 g) | 8 (8%) | 5 (13%) | 3 (5%) |
| SGA (weight ≤ 10th percentile) | 11 (12%) | 8 (20%) | 3 (6%)* |
| HC (cm) | 33.3 ± 1.5 | 33.4 ± 1.6 | 33.0 ± 1.3 |
| HC percentile | 36 ± 26 | 36 ± 26 | 32 ± 29 |
| Microcephaly (HC ≤ 3rd percentile) | 4 (12%) | 3 (10%) | 1 (20%) |
| Small head (HC ≤ 10th percentile) | 9 (27%) | 8 (28%) | 1 (20%) |
| Poor head growth | | 9 (32%) | NA |
| Poor somatic growth | | 18 (55%) | NA |

Poor growth was indicated if *z*-score decreased >0.67. Sample size varied by characteristic due to availability of data, e.g., only 34 patients (29 fetal and 5 neonatal) had an HC at birth.

EGA, estimated gestational age; HC, head circumference; HLHS, hypoplastic left heart syndrome; NA, not applicable; SGA, small for gestational age.

*$P < 0.05$ for difference between infants with fetal and neonatal data and infants with only neonatal data, *t*-test or $\chi^2$ analysis.

## RESULTS

### Study Population

A total of 157 HLHS patients were identified at Cincinnati Children's Hospital Medical Center over the 12-y period. Among the 104 patients who qualified for the study, there were 42 fetal diagnoses and anthropometric data were available for 38 (90% of fetal diagnoses, 37% of total). Mortality and G-tube placement data were available for 99 (94%) and 81 (78%) cases, respectively. A total of 53 infants were excluded for associated cardiac anatomy (29), prematurity (17), twinning (4), and genetic syndromes (3).

### Fetal Anthropometrics Are Normal at Midgestation

Mean gestational age at the time of fetal anthropometric measurements was 27 wk. Because gestational age varied among affected fetuses, anthropometric measures are reported as both an absolute measurement and a percentile (**Table 1**). Mean fetal head circumference (HC) of the HLHS patients was 242 ± 47 mm, corresponding to a gestational age–adjusted percentile of 46 ± 29. Mean estimated fetal weight was 1,211 ± 783 g, corresponding to a percentile of 61 ± 29. At midgestation, small head size (13%) and being small for gestational age (SGA) (9%) were observed at the anticipated frequency.

### Relative Cerebral Blood Flow Is Not Clearly Associated With Poor Head Growth

A subset of 22 fetuses had comprehensive fetal echocardiographic data (**Table 2**). Middle cerebral artery (MCA) Doppler velocities and pulsatility indexes (PIs) were normal for the entire cohort (20,21), including those with head growth abnormalities ($n = 4$). The mean MCA PI was 2.14 ± 0.30 in fetuses with normal growth and 2.32 ± 0.26 in fetuses with head growth abnormalities ($P$ = not significant). There was no difference between PIs and resistance indexes (data not shown). Of note, umbilical artery (UA) Doppler analysis was normal for all fetuses, suggesting that there were no cases with significant placental insufficiency, a common cause of growth restriction. The mean MCA:UA PI ratio, an index of relative cerebral blood flow, was 1.71 ± 0.30 in fetuses with normal growth and

**Table 2.** Fetal middle cerebral artery Doppler analysis

| | Normal head size and growth | Any growth abnormality | *P* value |
|---|---|---|---|
| *N* | 18 | 4 | |
| MCA PI | 2.14 ± 0.30 | 2.32 ± 0.26 | NS |
| UA PI | 1.71 ± 0.30 | 1.67 ± 0.05 | NS |
| MCA/UA PI | 1.30 ± 0.31 | 1.39 ± 0.18 | NS |

Significance determined by Fisher's exact test.

MCA, middle cerebral artery; NS, not significant; PI, pulsatility index; UA, umbilical artery.

1.67 ± 0.05 in fetuses with head growth abnormalities ($P$ = not significant), suggesting that blood flow perturbations did not affect head growth in this small sample.

### Small Head Size at Birth Is Not Dependent on Alterations in Somatic Growth

The average gestational age at birth was 39 wk and was slightly lower for the prenatally diagnosed group ($P < 0.05$). The mean birth HC was 33.3 cm ($z$-score = −0.54 ± 1.0). Small head size was evident in 9 patients (27%), of which microcephaly was observed in 4 (12%). The average birth weight was 3,205 g ($z$-score = −0.37 ± 0.86). SGA was present in 11 neonates (12%), and 8 (8%) demonstrated LBW. Among the 9 neonates with small head size, 4 (44%) were also SGA (symmetric growth restriction), whereas the remaining 5 (56%) had normal weight (asymmetric growth restriction).

### Fetal Head Growth Abnormalities Are Common at Birth

The mean HC and weight percentiles decreased from fetal to neonatal time points. In general, anthropometrics were normal at midgestation and followed a suboptimal growth trajectory through birth (**Figure 1**). During this time period, which was 13 wk on average, the HC $z$-score change was −0.46 ± 1.20, and the weight $z$-score change was −0.82 ± 0.72. The proportion exhibiting intrauterine growth restriction (SGA) increased from 9% *in utero* to 20% at birth. Poor head growth was observed in 32% of cases, and poor weight gain in 55% (**Table 1**).

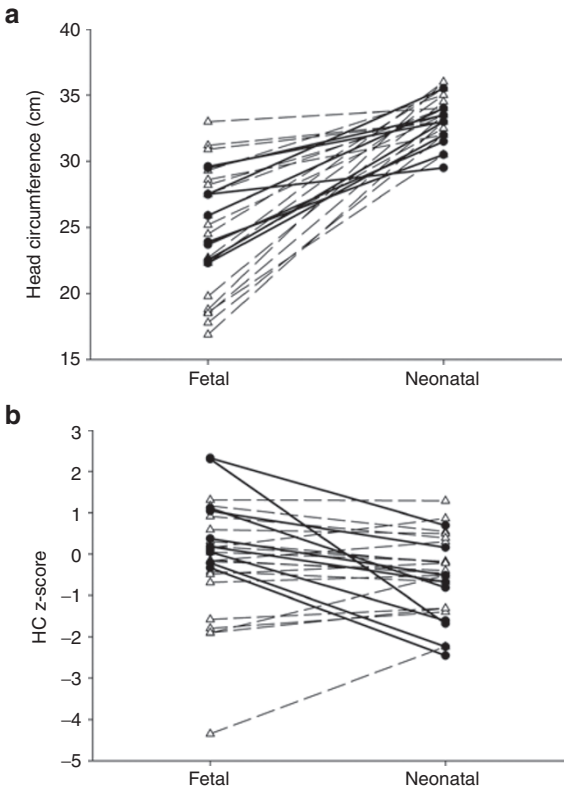


**Figure 1.** Fetal head growth in HLHS. Head growth from midgestation to birth is shown using (**a**) an absolute head circumference measurement and (**b**) a change in z-score comparison. Each line represents an individual fetus with either normal (triangles, dashed lines) or abnormal (circles, solid lines) head growth. HLHS, hypoplastic left heart syndrome.

### HLHS Continues to Be Associated With Substantial Adverse Clinical Outcomes

Overall all-cause mortality at 30 d was 31% ($n$ = 31) and at 1 y was 43% ($n$ = 43). Early adverse neurologic outcomes were present in 12 patients (12%), including clinical seizure activity (5), ischemia (3), hemorrhage (2), and unspecified injury (2). A G-tube was placed in 11 patients (14%). The average length of hospital stay (LOS) was 33.4 ± 33 d, censoring preoperative and postoperative day 0 deaths.

### Microcephaly Is Associated With Early Adverse Neurologic Outcomes but Not Mortality

The incidence of early adverse neurologic outcomes is significantly increased with microcephaly at birth (43 vs. 4%; $P$ = 0.02) and marginally increased with fetal microcephaly (33 vs. 3%; $P$ = 0.06), but not small fetal head size, poor fetal head growth, or small neonatal head size. Early adverse neurologic outcomes were also associated with lower gestational age ($P$ = 0.03) and being SGA ($P$ = 0.03). However, growth abnormalities were not associated with 1-y mortality (**Table 3**), G-tube placement (**Table 3**), or LOS (**Table 4**). Mortality analysis was also performed at day 30 of life, with similar results (data not shown). Surprisingly, neither LBW nor being SGA, established risk factors for early mortality, was associated with mortality, G-tube placement, or LOS in our study. Taken together, these findings suggest that microcephaly predicts early adverse neurologic outcomes in term nonsyndromic infants with HLHS.

## DISCUSSION

The findings of the current study indicate for the first time that fetuses or neonates with microcephaly are a high-risk subset of term nonsyndromic infants with HLHS for early adverse neurologic outcomes but do not appear to be at an elevated risk for mortality. Of note, the more common occurrences of small head size and poor fetal head growth are not associated with early adverse clinical outcomes in HLHS. Head growth abnormalities may have an additional impact on late outcomes, such as cognitive and behavioral deficits, as well as quality of life. Established shared factors regulating CNS and heart organogenesis and growth may impact long-term mortality and morbidity (**Figure 2**). As our understanding of the natural history of CNS abnormalities in the context of HLHS improves, we may be able to identify discrete subsets of HLHS fetuses that are associated with different early and late clinical courses. Identifying HLHS fetuses at higher risk of adverse outcomes may improve counseling efforts, advise decision making for potential fetal intervention, and inform postnatal surgical and clinical management.

Increasing evidence has identified CNS abnormalities in HLHS fetuses and neonates, identifying potential risk factors for short- and long-term clinical outcomes. Clinical seizure activity has long been viewed as an acquired injury due to

**Table 3.** Anthropometrics and clinical outcomes: mortality and G-tube placement

| | 1-y Mortality | | G-tube placement | |
|---|---|---|---|---|
| | Yes | No | Yes | No |
| Fetal measures | | | | |
| *N* | 16 | 21 | 5 | 27 |
| EGA (wk) | 26 (23, 31) | 26 (23, 28) | 27 (23, 27) | 26 (22, 31) |
| Weight z-score | 0.38 (−0.30, 1.5) | 0.46 (−0.32, 1.1) | 0.89 (0.70, 1.3) | 0.32 (−0.32, 1.1) |
| SGA | 1 (7%) | 2 (11%) | 1 (20%) | 2 (9%) |
| HC z-score | −0.06 (−0.84, 0.85) | −0.15 (−0.54, 0.19) | −0.14 (−0.42, 1.0) | −0.16 (−0.86, 0.31) |
| Microcephaly (HC ≤ 3rd percentile) | 0 (0%) | 3 (14%) | 1 (20%) | 2 (7%) |
| Small head (HC ≤ 10th percentile) | 2 (13%) | 3 (14%) | 1 (20%) | 4 (15%) |
| Birth measures | | | | |
| *N*[a] | 40 | 54 | 11 | 68 |
| EGA (wk) | 39 (38, 39) | 39 (38, 40) | 39 (38, 39.5) | 39 (38, 40) |
| Weight z-score | −0.5 (−0.82, −0.14) | −0.34 (−0.77, 0.04) | −0.43 (−0.58, 0.25) | −0.36 (−0.88, 0.06) |
| LBW (weight < 2,500 g) | 4 (10%) | 4 (7%) | 0 (0%) | 6 (9%) |
| SGA | 5 (14%) | 6 (12%) | 1 (13%) | 9 (14%) |
| HC z-score | −0.52 (−1.2, 0.19) | −0.34 (−1.3, 0.16) | −0.99 (−1.7, −0.46) | −0.39 (−1.3, 0.30) |
| Microcephaly (HC ≤ 3rd percentile) | 1 (6%) | 3 (17%) | 1 (17%) | 3 (14%) |
| Small head (HC ≤ 10th percentile) | 4 (25%) | 5 (28%) | 3 (50%) | 6 (27%) |
| Fetal growth | | | | |
| *N* | 14 | 18 | 5 | 23 |
| Change in weight z-score | −0.73 (−1.5, −0.4) | −0.96 (−1.3, −0.20) | −1.2 (−1.9, −0.08) | −0.59 (−1.1, −0.28) |
| Poor somatic growth | 7 (50%) | 11 (61%) | 3 (60%) | 11 (48%) |
| Change in HC z-score | −0.62 (−1.6, 0.17) | −0.25 (−0.53, 0.59) | −0.32 (−0.88, −0.25) | −0.16 (−0.74, 0.34) |
| Poor head growth | 6 (46%) | 3 (20%) | 2 (40%) | 5 (25%) |

Data are presented either as *N* (%) or as median (interquartile range). Sample size varied by characteristic due to availability of data. Significance tested using Fisher's exact test or Wilcoxon rank-sum test. There were no statistically significant differences identified.

EGA, estimated gestational age; G, gastrostomy; HC, head circumference; LBW, low birth weight; SGA, small for gestational age.

[a]*N* for birth head circumference measures differs from *N* with weight and gestational age (e.g., 1-year mortality: yes, $n = 16$; no, $n = 18$ have birth head circumference measurements).

neonatal cardiac surgery; for example, it has been shown that prolonged deep hypothermic circulatory arrest is associated with a higher incidence of seizures (15). Recently, however, the Pediatric Heart Network reported motor and cognitive developmental delays in HLHS survivors at the age of 14 mo that were independent of intraoperative management strategies (22). Furthermore, it has been shown that the CNS in term HLHS infants is immature and characterized by white matter injury (19,23). Microcephaly is an established risk factor for developmental delays in general, and in HLHS distinguishing primary from secondary microcephaly is challenging. In the current study, all neurologic outcomes occurred in the postoperative period, suggesting microcephaly may be a marker for an immature brain that is specifically vulnerable to CNS injury (e.g., hypoxia–ischemia) in the context of cardiac surgery. Taken together, brain findings and anthropometrics may inform ongoing efforts to develop new neuroprotection strategies.

Surprisingly, neither LBW nor SGA infants with HLHS demonstrated an increased frequency of adverse clinical outcomes, including mortality, at 1 y, which is contrary to previous reports (12,24,25). Although this may reflect an inadequate cohort size, a more compelling possibility is that by excluding prematurity and genetic syndromes (high-risk infants), two common causes of LBW, adverse outcomes are associated more strongly with gestational age rather than weight (26). Indeed, LBW infants with HLHS demonstrate an increased frequency of mortality after stage I surgery (27) but not before surgery (9). Whether identification of head growth abnormalities at midgestation can be used to predict early adverse clinical outcomes in *all* HLHS cases, including higher-risk infants, is yet to be determined.

In at least some cases, the fetus is identified as having aortic stenosis at midgestation, which then evolves into HLHS during the second half of pregnancy (28). Flow abnormalities have been implicated in CVM. In the context of HLHS, blood flow redirected across the foramen ovale due to aortic stenosis results in decreased blood flow through the left-sided structures and presumably contributes to subsequent hypoplasia. It has been suggested that decreased blood flow and/or decreased arterial pulsatility in the cerebral circulation results in poor head growth, analogous to placental abnormalities resulting

**Table 4.** Anthropometrics and clinical outcomes: LOS

| | LOS without growth abnormality | LOS with growth abnormality | LOS Spearman ρ correlation |
|---|---|---|---|
| Fetal measures | | | |
| EGA (wk) | | | −0.08 |
| Weight (z-score) | | | −0.36 |
| SGA | 19 (7, 34) | 65[a] | |
| HC (z-score) | | | 0.16 |
| Microcephaly (≤ 3rd percentile) | 18 (7, 30) | 65 (14, 116)[a] | |
| Small head (≤ 10th percentile) | 18 (7, 30) | 65 (14, 116)[a] | |
| Birth measures | | | |
| EGA (wk) | | | 0.04 |
| Weight (z-score) | | | 0.14 |
| LBW (<2,500 g) | 23 (15, 43) | 14 (6, 21) | |
| SGA (≤ 10th percentile) | 20 (15, 41) | 21 (12, 29) | |
| HC (z-score) | | | 0.08 |
| Microcephaly (≤ 3rd percentile) | 17.5 (7.5, 40) | 21 (9, 28.5) | |
| Small head (≤ 10th percentile) | 18 (8, 38) | 14 (4, 29) | |
| Fetal growth | | | |
| Change in weight z-score | | | 0.55* |
| Poor somatic growth | 38 (17, 65) | 17 (4, 24)* | |
| Change in HC z-score | | | 0.51* |
| Poor head growth | 19 (14, 42) | 4 (3, 29)* | |

Data are presented as median (interquartile range) or Spearman ρ correlation.

EGA, estimated gestational age; HC, head circumference; LBW, low birth weight; LOS, length of hospital stay; SGA, small for gestational age.

[a]Unstable estimate based on $n < 5$. *$P < 0.05$ from Wilcoxon rank-sum (categorical) or Spearman ρ correlation (continuous) tests.

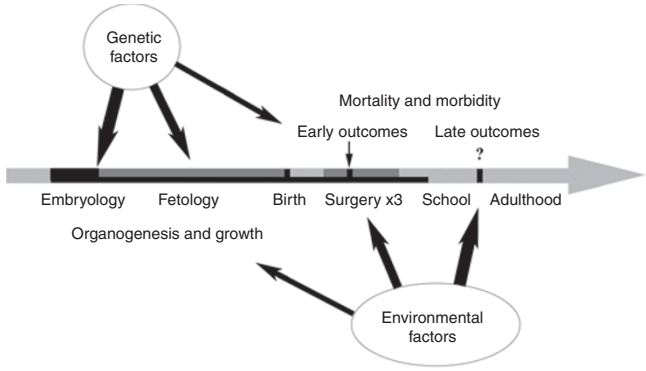


**Figure 2.** Model of factors contributing to growth and outcomes in HLHS. Prenatal and postnatal genetic and environmental factors contribute to the development of HLHS and associated CNS abnormalities over time (gray arrow). Shared genetic factors contribute to organogenesis, which occurs during embryonic development for heart (black box) and extends through fetal development and early postnatal life for brain (long black line). Because CNS development is significantly longer than cardiac development, the CNS is more vulnerable to ongoing insults. This study examined the impact of fetal growth on early outcomes (1 y), but any association with late outcomes remains unknown (question mark). Genetic factors include heritable germ-line variation, epigenetic variation, and somatic mutation; these have a disproportionate effect on early organ development (big black arrow at the top). Environmental factors include maternal–fetal interactions, medications, and nutrition; these have a disproportionate effect on later tissue growth (big black arrow at the bottom). Specific factors that potentially impact CNS structure and function and are due to the CVM itself include altered hemodynamics and low cardiac output (early), as well as chronic cyanosis and thromboembolism (late). CNS, central nervous system; CVM, cardiovascular malformation; HLHS, hypoplastic left heart syndrome.

in intrauterine growth restriction. Abnormal MCA blood flow has been observed in fetuses with evolving HLHS (21,29,30), but the clinical significance of this finding is unclear. Currently, some centers advocate fetal intervention in fetuses with severe valvar aortic stenosis in order to improve antegrade blood flow across the aortic valve, postulating that this forward flow may prevent the progression of aortic stenosis to HLHS and result in better neurodevelopmental outcomes (31). The specificity of the resulting multivariable threshold scoring system based on quantitative cardiac findings is modest, suggesting that the addition of noncardiac parameters, such as anthropometrics, may improve identification of those fetuses that will develop HLHS.

There is considerable evidence that a combination of genetic (32,33) and maternal–fetal environmental factors (34) contribute to HLHS and associated CNS abnormalities during embryonic and fetal development (**Figure 2**). The prevailing view is that CNS abnormalities arise as a result of the CVM, but one plausible alternative is that both arise concomitantly as a result of shared etiologic factors and the CNS abnormalities worsen as a result of the early hemodynamic changes due to the CVM. Multiple chromosomal abnormalities are associated with HLHS, including trisomy 13, trisomy 18, and Turner and Jacobsen syndromes, suggesting that the etiology of HLHS is at least in part genetic (35). Of note, these genetic syndromes are also characterized by head growth and CNS abnormalities, consistent with the idea that there may be a common genetic basis. A growing body of evidence shows that there is a major genetic contribution underlying nonsyndromic HLHS (33,36). Although the designation of HLHS as a "syndrome" is thought to be a misnomer from a human genetics perspective, extracardiac anomalies are frequently encountered, including CNS and renal abnormalities (5,35,37). The shared genetic basis of these findings suggests the possibility that HLHS is a true heart–brain syndrome.

This study has notable limitations. First, this involved a relatively small sample size, and we were underpowered to perform several analyses. Paired fetal and birth anthropometrics were available for only 34% of the sample, suggesting that these findings may not be easily generalizable. However, we noted similarity between neonates who had been diagnosed prenatally, and thus had fetal measurements, and those with only neonatal measurements, indicating that the potential for bias was small. Exclusion of subjects with previously established high-risk factors such as prematurity and/or genetic

syndromes creates a relatively low-risk cohort, leading to a relatively low power to detect associations with infrequent outcomes. Finally, interpreting growth using ultrasound estimates at the fetal time point and actual measurements at the neonatal time point creates a challenging problem (26) and should be acknowledged in the interpretation of these findings. A large multicenter study may allow stratification of the cohort to confirm and expand these observations, and assessing the effects of abnormal fetal growth may inform longitudinal adult quality-of-life evaluations (38).

In summary, this study found that microcephaly is associated with early adverse neurologic outcomes but not all-cause 30-d or 1-y mortality, in term nonsyndromic infants with HLHS. Therefore, assessing abnormal fetal growth may also inform late adverse clinical outcomes, including cognitive, behavior, and quality-of-life deficits. A better understanding of fetal growth promises to significantly impact clinical care. Clinical implications of detecting early growth abnormalities may include an indication for genetic testing *in utero*, a more informed composite assessment for potential fetal intervention, and enhanced neurodevelopmental surveillance and counseling.

## METHODS

### Study Population

Neonates (<28 d of age) diagnosed with HLHS between January 1998 and June 2010 were identified from the databases within The Heart Institute at Cincinnati Children's Hospital Medical Center. HLHS was strictly defined as atresia or stenosis of the aortic and mitral valves, and hypoplasia of the left ventricle and ascending aorta. All patients had an intact ventricular septum and normally related great arteries. Exclusion criteria were (i) established risk factors for poor outcomes, including known genetic syndromes (e.g., Turner syndrome), (ii) prematurity (<37 wk gestation), and (iii) multiple-gestation pregnancies. The Cincinnati Children's Hospital Medical Center's Institutional Review Board approved this study, and a waiver of consent was given due to the retrospective nature of the study.

### Anthropometric Data

Fetal biometry and anthropometrics were assessed using the earliest available obstetric ultrasound reports and the Hadlock standard (39). HC, estimated fetal weight, and gestational age data were collected. Neonatal anthropometrics included HC and weight at birth. Fetal and neonatal HC percentiles were evaluated using a contemporary standard based on growth curves derived from a large, racially diverse US sample adjusted for gestational age and gender (40). Microcephaly was defined as birth HC ≤3rd percentile. Small head size was defined as birth HC ≤10th percentile. SGA was defined as estimated fetal weight (intrauterine growth restriction) or birth weight ≤10th percentile using the Olsen standard (40). LBW was considered to be <2,500 g. Poor head growth and poor weight gain were defined as trajectory decreases over time in HC or weight *z*-score, respectively, >0.67 (equivalent to crossing two major percentile lines) from midgestation to birth using Olsen standards (40,41).

### Echocardiographic Data

Diagnosis of HLHS was determined by neonatal echocardiogram. HLHS phenotype was classified according to patency of the aortic and mitral valves (atresia vs. stenosis) (1). Fetal echocardiograms were performed on either Sequoia C512 (Siemens, Mountain View, CA) or Vivid 7 (General Electric, Milwaukee, WI) ultrasound systems with either a 2–6 MHz curvilinear or 3–5 MHz phased array transducer. Complete two-dimensional, color flow, and spectral Doppler studies were performed according to accepted standards (23). In recent years, Doppler profiles of the UA and MCA were included in the analysis, and this information is available for a subset of the cohort ($n$ = 22). When performed, the peak systolic, end diastolic, and mean velocities were measured off-line to calculate resistance index and PI. MCA:UA ratios were also calculated. All measurements were performed in triplicate and averaged to take into account the beat-to-beat variation.

### Clinical Outcome Data

Early clinical outcomes were obtained through Cincinnati Children's Hospital Medical Center Heart Institute's medical record databases. Outcomes were studied in the first year of life, including mortality, G-tube placement, and LOS following stage I palliation (Norwood procedure). Adverse neurologic outcomes were studied from birth through the first hospitalization only. Mortality was classified into two categories: death at any time from birth through 1 y of life ("all-cause mortality") and the subgroup representing death at any time in the first month of life ("30-d mortality"). Adverse neurologic outcomes included clinical seizure activity, ischemia, hemorrhage, and injury not otherwise specified. LOS calculations excluded patients who died on the day of surgery ($n$ = 13).

### Statistical Analyses

Descriptive statistics (mean ± SD or frequency ($n$) and percentage(%)) were generated for all relevant variables in the data set, with emphasis on anthropometrics and clinical outcomes. Because of small sample sizes and nonnormal distributions, outcome analyses were conducted using nonparametric methods, with median (interquartile range) or $n$ (%) reported. Relevant characteristics were compared between groups diagnosed at the fetal or neonatal stages using unpaired $t$-tests (continuous) or $\chi^2$ analyses (categorical). The Wilcoxon rank-sum test was used to compare medians between patients with and without a specific clinical outcome, and Fisher's exact tests were used to compare proportions. Spearman rank correlations were used to assess association between LOS and continuous variables (e.g., change in HC $z$-score). All available data were used in each analysis, so sample size varied by test. All statistical analyses were conducted using SAS v9.2 (SAS Institute, Cary, NC). A $P$ value < 0.05 was considered statistically significant.

### STATEMENT OF FINANCIAL SUPPORT

This project was supported by a Summer Undergraduate Research Fellowship (P.T.H.) and National Institutes of Health grant HL085122 (R.B.H.).

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