

Hypoplastic Left Heart Syndrome Is Heritable

Robert B. Hinton, JR, MD,* Lisa J. Martin, PhD,† Meredith E. Tabangin, MPH,†
Mjaye L. Mazwi, MD,* Linda H. Cripe, MD,* D. Woodrow Benson, MD, PhD*
Cincinnati, Ohio

- Objectives** This study sought to determine the size of the genetic effect (heritability) in families identified by a hypoplastic left heart syndrome (HLHS) proband.
- Background** Hypoplastic left heart syndrome is a severe form of cardiovascular malformation (CVM), and it remains a leading cause of infant mortality and childhood morbidity. Familial clustering of HLHS and bicuspid aortic valve (BAV) has been observed, and pedigree analysis has suggested recessive inheritance. The genetic significance of these observations is unknown.
- Methods** In 38 probands with HLHS, a 3-generation family history was obtained; using a sequential sampling strategy, echocardiograms on family members were performed. A total of 235 participants were recruited. Heritability (h^2) of HLHS and associated CVM was estimated using maximum-likelihood-based variance decomposition.
- Results** All HLHS probands had aortic valve hypoplasia and dysplasia; dysplasia of the mitral (94%), tricuspid (56%), and pulmonary (11%) valves was also noted. Overall, 21 of 38 (55%) families had more than 1 affected individual, and 36% of participants had CVM, including 11% with BAV. The heritability of HLHS alone and with associated CVM were 99% and 74% ($p < 0.00001$), respectively. The sibling recurrence risk for HLHS was 8%, and for CVM was 22%.
- Conclusions** The high heritability of HLHS suggests that it is determined largely by genetic factors. The frequent occurrence of left- and right-sided valve dysplasia in HLHS probands and the increased prevalence of BAV in family members suggests that HLHS is a severe form of valve malformation. (J Am Coll Cardiol 2007;50:1590-5) © 2007 by the American College of Cardiology Foundation

Hypoplastic left heart syndrome (HLHS) (MIM#241550) remains a leading cause of infant death due to cardiovascular malformation (CVM) and is associated with significant long-term morbidity. Despite remarkable advances in diagnosis and therapy, little is known of the etiology of HLHS (1,2). Several observations suggest a genetic basis. Based on

See page 1596

pedigree analysis of multiplex families in a consanguineous population, autosomal recessive inheritance has been proposed (3). The idea that left-sided lesions, namely bicuspid aortic valve (BAV) (MIM109730), aortic coarctation, and HLHS, are causally related was based on epidemiology studies that identified BAV in first-degree relatives of

HLHS probands (4,5). Bicuspid aortic valve frequently underlies aortic stenosis in patients of all ages, and longitudinal studies in the fetus have identified HLHS as part of the in utero natural history of aortic stenosis, suggesting that these defects may share a common etiology (6,7). Several studies have noted an increased prevalence of CVM in families identified by an HLHS proband (4,5,8). Familial clustering of BAV has been documented (5,9,10), and both BAV and left-sided lesions taken together have been shown to be heritable (11,12). However, the size of the genetic effect of HLHS remains unknown.

Classification of HLHS, based on the pathologic anatomy of the aortic and mitral valves, has been widely accepted (13-15). In this scheme, HLHS is defined as atresia or stenosis of the aortic and mitral valves and hypoplasia of the left ventricle and ascending aorta. Hypoplastic left heart syndrome pathology series have shown that aortic valve malformation is universal and mitral valve abnormalities are common (16,17); however, despite a conventional emphasis on left-sided heart structures, both tricuspid and pulmonary valve dysplasia have been reported (16,18,19). It is unclear to what extent aortic valve morphology and right-sided valve involvement can be used to further refine the HLHS phenotype.

From the Divisions of *Cardiology and †Biostatistics and Epidemiology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio. Supported by a Trustee Award from Children's Hospital Research Foundation (to Dr. Hinton), Cincinnati, Ohio, and grants from the National Institutes of Health HD43005 (to Dr. Hinton), HL085122 (to Dr. Hinton), MH059490 (for Sequential Oligogenic Linkage Analysis Routines software package, San Antonio, Texas), HL069712 (to Dr. Benson), and HL074728 (to Drs. Martin, Cripe, and Benson), Bethesda, Maryland.

Manuscript received April 4, 2007; revised manuscript received June 28, 2007, accepted July 1, 2007.

In this study, we estimated heritability to determine the size of the genetic effect of HLHS. We found that HLHS is determined largely by genetic factors. In addition, we identified a high incidence of left-sided and right-sided valve dysplasia in probands and an increased prevalence of BAV in family members.

Methods

Proband recruitment and sequential sampling. Probands with HLHS were recruited from the Division of Cardiology at Cincinnati Children's Hospital Medical Center. Family members were recruited using a sequential sampling strategy (11). Briefly, each proband's first-degree relatives were evaluated, and for every new affected individual identified, all of that individual's first-degree relatives were evaluated. When the family history identified affected second-degree relatives, sampling was extended to include the first-degree relatives. Informed consent was obtained from each participant or participant's parent or guardian. Assent was obtained from pediatric participants when appropriate. This study was approved by the Institutional Review Board at Cincinnati Children's Hospital Medical Center.

Proband inclusion and exclusion criteria. Hypoplastic left heart syndrome was strictly defined as atresia or stenosis of the aortic and mitral valves and hypoplasia of the left ventricle and ascending aorta with intact ventricular septum (15). Patients with complex CVM and left ventricular hypoplasia (e.g., variants of unbalanced atrioventricular septal defects, double-outlet right ventricle, or Shone anomaly) were excluded. Patients with known genetic syndromes (e.g., trisomy 18 or Turner syndrome) also were excluded.

Echocardiographic analysis. Cross-sectional 2-dimensional and Doppler transthoracic echocardiography was performed on all participants using Hewlett-Packard Sonos 5500 (Philips, Eindhoven, the Netherlands), Vivid 5 or Vivid 7 systems (General Electric, Fairfield, Connecticut). A detailed echocardiographic protocol previously described was used to assess cardiovascular structures (11). A single experienced echocardiographer (L.H.C.) interpreted all echocardiograms. To assess valve anatomy in HLHS probands, echocardiographic studies performed in the newborn period before surgical intervention were reviewed. Valve hypoplasia was defined as an annulus dimension z score < -2 . Valve dysplasia was defined as cusp or leaflet thickening, redundancy, or immobility. Bicuspid aortic valve was defined as a bicommissural appearance in the parasternal short-axis echocardiographic view and was classified as right-left or anterior-posterior (11,20,21). Unicuspid aortic valve morphology was classified as unicommissural or acommisural based on appearance in the parasternal short-axis view (20,22).

Statistical analysis: heritability of HLHS. To determine the extent to which genes influence the development of HLHS, we used variance component methodology implemented in the computer package Sequential Oligo-

genic Linkage Analysis Routines (SOLAR, San Antonio, Texas). The phenotypic variance (σ^2_P) is derived from the additive genetic (σ^2_G) and residual or epigenetic (σ^2_E) effects. The heritability (h^2) or overall genetic effect is estimated as $h^2 = \sigma^2_G/\sigma^2_P$. This nonparametric approach does not require a model of inheritance and allows analysis of all relative pairs (23).

The recruitment strategy resulted in a population enriched for HLHS and associated CVM. Ascertainment is necessary to identify sufficient numbers of affected individuals when studying a rare disease. Accordingly, the appropriate population prevalence is constrained to correct for ascertainment bias. A population prevalence of 0.02% for HLHS and 2% for HLHS and associated CVM was used (conservative estimate) (4,24,25). In light of recent evidence reporting an increased prevalence of CVM, and the increased incidence of CVM in utero (24,26-29), heritability estimates also were made using an HLHS prevalence of 0.08% and an HLHS and associated CVM prevalence of 5% (liberal estimate). Heritability was estimated as a continuous trait using a semiquantitative method (HLHS = 2, BAV or other CVM = 1, and normal = 0) based on the assumption that HLHS is inherited as an autosomal recessive trait (30). The mean in this model was constrained to equal the population prevalence. Therefore, we estimated heritability: 1) using HLHS as either a discrete or a continuous trait, and 2) using a BAV and other CVM prevalence of either 2% or 5%. Values of $p < 0.05$ were considered significant for additive genetic effects. The 95% confidence interval (CI) was determined empirically for heritability estimates using previously described methods (31).

Statistical analysis: recurrence risk ratio. Using a family-based approach, we calculated recurrence risk ratios for HLHS and associated CVM for first-degree relatives and siblings in the context of both unaffected parents and 1 parent with CVM. Recurrence risk ratio (λ_R) was defined as $\lambda_R = \text{frequency (relatives)}/\text{frequency (population)}$, where the frequency in relatives is the number of first-degree relatives of a proband over the total number of first-degree relatives, and the frequency in the population is the prevalence in the general population (0.02% and 2% for HLHS and CVM, respectively) (32).

Results

Study population. From October 2004 through October 2006, 44 potential recruits were identified; 38 (86%) HLHS probands and their families were recruited (Fig. 1). Four families declined to participate, 1 proband was

Abbreviations and Acronyms

BAV = bicuspid aortic valve
CI = confidence interval
CVM = cardiovascular malformation
HLHS = hypoplastic left heart syndrome
SOLAR = Sequential Oligogenic Linkage Analysis Routines

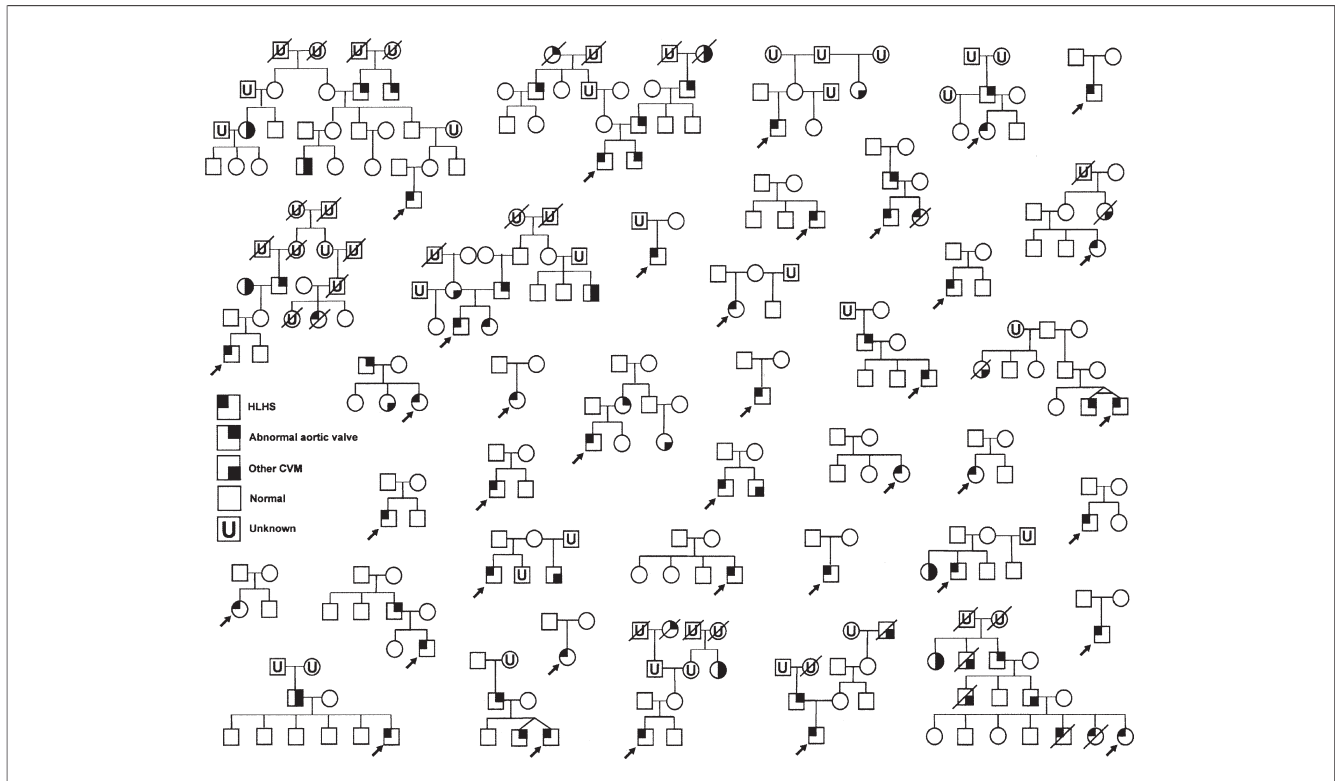


Figure 1 Pedigrees Depicting the 38 HLHS Kindreds

Cardiovascular phenotype is represented by a **box in the left upper quadrant** for hypoplastic left heart syndrome (HLHS), in the **right upper quadrant** for an abnormal aortic valve (including bicuspid aortic valve), and in the **right lower quadrant** for other cardiovascular malformation (CVM). An **empty symbol** represents a normal cardiovascular phenotype, and a **U** denotes an individual not evaluated (unknown). **Circles** = female patients; **squares** = male patients. Probands indicated by **arrows**.

excluded for medical reasons, and 1 family repeatedly missed appointments. Among the 38 probands, 36 were Caucasian and 2 were African American; there were 28 male and 10 female subjects (2.8:1), consistent with studies that have shown differences in rates of CVM by race or gender (28,29). A total of 235 family members ranging in age from 3 days to 74 years (130 male and 105 female subjects with a gender ratio of 1.24:1 male:female) were evaluated, including 126 first-degree relatives. A total of 193 blood relatives of the probands were evaluated (Table 1); 4 participants were not genetically related to the probands.

Table 1 Relatives of 38 HLHS Probands Affected With CVM

Relationship	Normal	Affected	Total
Fourth-degree plus	12	5	17
Third-degree	8	12	20
Grandparent	15	4	19
Avuncular	9	2	11
Half sibling	5	1	6
Parent	63	12	75
Sibling	35	10	45
Total	147	46	193*

*Excludes participants not genetically related to the proband.

CVM = cardiovascular malformation; HLHS = hypoplastic left heart syndrome.

Phenotype of probands. Aortic and mitral valve anatomy varied among the 38 HLHS probands. Three combinations were observed: aortic atresia and mitral atresia (11), aortic atresia and mitral stenosis (11), and aortic stenosis and mitral stenosis (16). No cases of aortic stenosis and mitral atresia were observed. All aortic valves were both hypoplastic and dysplastic (Table 2). Aortic atresia was present in 22 probands. Among 16 HLHS probands with aortic stenosis, assessment of aortic valve morphology identified BAV in 6 of 16 (38%) and unicuspid aortic valve in 10 of 16 (62%) (Figs. 2A and 2B). Of those with BAV, aortic valve morphology was anterior-posterior in 5 and right-left in 1, consistent with relative morphology frequencies in isolated BAV (33). Of those with unicuspid aortic valves, aortic valve morphology was unicommissural in 4 and aocommissural in 6. In addition, HLHS probands had dysplasia of the

Table 2 Valve Phenotype in 38 HLHS Probands

Valve	Hypoplasia (%)	Dysplasia (%)
Aortic	100	100
Mitral	100	94
Pulmonary	0	11
Tricuspid	0	56

HLHS = hypoplastic left heart syndrome.

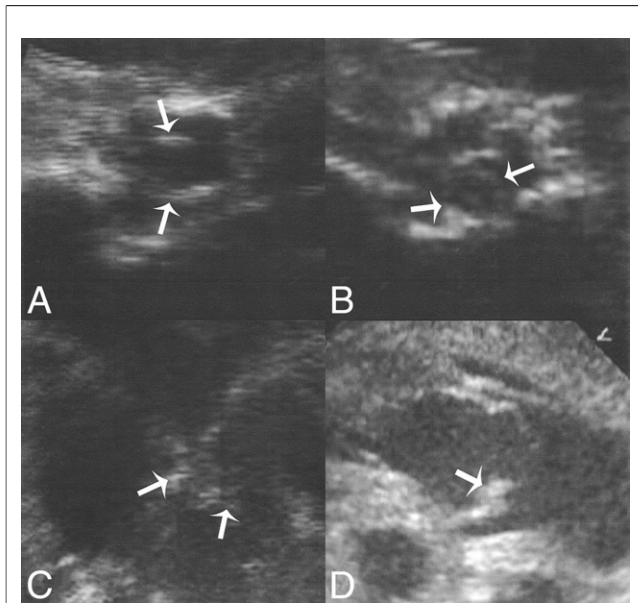


Figure 2 Valve Phenotype in HLHS Probands

All aortic valves were both dysplastic and hypoplastic. Aortic valve morphology (A, B) showing the effective orifice of an anterior posterior bicuspid aortic valve (arrows, A) and unicommissural unicuspid aortic valve (arrows, B) in the parasternal short axis. Right-sided valve involvement (C, D) showing dysplasia of the tricuspid (arrows, C) and pulmonary (arrow, D) valves in the apical 4-chamber and parasternal long axis, respectively. Abbreviations as in Figure 1.

mitral (94%), tricuspid (56%), and pulmonary (11%) valves (Figs. 2C and 2D, Table 2).

Phenotype of family members. In addition to probands, 193 family members were evaluated; 46 (24%) had CVM (Table 1). In 21 families (55%), there were 2 or more affected individuals. Cardiovascular malformation was documented in 11 of 51 (22%) siblings and 23 of 126 (18%) first-degree relatives, including BAV (12, 10%), HLHS (4), aortic root dilation (2), and 1 each with BAV and aortic root dilation, aortic coarctation, atrioventricular septal defect, ventricular septal defect, and left superior vena cava. In addition, CVM was documented in 23 second-degree or higher order relatives, including aortic valve malformation together with BAV (14), aortic root dilation (3), aortic coarctation (3), atrial septal defect (3), ventricular septal defect (2), fatal unspecified cyanotic CVM as a newborn infant (2), and 1 each with HLHS, mitral valve abnormality, and truncus arteriosus. Among the 38 kindreds, first-degree (1) and second-degree (5) relatives required aortic valve replacement. Some individuals had multiple CVMs. Interestingly, screening echocardiography resulted in the identification of previously unknown cases of BAV (10) and aortic root dilation (2). These findings underscore the importance of screening first-degree relatives of HLHS patients to identify those at risk for latent disease, infective endocarditis, and aortic dissection.

Heritability. The sequential sampling strategy resulted in information from 652 relative pairs distributed nearly

equally between first-degree and higher-order relatives (Table 1). The prevalence was initially constrained to 0.02%; however, because the initial estimate was bound at 1.0, the prevalence was liberated to 0.08% to achieve an unbound estimate. The heritability of HLHS was estimated as 0.99 ± 0.002 (95% CI 0.59 to 1.0, $p < 0.00001$). When the prevalence of HLHS and associated CVM was constrained to 2% and 5%, the heritability was estimated as 0.96 ± 0.08 (95% CI 0.76 to 1.0, $p < 0.00001$) and 0.74 ± 0.004 (95% CI 0.5 to 0.96, $p < 0.00001$), consistent with previous findings in an independent population (11). Heritability estimates using male-female gender ratios of 1:1 (general population) or 2.8:1 (study population) were not significantly different (data not shown). Using a semiquantitative method, the heritability for HLHS and associated CVM was 0.32 ± 0.10 (95% CI 0.13 to 0.53, $p = 0.0008$) (2% prevalence) and 0.28 ± 0.10 (95% CI 0.09 to 0.49, $p < 0.0008$) (5% prevalence) confirming significant heritability. The reduction in heritability estimates using the semiquantitative method questions the assumption that HLHS is inherited as a simple Mendelian autosomal recessive condition, and implicates HLHS as a complex trait.

Recurrence risk. The frequency of first-degree relatives with HLHS or CVM was 3.5% and 18.3%, consistent with previous findings (8). Siblings of HLHS probands were diagnosed with HLHS in 8% (4 of 51) of cases and any CVM in 22% (11 of 51) of cases. Based on population frequencies, recurrence risk ratios for both first-degree relatives and siblings were significantly increased. If 1 parent was affected with CVM, the frequency and recurrence risk ratios of siblings with either HLHS or CVM increased substantially (Table 3).

Discussion

The present study provides the first estimate of the heritability of HLHS using variance decomposition methodology; the finding of high heritability suggests that HLHS is determined largely by genetic factors. The heritability of HLHS and associated CVM is similar to the heritability of left-sided lesions reported by McBride et al. (12); however, HLHS composed only 23% of their study population. Because HLHS is a rare disease and kindreds identified by a HLHS proband are enriched for CVM, we also estimated

Table 3 Hypoplastic Left Heart Syndrome and CVM λ_R

	HLHS	CVM
Frequency in first-degree relatives	3.5%	18.3%
Frequency in siblings	8%	22%
Frequency in siblings with 1 affected parent	21%	26%
First-degree relative λ_R	175	9
Sibling λ_R	515	11
Sibling λ_R with 1 affected parent	1,050	13

CVM = cardiovascular malformation; HLHS = hypoplastic left heart syndrome; λ_R = recurrence risk ratio.

heritability as a continuous trait. This analysis also showed that HLHS is heritable and implicates HLHS as a complex trait.

Concepts of cardiac development have greatly influenced our understanding of the formation of the mesoderm-derived, 4-chambered vertebrate heart. Human genetic studies have identified mutations in genes important for early heart formation that cause congenital CVM, supporting the idea that these birth defects are caused by alterations during cardiogenesis (34–38). There has been considerable interest in human conditions such as HLHS, in which individual chambers or valves of the developing heart are selectively impaired (34). A widely accepted hypothesis is that HLHS develops as a result of embryonic alterations in blood flow, such as premature narrowing of the foramen ovale (13) and aortic stenosis (39). This hypothesis is supported by studies in the embryonic chick, in which ligation of the left atrium results in diminished flow through the left ventricle, resulting in left ventricular hypoplasia (40). In addition, an alternative hypothesis for the etiology of HLHS is being viewed as the summation of separate genetic modules. Recent studies have investigated chamber-specific regulatory mechanisms (e.g., *TBX5* and *IRX1*) leading to formation of morphologically, functionally, and molecularly distinct cardiac chambers (35,37). In this context, it has been suggested that left ventricular hypoplasia may result from a primary defect in myocardial growth during development. Unfortunately, there are presently no experimental models of HLHS to elucidate the relative contribution of these 2 hypotheses.

The definition of HLHS we used was not based on left ventricular hypoplasia alone (15); this is important because left ventricular hypoplasia is a feature of several different CVMs (e.g., unbalanced atrioventricular septal defect) believed to have diverse developmental origins. In the current study, we used echocardiography to characterize the phenotype of HLHS probands and family members. This detailed phenotyping identified the frequent occurrence of left-sided and right-sided valve dysplasia in HLHS probands and the increased prevalence of BAV in probands and family members. The latter observation and the identification of discordant phenotypes (HLHS and BAV) in one set of identical twins provides further support for the idea that HLHS is allelic to BAV. Based on these findings, it can be speculated that the left ventricular hypoplasia present in HLHS is secondary to a morphogenetic valve defect rather than a primary myocardial problem.

Previous studies have shown that the recurrence risk of HLHS is increased compared with other forms of CVM; however, these approximations may be underestimated because they are based on epidemiologic and fetal studies that did not perform echocardiographic screening for subclinical BAV or other CVM (26,41,42). The recurrence risk ratios calculated in this study are significantly higher than previously reported, particularly in the context of an affected parent. There is a potential sampling

bias for families with multiple affected children; however, in this study, ascertainment was not based on family history. These findings may represent more accurate estimations than previously reported in part because they include definitive phenotyping on all first-degree relatives. These new estimates support the idea that HLHS is primarily caused by genetic factors and should be considered when counseling families.

In summary, we have shown that HLHS has high heritability, suggesting that it is almost entirely caused by genetic effects. Therefore, using family-based linkage analysis, it should be possible to identify loci harboring HLHS-causing genes, especially in light of the proportion of multiplex kindreds. The findings of left- and right-sided valve dysplasia in HLHS probands and associated BAV in family members suggest that HLHS is a severe form of valve malformation. Therefore, we anticipate that HLHS-causing genes will be involved in valve development (e.g., transcription factors, signaling molecules, or extracellular matrix proteins) (36,38). Elucidation of the genetic basis of HLHS has significant potential clinical implications; for example, genotype stratification of HLHS probands may provide indications for fetal intervention or specific postoperative pharmacologic strategies, as well as long-term prognostic information such as the risk for right ventricular failure or neurocognitive deficit.

Acknowledgments

The authors thank the families who participated in this study, and Kerry Shooner, Wendi Long, Sandy Witt, Betty Glascock, and Vicki Moore for providing assistance.

Reprint requests and correspondence: Dr. D. Woodrow Benson, Division of Cardiology, MLC 7042, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, Ohio 45229-3039. E-mail: Woody.Benson@cchmc.org.

REFERENCES

1. Tworetzky W, McElhinney DB, Reddy VM, Brook MM, Hanley FL, Silverman NH. Improved surgical outcome after fetal diagnosis of hypoplastic left heart syndrome. *Circulation* 2001;103:1269–73.
2. Tweddell JS, Hoffman GM, Mussatto KA, et al. Improved survival of patients undergoing palliation of hypoplastic left heart syndrome: lessons learned from 115 consecutive patients. *Circulation* 2002;106:182–9.
3. Shokeir MH. Hypoplastic left heart. Evidence for possible autosomal recessive inheritance. *Birth Defects Orig Artic Ser* 1974;10:223–7.
4. Ferenc C, Rubin JD, McCarter RJ, et al. Congenital heart disease: prevalence at livebirth. The Baltimore-Washington Infant Study. *Am J Epidemiol* 1985;121:31–6.
5. Brenner JI, Berg KA, Schneider DS, Clark EB, Boughman JA. Cardiac malformations in relatives of infants with hypoplastic left-heart syndrome. *Am J Dis Child* 1989;143:1492–4.
6. Hornberger LK, Sanders SP, Rein AJ, Spevak PJ, Parness IA, Colan SD. Left heart obstructive lesions and left ventricular growth in the midtrimester fetus. A longitudinal study. *Circulation* 1995;92:1531–8.
7. Makikallio K, McElhinney DB, Levine JC, et al. Fetal aortic valve stenosis and the evolution of hypoplastic left heart syndrome: patient selection for fetal intervention. *Circulation* 2006;113:1401–5.

8. Loffredo CA, Chokkalingam A, Sill AM, et al. Prevalence of congenital cardiovascular malformations among relatives of infants with hypoplastic left heart, coarctation of the aorta, and d-transposition of the great arteries. *Am J Med Genet A* 2004;124:225-30.
9. Huntington K, Hunter AG, Chan KL. A prospective study to assess the frequency of familial clustering of congenital bicuspid aortic valve. *J Am Coll Cardiol* 1997;30:1809-12.
10. Martin LJ, Ramachandran V, Cripe LH, et al. Evidence in favor of linkage to human chromosomal regions 18q, 5q and 13q for bicuspid aortic valve and associated cardiovascular malformations. *Hum Genet* 2007;121:275-84.
11. Cripe L, Andelfinger G, Martin LJ, Shoener K, Benson DW. Bicuspid aortic valve is heritable. *J Am Coll Cardiol* 2004;44:138-43.
12. McBride KL, Pignatelli R, Lewin M, et al. Inheritance analysis of congenital left ventricular outflow tract obstruction malformations: segregation, multiplex relative risk, and heritability. *Am J Med Genet A* 2005;134:180-6.
13. Lev M. Pathologic anatomy and interrelationship of hypoplasia of the aortic tract complexes. *Lab Invest* 1952;1:61-70.
14. Noonan JA, Nadas AS. The hypoplastic left heart syndrome; an analysis of 101 cases. *Pediatr Clin North Am* 1958;5:1029-56.
15. Tchervenkov CI, Jacobs JP, Weinberg PM, et al. The nomenclature, definition and classification of hypoplastic left heart syndrome. *Cardiol Young* 2006;16:339-68.
16. Bharati S, Lev M. The surgical anatomy of hypoplasia of aortic tract complex. *J Thorac Cardiovasc Surg* 1984;88:97-101.
17. Aiello VD, Ho SY, Anderson RH, Thiene G. Morphologic features of the hypoplastic left heart syndrome—a reappraisal. *Pediatr Pathol* 1990;10:931-43.
18. Stamm C, Anderson RH, Ho SY. The morphologically tricuspid valve in hypoplastic left heart syndrome. *Eur J Cardiothorac Surg* 1997;12:587-92.
19. Bharati S, Nordenberg A, Brock RR, Lev M. Hypoplastic left heart syndrome with dysplastic pulmonary valve with stenosis. *Pediatr Cardiol* 1984;5:127-30.
20. Roberts WC, Ko JM. Frequency by decades of unicuspid, bicuspid, and tricuspid aortic valves in adults having isolated aortic valve replacement for aortic stenosis, with or without associated aortic regurgitation. *Circulation* 2005;111:920-5.
21. Roberts WC. The congenitally bicuspid aortic valve. A study of 85 autopsy cases. *Am J Cardiol* 1970;26:72-83.
22. Novaro GM, Mishra M, Griffin BP. Incidence and echocardiographic features of congenital unicuspid aortic valve in an adult population. *J Heart Valve Dis* 2003;12:674-8.
23. Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet* 1998;62:1198-211.
24. Hoffman JL, Kaplan S. The incidence of congenital heart disease. *J Am Coll Cardiol* 2002;39:1890-900.
25. Hoffman JI. Congenital heart disease: incidence and inheritance. *Pediatr Clin North Am* 1990;37:25-43.
26. Ferencz C, Rubin JD, Loffredo CA, Wilson PD. Genetic and environmental risk factors of major cardiovascular malformations: The Baltimore-Washington Infant Study 1981-1989. In: Anderson RH, editor. *Perspectives in Pediatric Cardiology, Volume 5*. Mount Kisco, NY: Futura Publishing, 1997.
27. Hoffman JI. Incidence of congenital heart disease: II. Prenatal incidence. *Pediatr Cardiol* 1995;16:155-65.
28. Pradat P, Francannet C, Harris JA, Robert E. The epidemiology of cardiovascular defects, part I: a study based on data from three large registries of congenital malformations. *Pediatr Cardiol* 2003;24:195-221.
29. Botto LD, Robert-Gnansia E, Siffel C, Harris J, Borman B, Mastroiacovo P. Fostering international collaboration in birth defects research and prevention: a perspective from the International Clearinghouse for Birth Defects Surveillance and Research. *Am J Public Health* 2006;96:774-80.
30. Duggirala R, Williams JT, Williams-Blangero S, Blangero J. A variance component approach to dichotomous trait linkage analysis using a threshold model. *Genet Epidemiol* 1997;14:987-92.
31. Towne B, Czerwinski SA, Demerath EW, Blangero J, Roche AF, Siervogel RM. Heritability of age at menarche in girls from the Fels Longitudinal Study. *Am J Phys Anthropol* 2005;128:210-9.
32. Risch N. Linkage strategies for genetically complex traits. I. Multilocus models. *Am J Hum Genet* 1990;46:222-8.
33. Fernandes SM, Sanders SP, Khairy P, et al. Morphology of bicuspid aortic valve in children and adolescents. *J Am Coll Cardiol* 2004;44:1648-51.
34. Bruneau BG. The developing heart and congenital heart defects: a make or break situation. *Clin Genet* 2003;63:252-61.
35. Fishman MC, Olson EN. Parsing the heart: genetic modules for organ assembly. *Cell* 1997;91:153-6.
36. Hinton RB, Yutzey KE, Benson DW. Congenital heart disease: genetic causes and developmental insights. *Prog Pediatr Cardiol* 2005;20:101-11.
37. Small EM, Krieg PA. Molecular regulation of cardiac chamber-specific gene expression. *Trends Cardiovasc Med* 2004;14:13-8.
38. Armstrong EJ, Bischoff J. Heart valve development: endothelial cell signaling and differentiation. *Circ Res* 2004;95:459-70.
39. Fishman NH, Hof RB, Rudolph AM, Heymann MA. Models of congenital heart disease in fetal lambs. *Circulation* 1978;58:354-64.
40. deAlmeida A, McQuinn T, Sedmera D. Increased ventricular preload is compensated by myocyte proliferation in normal and hypoplastic fetal chick left ventricle. *Circ Res* 2007;100:1363-70.
41. Allan LD, Crawford DC, Chita SK, Anderson RH, Tynan MJ. Familial recurrence of congenital heart disease in a prospective series of mothers referred for fetal echocardiography. *Am J Cardiol* 1986;58:334-7.
42. Whittemore R, Wells JA, Castellsague X. A second-generation study of 427 probands with congenital heart defects and their 837 children. *J Am Coll Cardiol* 1994;23:1459-67.