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## Evaluation of Recurrence Risks for Left-Sided Cardiac Lesions

Sarah Swain

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EVALUATION OF RECURRENCE RISKS FOR LEFT-SIDED CARDIAC LESIONS

by

*Sarah Elizabeth Swain*

APPROVED:

---

Supervisory Professor  
Laura E. Mitchell, PhD

---

Elizabeth Goldmuntz, MD

---

Jacqueline T. Hecht, PhD

---

Philip Lupo, PhD

---

Sarah G. Seth, MPH, CGC

---

Jennifer Czerwinski, MS, CGC

APPROVED:

---

Dean, The University of Texas  
Graduate School of Biomedical Sciences at Houston

EVALUATION OF RECURRENCE RISKS FOR LEFT-SIDED CARDIAC LESIONS

A  
THESIS

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by

Sarah Elizabeth Swain, B.S.  
Houston, TX

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# EVALUATION OF RECURRENCE RISKS FOR LEFT-SIDED CARDIAC LESIONS

Publication No. \_\_\_\_\_

Sarah Elizabeth Swain, B.S.

Supervisory Professor: Laura E. Mitchell, PhD

It is widely accepted that hypoplastic left heart syndrome (HLHS), aortic valve stenosis with or without bicuspid aortic valve (AS/BAV) and coarctation of the aorta (CoA) occur in families more commonly with each other than with any other congenital heart defect (CHD). Genetic counseling for CHDs is currently based on empiric risk estimates derived from data collected on all types of CHDs between 1968 and 1990. Additionally, for the specific group of defects described above, termed left-sided lesions, estimates are available for sibling recurrence. Utilizing family history data from 757 probands recruited between 1997 and 2007 from The Children's Hospital of Philadelphia, this study reassessed the pre/recurrence risks for LSLs specifically. Sibling pre/recurrence risks for HLHS (5.5%, 95% CI: 3.1%-8.9%), CoA (4.0%, 95% CI: 2.1%-6.7%), and AS/BAV (6.0%, 95% CI: 3.3%-9.8%) were higher than currently quoted risks based on sibling data for individual LSLs. Additionally, the prevalence of BAV in 202, apparently unaffected, parents of 134 probands was assessed by echocardiography. BAV, which occurs at a frequency of 1% in the general population, was found to occur in approximately 10% of parents of LSL probands. Lastly, among affected first-degree relative pairs (i.e. siblings, parent-offspring), the majority (65%-70%) were both affected with a LSL. Defect specific concordance rates were highest for AS/BAV. Together, these findings suggest that over the past 20 years with

changing diagnostic capabilities and environmental/maternal conditions (e.g. folic acid fortification, increased maternal diabetes and obesity) recurrence risks may have increased, as compared to current LSL specific risk estimates. Based on these risk estimate increases and prior studies, a protocol for screening first-degree relatives of LSL probands should be devised.

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## **INTRODUCTION**

Every year an estimated 7.9 million children, or 6 percent of total births worldwide, are born with a serious birth defect of genetic or partially genetic origin (Christianson, Howson, & Modell, 2006). Hundreds of thousands more are born with serious birth defects of post-conception origin. Congenital heart defects (CHD) are the most common birth defect worldwide, followed by neural tube defects, hemoglobinopathies, Down Syndrome and glucose-6-phosphate dehydrogenase deficiency (Christianson, et al., 2006).

Significant advances in diagnosis, cardiac care and surgery have dramatically increased survival of individuals born with a CHD and there are now greater than one million CHD survivors in the United States (Pierpont et al., 2007). As more individuals with CHD reach reproductive age, questions about genetic contribution to disease and risk of transmission have moved to the forefront. Although the precise causes of CHDs remain largely unknown, they are thought to have a multifactorial inheritance pattern where both genetic and environmental factors contribute to disease (Nora, Berg, & Nora, 1991).

CHDs are anatomically, clinically, epidemiologically and developmentally heterogeneous (Botto, Lin, Riehle-Colarusso, Malik, & Correa, 2007). However, subgroups of CHDs have been identified (Ferencz, Rubin, Loffredo, & Magee, 1993). These subgroups are based on underlying developmental mechanisms, epidemiological evidence and clinical considerations. One such subgroup encompasses defects of the left side of the heart, termed left-sided obstructive lesions (LSLs). The Baltimore Washington Infant Study clearly showed that LSLs, including hypoplastic left heart syndrome, aortic valve stenosis and coarctation of the aorta occur in families much more commonly with each other than with any other CHD (Boughman et al., 1987). In general, genetic counseling for these CHDs is currently based on empiric risk estimates, derived from data

collected on all types of CHDs between 1968 and 1990. However, sibling risk estimates for specific LSLs are available.

#### *Definition of left-sided cardiac lesions*

The LSLs account for 15-20% of CHDs and include hypoplastic left heart syndrome (HLHS), coarctation of the aorta (CoA), aortic valve stenosis (AS), and bicuspid aortic valve (BAV) (Towbin & Belmont, 2000). The various LSLs differ considerably with respect to morbidity and mortality. BAV (i.e. an aortic valve with two rather than three leaflets) is the most common cardiovascular malformation with an incidence of 1-2% in the general population. BAV has been shown to occur with increased frequency in asymptomatic parents and other first-degree relatives of probands with LSLs (Cripe, Andelfinger, Martin, Shooner, & Benson, 2004; Loffredo et al., 2004; Ward, 2000). Although BAV is often considered a benign lesion early in life, complications, including aortic stenosis and/or insufficiency, infective endocarditis and aortic dilation and dissection, can result in morbidity and mortality later in life (Cripe, et al., 2004; Ward, 2000). On the other end of the spectrum, HLHS, a condition where the left side of the heart, including the left ventricle, aorta, mitral valve and aortic valve, is severely underdeveloped is fatal in infancy without surgical intervention.

#### **BIRTH PREVALENCE**

The birth prevalence of CHDs is 5 to 10 per 1000 live births (Oyen et al., 2009). Approximately 40,000 children are born each year in the United States with a clinically significant heart defect and at least another 40,000 are born annually with subclinical malformations that result in heart disease later in life (Shieh & Srivastava, 2009). A comparison of several studies conducted during the second half of the twentieth century revealed a range of prevalence estimates from various populations and at different time

frames within the same population (Table 1). This range may be due to differing methods of case identification and/or the evolution of diagnostic techniques, as well as environmental and temporal variation.

**Table 1. Prevalence of congenital heart defects in defined populations**

<b>Population</b>	<b>Cases/1000</b>	<b>Time Frame</b>
Sweden, Gothenburg	6.4	1941-1950
USA, NIH Collaborative	7.7	1956-1965
USA, California-Kaiser	11.7	1960-1966
Denmark	6.1	1963-1973
USA, New England	2.1	1969-1974
EUROCAT	1.9-10.8	1979-1982
European Collaborative	6.04	1986
Switzerland	4.0	1986
Japan	10.6	1985

Adapted from Nora, et al., 1991

A study conducted using data collected by the Metropolitan Atlanta Congenital Defects Program from 1968 to 1997 aimed to determine racial variations in the prevalence of heart defects (Botto, Correa, & Erickson, 2001). Although this study found an overall greater prevalence of CHDs in the black population, this increase was not observed for all defect categories. For example, LSLs tended to occur more frequently in whites, whereas peripheral pulmonary stenosis occurred more frequently in blacks (Table 2).

**Table 2. Prevalence of LSLs by race (per 10,000 births)**

<b>Defect Type</b>	<b>Whites</b>	<b>Blacks</b>	<b>Rate Ratio</b> Rate <sup>Blacks</sup> /Rate <sup>Whites</sup>
<b>HLHS</b>	1.96	2.24	1.14
<b>Coarctation of the aorta</b>	3.33	2.36	0.71
<b>Aortic valve stenosis</b>	1.32	0.49	0.38
<b>Peripheral Pulmonary Stenosis</b>	4.1	8.0	2.18

Adapted from Botto, Correa, & Erickson, 2001

## **NORMAL AND ABNORMAL DEVELOPMENT OF THE CARDIOVASCULAR SYSTEM**

In order to understand the left-sided cardiac defects, it is important to examine the normal development of the heart. In LSLs, all the essential components of the heart are in place but are malformed or not working properly.

### *Normal cardiovascular development*

As described in *The Developing Human*, the primordial heart and vascular system appear in the middle of the third week of gestation, and the cardiovascular system is the first major system to function in the embryo (Moore & Persaud, 2003). Cardiac function at this early stage is necessary because the rapid rate of growth in the embryo requires an efficient system for acquiring oxygen and nutrients from maternal blood and disposing of carbon dioxide and waste. Thus cardiac function must begin even as cardiac development progresses.

The cardiovascular system has three main derivations; splanchnic mesoderm, paraxial and lateral mesoderm, and neural crest cells. The earliest sign of the heart are paired angioblastic cords in the cardiogenic mesoderm that appear during the third week. These

paired cords canalize to form thin heart tubes that subsequently fuse, beginning at the cranial end and extending caudally, as lateral embryonic folding occurs. This primitive heart begins to beat at 22 to 23 days gestation and blood flow begins during the fourth week gestation.

With cell growth and proliferation, the heart tube starts to bend upon itself, forming a U-shaped bulboventricular loop. As the primordial heart bending continues, the sinus venosus and atrium come to lie dorsal to the truncus arteriosus, bulbus cordis and ventricle, creating the correct anatomical position of the adult heart in which the atria lie dorsal to the ventricles, pulmonary trunk and aorta.

Initially, circulation through the heart is an ebb and flow. However, by the end of the fourth week, coordinated contractions result in unidirectional flow. Blood from the embryo, developing placenta and umbilical vesicle enters the sinus venosus through three paired veins. The flow, which is controlled by the sinuatrial valves, then travels to the primordial atrium. It passes through the atrioventricular canals into the primordial ventricle. When the ventricle contracts, blood is pumped through the bulbous cordis and truncus arteriosus to the aortic sac. From the aortic sac, it is distributed to the pharyngeal arch arteries and then passes into the dorsal aortas for distribution to the embryo, umbilical vesicle and placenta.

Partitioning of the primordial heart, including partitioning of the atrioventricular canal, primordial atrium and ventricle, begins around the middle of the fourth week and is essentially complete by the end of the eighth week. Endocardial cushions form on the dorsal and ventral surfaces of the atrioventricular canal. As the endocardial cushions approach each other and fuse, the atrioventricular canal divides into right and left canals. These canals function as atrioventricular valves and partially separate the primordial atrium

from the primordial ventricle. Concurrently, the common atrium is partitioned into left and right atria by the formation and modification of the septum primum and the septum secundum.

At the end of the fourth week, the division of the primordial ventricle is indicated by a ridge, the muscular interventricular septum, in the floor of the ventricle. The partition is complete at the end of the seventh week, when the endocardial cushions and conotruncal ridges fuse. The membranous part of the interventricular septum is derived from an extension of tissue from the endocardial cushion to the muscular interventricular septum. This then merges with the aorticopulmonary septum, which divides the truncus arteriosus into the pulmonary trunk and ascending aorta. When closure is complete, the pulmonary trunk communicates with the right ventricle, while the aorta communicates with the left ventricle.

At five weeks, the aorticopulmonary septum undergoes a spiraling, accounting for the twisting of the pulmonary trunk around the ascending aorta. When this partition and twisting are nearly complete, the pulmonic and aortic valves begin to develop from three swellings of subendocardial tissue around the openings of the aorta and pulmonary trunk. These swellings form three thin-walled cusps. The tricuspid and mitral valves are formed similarly from proliferations of tissue around the atrioventricular canals.

In the normal adult heart, deoxygenated blood enters the right atrium through the superior and inferior vena cava and moves to the right ventricle through the tricuspid valve where it is then pumped to the lungs through the pulmonic valve and pulmonary arteries. Oxygenated blood then returns to the heart via the pulmonary veins and enters the left atrium; blood moves to the left ventricle via the mitral valve and is pumped through the aortic valve and aorta to the rest of the body.

### *Development of left-sided lesions*

The development of the left side of the heart and the aortic outflow tract may be affected by obstruction and subsequent reduction in blood flow. Most cases of LSLs are isolated defects. There are few known genetic syndromes characterized by LSLs and few individuals with an LSL have a recognized genetic syndrome. This lack of knowledge has made it difficult to elucidate the pathogenesis of LSLs.

### *Hypoplastic left heart syndrome (HLHS)*

First described in 1952 by Lev, HLHS is the most severe LSL. It occurs when parts of the left side of the heart, including the mitral valve, left ventricle, aortic valve and aorta, do not develop completely. Before 1980, HLHS was deemed inoperable and was almost always fatal in infancy with extremely rare cases of survival into childhood (Studer & Justino, 2010). In patients with HLHS, the left side of the heart is unable to send enough blood to the body such that the right side of the heart must maintain the circulation for both the lungs and the body. Because the aorta and left ventricle are so underdeveloped, the systemic circulation is dependent on a patent ductus arteriosus, a shunt allowing the mixed oxygenated/unxygenated blood to cross from the pulmonary artery to the aorta and be pumped to the rest of the body.

It is generally believed that HLHS develops as a result of an embryonic alteration in blood flow (Ferencz, Loffredo, Correa-Villasenor, & Wilson, 1997). Specifically, cardiac morphogenesis is thought to require both intrinsic processes of pattern formation and extrinsic forces of blood-flow mediated remodeling (Towbin & Belmont, 2000). As intracardiac blood flow begins before ventricular septation is complete, it may play a role in modeling the chambers of the heart (Ferencz, et al., 1997).

### *Coarctation of the Aorta (CoA)*

CoA is defined as a constriction in the aortic isthmus between the origin of the left subclavian artery and the ductus arteriosus, resulting in flow obstruction. This lesion is the most common anomaly of the aortic arch, occurring in 5-8% of children with CHDs (O'Brien, 2010; Towbin & Belmont, 2000). The clinical presentation of CoA varies, depending on the age of the patient and the severity of the obstruction, from systemic hypertension to congestive heart failure. Often, femoral pulses will be weaker than brachial pulses.

The underlying mechanism causing CoA is not entirely understood, however it may involve an abnormality in the tissue arising from the fourth or sixth aortic arches or from reduced blood flow in the aortic arch during development in utero (O'Brien, 2010).

### *Aortic Valve Malformations*

The most common left ventricular outflow tract obstruction in the pediatric population is valvular aortic stenosis (AS), which accounts for approximately three-quarters of all LSLs (Kitchiner et al., 1994). The underlying abnormality of AS is most commonly restricted leaflet motion of the aortic valve, resulting in obstruction to left ventricular outflow (O'Brien, 2010). The most frequent etiology of restricted leaflet motion is a bicuspid aortic valve (BAV), caused by fusion of two of the three valve leaflets. Studies suggest that approximately 1% of the general population have BAV (Roberts, 1970). Some neonates and infants with aortic valve malformations present with severely stenotic, unicuspid aortic valves that require immediate intervention. Although most children with BAV are asymptomatic, development aortic stenosis and/or regurgitation, infective endocarditis and aortic dissection may occur later in life (O'Brien, 2010).

According to Towbin and Belmont (2000), the formation of the valve leaflets requires

transformation of a subset of endothelial cells of the endocardium into mesenchyme; when this transformation goes awry, valvular malformations arise.

## **DIAGNOSIS, TREATMENT AND PROGNOSIS**

Approximately one-third of congenital heart disease is considered critical or cyanotic and requires surgery or cardiac catheterization to assure survival (Botto, et al., 2001). Children with critical CHD are diagnosed using a variety of methods. Fetuses may be diagnosed on a routine level II targeted anatomy ultrasound at 18-20 weeks gestation. However, it is estimated that only 20-40% of cases of critical CHDs are diagnosed prenatally (Montana et al., 1996). In newborns, the diagnosis may be made on physical exam with findings of a murmur, tachypnea or cyanosis (Koppel & Mahle, 2010). These findings, however, are not always present prior to hospital discharge at 48 hours of life. Consequently, diagnosis may be delayed until the neonate demonstrates features of cardiogenic shock, poor growth, poor feeding, respiratory distress or a murmur prompting a diagnostic workup (Koppel & Mahle, 2010).

Noninvasive imaging has become the cornerstone for the diagnosis of CHDs. The introduction of cardiac catheterization and echocardiography has greatly improved diagnostic capabilities prior to surgical intervention (Phoon, Chun, & Srichai, 2010). Currently, many techniques, such as x-ray, echocardiography, cardiac catheterization and simple clinical observation, are used in the diagnosis of congenital heart defects.

While the spectrum of outcomes for LSLs is wide, most of the left-sided obstructive lesions presenting in the neonate or infant require surgical correction. HLHS has a greater than 95% mortality rate in the first month of life if left untreated. In contrast, AS/BAV is frequently a progressive disorder with a risk of arrhythmia or sudden death and only 20% of

neonates require intervention (Wyszynski, Correa-Villaseñor, & Graham, 2010).

Before the advent of cardiac surgery in the mid-1940's, fewer than 25% of infants born with complex CHDs survived past their first year of life. Today, in the beginning of the 21<sup>st</sup> century, over 90% of infants with CHDs are expected to live into adulthood (Wu & Landzberg, 2010). Despite improvements in life expectancy, no adult survivors are ever really cured of their disease. Often, one problem is traded for a new set of problems after surgical repair or transplant, as evidenced by data showing that adult survivors of CHDs have significantly higher utilization of health care services than their peers (Mackie, Pilote, Ionescu-Ittu, Rahme, & Marelli, 2007).

### *Echocardiography*

In 1954, Edler and Hertz first reported recordings of ultrasound reflections from the heart. By the 1970's, two-dimensional echocardiography was in widespread use and revolutionized diagnostic abilities in pediatric cardiology (Phoon, et al., 2010). Rapid improvement in the technology, the addition of color Doppler flow mapping, and the introduction of transesophageal (as opposed to transthoracic) and fetal echocardiography put ultrasound imaging in its current position as the primary diagnostic tool for both children and adults with known or suspected heart disease.

An echocardiogram, or sonogram of the heart, employs standard ultrasound techniques to image the heart and produce an accurate assessment of the velocity and direction of blood flow using Doppler ultrasound. This technique allows for non-invasive assessment of the size and shape of the heart, pumping capacity, evaluation of the valves, abnormalities in the pattern of blood flow and any abnormal communication between the left and right side of the heart. In addition to the myocardium, chambers and valvular structures, this noninvasive technique also evaluates the outflow tracts, coronary arteries,

great arteries, aortic arch, systemic arteries and systemic and pulmonary veins (Phoon, et al., 2010).

In assessing individuals with valve disease, echocardiography is the primary imaging modality used both for initial assessment and for long-term follow-up. Information regarding valve structure and function, cardiac chamber size, wall thickness, and ventricular function can be readily obtained and utilized to assess the severity of valve disease (Shah, 2010). For quality echocardiograms, it is essential to obtain the best possible images and highly skilled interpretation based on training, experience and knowledge (Phoon, et al., 2010). In some instances, body habitus or the presence of coexisting lung disease may result in suboptimal studies, which are difficult to read (Shah, 2010).

As recently reviewed by O'Brien (2010), 2-D echocardiography is an excellent method of evaluating the morphology of the aortic valve. The number of leaflets, whether or not these leaflets are partially or completely fused, the size of the leaflets and thickening of the leaflets are all examined from the parasternal short axis view. The dimension of the aortic valve annulus and leaflet mobility is best demonstrated from the parasternal long axis view. Color Doppler will reveal flow turbulence and aortic regurgitation.

### *Costs*

CHDs are a heterogeneous group of serious birth defects that contribute to half of all infant deaths each year and one-third of hospitalizations due to congenital anomalies in the United States (Rosano, Botto, Botting, & Mastroiacovo, 2000). The cost of CHDs encompass direct costs of healthcare as well as indirect costs of reduced economic productivity of individuals with a CHD and reduced productivity of their caregivers. The indirect costs are generally difficult to quantify and therefore, many studies focus only on the direct costs with the acknowledgement that the true overall cost is likely greater than

that measured. An analysis conducted using data available through HCUPNet, the online search tool for all Health Care Cost Utilization Project databases including the Nationwide Inpatient Sample (NIS), found that the mean cost of healthcare for a child with CHD is 25 times greater than those for a child with no CHD (Boulet, Grosse, Riehle-Colarusso, & Correa-Villaseñor, 2010).

### *Genetic Counseling*

Genetic counseling, as defined by the National Society of Genetic Counselors, is a process that helps people “understand and adapt to the medical, psychological, and familial implications of the genetic contributions to disease” (Resta, 2006). Questions for genetic professionals will arise no matter when a CHD is diagnosed, whether in a fetus, toddler, or adult. Parents and patients will have questions regarding how/why this happened and will wonder whether it will happen again. They may think about ways they can prevent this in the future and the impact it will have on their own and/or their child’s life. Genetic counseling for CHDs requires an understanding of the cardiac anatomy and the mechanism of the defect; the ability to identify associated anomalies or syndromes; delineation of a careful family history for risk assessment and ascertainment of other affected family members; and information regarding the options for prenatal diagnosis (Lin & Garver, 1988).

Prenatal diagnosis for CHDs is steadily improving in frequency and accuracy. The detection of a CHD may appear on a level I ultrasound as part of the general survey for birth defects, pregnancy dates and placental exam. Later in pregnancy, the CHD may be detected on the level II targeted anatomy scan conducted between 18-20 weeks gestation. If a cardiac abnormality is suspected on ultrasound, follow-up with fetal echocardiography will often be recommended. One study found that among infants undergoing cardiac

surgery, 57% had been diagnosed prenatally by fetal echocardiography (Mohan, Kleinman, & Kern, 2005).

Another role of a genetic professional is emotional support for the individual and/or parents of a fetus or child with a CHD. Because CHDs are often an isolated, internal malformation, as opposed to an externally visible defect, there may be a tendency to underestimate the impact on the family (Lin & Garver, 1988). Families should be empowered with the tools to access the language, information, emotional and peer support necessary to become active members in their child's care and decision-making conversations.

## **ETIOLOGY AND RISK FACTORS**

### *Hypothesized etiologies*

A multifactorial etiology, with environmental and genetic factors playing important roles, is the most widely accepted hypothesis for the etiology of CHDs. It is well known that environmental exposures during fetal development, such as maternal infections, diabetes, and certain medications, increase the risk of CHDs. Further, the association of CHDs with chromosome abnormalities and single-gene disorders demonstrates the influence of genetic factors. Although the majority of CHD cases are non-syndromic, there is evidence that these conditions aggregate within families and thus are likely to involve genetic factors.

### *Evidence for genetic etiology*

Pedigrees with more than one family member affected with a CHD appeared very sporadically in the literature until the first large scale studies were undertaken in the 1950s (Nora, et al., 1991). However, over the past 60 years, many studies have attempted to

quantify the risks conferred by a family history of CHD and environmental exposures. Most studies support a multifactorial origin for CHDs in which a parent passes on a genetic predisposition to disease and disease only manifests in individuals whose susceptibility exceeds a threshold value (Shieh & Srivastava, 2009).

Between 1968 and 1990, 16 studies were conducted in an attempt to determine recurrence of any congenital cardiac defect as well as recurrence of specific subtypes of CHDs in the relatives of affected individuals (Anderson, 1976; Boughman, et al., 1987; W. Fuhrmann, 1968; Walter Fuhrmann & Vogel, 1969; Jorgensen, Beuren, & Stoermer, 1971; Mori, Ando, & Takao, 1973; Morris, Outcalt, & Menashe, 1990; Nora, 1968; Nora & Nora, 1978, 1988; Pierpont, Gobel, Moller, & Edwards, 1988; Sanchez-Cascos, 1978; Williamson, 1969; Zoethout, Carter, & Carter, 1964). Nora, Berg, and Nora (1991), combined risk estimates of these published data and generated the recurrence risks for specific cardiac defects that are used today when counseling families (Table 3). Based on these data the sibling recurrence risk for HLHS is quoted at 3%, while for CoA and AS this risk is estimated at 2%. Using these same combined risk estimates, Nora, Berg, and Nora (1991) reported a higher offspring recurrence risk for AS (5-18%) and CoA (3-4%).

**Table 3. Recurrence risks in sibs for any congenital heart defect: data published during two decades from European and North American populations**

<b>Proband Defect</b>	<b>1968-1990 Risk(%)</b>
<b>Ventricular septal defect</b>	3.2
<b>Hypoplastic left heart</b>	3.2
<b>Patent ductus</b>	3.1
<b>Atrial septal defect</b>	2.7
<b>Endocardial cushion defect</b>	2.5
<b>Tetralogy of Fallot</b>	2.4
<b>Pulmonary stenosis</b>	2.2
<b>Coarctation of aorta</b>	2.1
<b>Aortic stenosis</b>	2.0
<b>Transposition</b>	1.4

Adapted from Nora, Berg, and Nora (1991)

Findings from a pilot study of HLHS showed that the first-degree relatives of HLHS probands have an increased risk for subclinical cardiac defects. Further, this study demonstrated that HLHS, CoA and AS are more likely to co-occur with each other within a family than they are to co-occur with other CHDs (Brenner, Berg, Schneider, Clark, & Boughman, 1989). This suggests that these three conditions are more closely related to each other than they are to other CHDs.

#### *Left-sided lesions and subclinical BAV*

Several groups have proposed that BAV represents a reduced or mild expression of the more severe forms of LSLs, such as HLHS, and have sought to determine the prevalence of BAV in the first-degree relatives of probands with LSLs. In 1989, Brenner et al. performed echocardiograms on 41 first degree relatives of 11 children with HLHS and observed 5 of 41 (12%) with previously unrecognized BAV. Using a larger cohort, Huntington et al performed echocardiograms on 186 first degree relatives of 30 adults with BAV and found 17 of 186 (9%) to have previously unidentified BAV (Huntington, Hunter, & Chan, 1997). Lewin et al performed echocardiograms on 278 first degree relatives of 113 probands with a diagnosis of AS, BAV, CoA, HLHS or aortic hypoplasia with mitral valve atresia and found 21 of 278 (7.5%) to have aortic valve anomalies (Lewin et al., 2004). Finally, Cripe et al performed echocardiograms on 259 first degree relatives of 50 probands with BAV and found 24 of 259 (9.3%) to have BAV (Cripe, et al., 2004). Using information on more than 800 relative pairs, Cripe et al found BAV to be strongly determined by additive genetic effects with a heritability estimate of 89% (2004).

#### *Environmental risk factors*

It is generally believed that non-syndromic CHDs occur as a combination of genes and environment, meaning that non-genetic risk factors also exist for CHDs.

Characterizing the non-genetic risk factors for CHDs has, however, been challenging and for many potential risk factors the current evidence linking them to CHDs is limited or inconclusive (Jenkins et al., 2007). However, there are some risk factors that are well-established causes for CHDs, such as maternal pre-gestational diabetes and maternal use of retinoic acid. Other potential risk factors include maternal obesity and low maternal folate status. As the prevalence of these latter two factors has changed substantially over the last 20 years, it is possible that recurrence risks estimated from previous time periods may not provide accurate risks for contemporary populations. For example, if maternal obesity accounts for an increasing proportion of CHDs, the relative importance of genetic risk factors may be on the decline.

### *Folic Acid*

In 1992, the Centers for Disease Control made a recommendation that “all women of childbearing age in the United States who are capable of becoming pregnant should consume 0.4mg of folic acid per day...” (“Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects,” 1992). Four years later, the U.S. Food and Drug Administration’s (FDA) final rule on folic acid fortification was published and implemented nationwide in 1998. The final rule states:

...based on the totality of the publicly available scientific evidence, there is significant scientific agreement among qualified experts that, among women of childbearing age in the general U.S. population, maintaining adequate folate intake, particularly during the periconceptional interval, may reduce the risk of a neural tube birth defect-affected pregnancy. (Kessler, 1996)

This rule created near ubiquitous exposure to folic acid in the U.S.; it is now found in items such as pasta, rice, and cereals.

Studies conducted on periconceptional folic acid supplementation and on post-fortification populations have shown ~25% reduction in the prevalence of any CHD as well

as specific subgroups of CHDs including conotruncal defects, ventricular septal defects, and possibly CoA (Botto, Mulinare, & Erickson, 2000; Canfield et al., 2005; Ionescu-Ittu, Marelli, Mackie, & Pilote, 2009; van Beynum et al., 2010). While the findings of folic acid conferring a possible protective effect for CHDs are encouraging, they are not conclusive given mixed results in a limited number of studies (Jenkins, et al., 2007).

### *Maternal Conditions*

Maternal factors that have increased in prevalence in recent years include obesity and type II diabetes in the United States. According to the National Health and Nutrition Examination Survey (NHANES), obesity has increased 37% while diabetes has increased 60% since 1990.

### *Diabetes*

Maternal pre-gestational diabetes is known to cause multiple congenital anomalies and have a teratogenic effect on the cardiovascular system with a reported relative risk of CHD of 1.7-4.0 (Becerra, Khoury, Cordero, & Erickson, 1990; Ferencz, Rubin, McCarter, & Clark, 1990; Mills et al., 1988; Mitchell, Sellmann, Westphal, & Park, 1971; Pedersen, Tygstrup, & Pedersen, 1964; Rowland, Hubbell, & Nadas, 1973; Wren, Birrell, & Hawthorne, 2003). The most commonly reported defects are laterality defects (e.g. heterotaxy, situs inversus), conotruncal defects and less commonly, some LSLs (Becerra, et al., 1990; Ferencz, et al., 1997; Rowland, et al., 1973; Wren, et al., 2003).

### *Obesity*

Significant associations between CHDs and maternal body mass index (BMI), which is defined as weight in kilograms divided by the square of height in meters, have been found in many studies. These studies have shown significant increases in the occurrence of any heart defect in children of overweight (BMI 25-<30 kg/m<sup>2</sup>), obese (BMI 30-35 kg/m<sup>2</sup>),

and severely obese (BMI >35 kg/m<sup>2</sup>) mothers (Cedergren & Kallen, 2003; Gilboa et al., 2010; Watkins, Rasmussen, Honein, Botto, & Moore, 2003) (Table 4). Further, Gilboa, et. al. (2010) found a significant increase in the occurrence of LSLs as a group, as well as a specific increase in HLHS, in infants of obese mothers.

**Table 4. Risk of CHD in children of overweight and obese mothers**

	<b>Defect(s) Studied</b>	<b>Overweight (BMI 25-29.9)</b>	<b>Obese (BMI 30-35)</b>	<b>Severely Obese (BMI &gt;35)</b>
		OR (95% CI)	OR (95% CI)	OR (95% CI)
<b>Watkins et al, 2003</b>	All CHD	<b>2.0 (1.2-3.1)</b>		
	LSL	<b>3.3 (1.6-6.7)</b>		
<b>Cedergren and Kallen, 2003</b>	All CHD	1.03 (0.96-1.11)	<b>1.18 (1.09-1.27)</b>	<b>1.41 (1.22-1.64)</b>
	HLHS	1.41 (0.90-2.21)		
<b>Gilboa et al, 2010</b>	All CHD	<b>1.16 (1.05-1.29)</b>	<b>1.15 (1.00-1.32)</b>	<b>1.31 (1.11-1.56)</b>
	LSL	1.14 (0.94-1.40)	<b>1.34 (1.03-1.73)</b>	0.85 (0.58-1.26)
	HLHS	1.27 (0.94-1.73)	<b>1.51 (1.03-2.22)</b>	1.21 (0.72-2.06)

Current recurrence risk estimates for LSLs, for relatives other than siblings, are based on data collected on all types of CHDs and published more than 20 years ago. Utilizing these same data, recurrence risk estimates were generated for siblings of probands with specific LSLs. There is evidence to show that CHDs are anatomically, clinically, epidemiologically and developmentally heterogeneous. Further, potential risk factors for CHD, such as maternal obesity and maternal folate status, have changed over the past 20 years. Finally, not all CHDs are clinically significant and failure to include sub-clinical findings will underestimate recurrence risks. Therefore, it is prudent to reassess the risk estimates for left-sided lesions in a contemporary population with the inclusion of sub-clinical findings.

## **MATERIALS AND METHODS**

### *IRB Approval*

This study was reviewed and approved by the Institutional Review Boards for the Protection of Human Subjects at the University of Texas Health Science Center (HSC-MS-10-0469) and The Children's Hospital of Philadelphia (IRB #1995-1029).

### *Study Population*

This study is based on data from the families of 757 probands recruited between 1997 and 2007 from the Cardiac Center at The Children's Hospital of Philadelphia. Study probands were comprised of those with LSLs, including HLHS, CoA, AS/BAV, and isolated mitral valve anomalies. Males and females of any racial/ethnic group were eligible to participate in the study. Patients with maligned atrioventricular canal defects or double outlet right ventricle with mitral valve atresia and those with a recognized genetic syndrome or chromosome anomaly, including those with Turner syndrome, were excluded from this study.

### *Data Collection*

Medical records, including, when necessary, original imaging studies were reviewed to confirm the cardiac diagnosis. In addition, a brief in-person medical interview, usually with the mother of the proband, that included a three-generation pedigree was completed by a genetic counselor at The Children's Hospital of Philadelphia. Data collected as part of the pedigree included whether each relative had a congenital heart defect (CHD) and, when available, the specific type of CHD.

One or both parents of 134 probands underwent echocardiography at the Cardiac Center at The Children's Hospital of Philadelphia. Standard parasternal short and long axis views were completed to define aortic valve anatomy and function. Both 2D imaging as

well as color, pulse wave and continuous wave Doppler analysis was performed to: (1) define aortic valve anatomy, (2) assess aortic valve leaflet excursion or thickness, (3) detect turbulent and/or accelerated flow, and (4) detect aortic valve insufficiency. A single pediatric cardiologist specializing in echocardiography reviewed and characterized all studies for: (1) the ability to interpret the study, (2) the presence of a tri-, bi or unicuspid aortic valve, (3) the presence of aortic valve stenosis as defined by thickened leaflets, limited leaflet excursion, turbulent antegrade flow, and/or accelerated antegrade flow, and (4) the presence of aortic valve insufficiency. Results were summarized as normal or abnormal.

### *Statistical Analysis*

The characteristics of the probands were summarized using counts and proportions. Precurrence and recurrence risks were calculated as the proportion of relatives of a particular type that had any type of CHD. Pre/recurrence risks were calculated separately for parents, siblings, second (aunts/uncles) and third (cousins) degree relatives, and within subgroups of these relatives defined by the sex of the proband, sex of the relative or the proband's lesion. For parents, precurrence risks were initially calculated counting as affected only those parents reported as affected in the pedigree and then including as affected both those reported by family history and those identified by echocardiography. Pre/recurrence risks were estimated as binomial proportions and exact 95% confidence intervals were calculated using an online Java script calculator (<http://statpages.org/confint.html>). Risks to different groups of relatives (e.g. mothers and fathers) were compared using odds ratios and their associated 95% confidence intervals. Concordance rates were calculated for affected proband-relative pairs, separately for each

type of relative (i.e. parent, sib, aunt/uncle, cousin). Concordance rates for the same LSL and for a different LSL were estimated. Unless otherwise noted, all statistical analyses were conducted using SAS version 9.2.

## RESULTS

Characteristics of the study probands are presented in Table 5. Briefly, the majority of the probands were White (85%) and there was a predominance of males (65%). Approximately, 40% of the probands had HLHS, 35% had CoA, 23% had AS/BAV and the remainder had isolated mitral valve abnormalities or HLHS variants.

Pre/recurrence risks, i.e., the risk of a CHD to relatives born before the study proband or the risk to relatives born after the proband, for mothers, fathers, brothers, sisters, maternal aunts/uncles, paternal aunts/uncles, maternal cousins and paternal cousins of 757 LSL probands are summarized in Table 6. Based on the family history data, the overall risk of any CHD among the parents of probands was 1.67% (95% CI: 1.08%-2.35%), and was higher for fathers (1.86%, 95% CI: 1.02%-3.10%) than mothers (1.21%, 95% CI: 0.55%-2.28%). The risk to fathers was also higher than the risk to mothers for each of the three major lesion categories: HLHS (1.99% versus 1.36%), CoA (1.53% versus 0.78%), and AS/BAV (1.81% versus 1.19%). For both mothers and fathers, risk varied based on the LSL phenotype of the child, with the highest risk observed for mothers and fathers of probands with HLHS (1.36% and 1.99%, respectively).

The overall risk to siblings was markedly higher than that to parents (5.13%, 95% CI: 3.76%-6.83%). The overall risk to brothers (6.64%, 95% CI: 4.52%-9.34%) was approximately twice that of sisters (3.46%, 95% CI: 1.90%-5.73%). Further, the risk to brothers exceeded that to sisters for each of the three major categories of LSLs in the

**Table 5. Characteristics of the study probands and families**

<b>Proband Gender</b>	
Male	493 (65%)
Female	264 (35%)
<b>Proband CHD</b>	
HLHS	299 (39%)
CoA	263 (35%)
AS/BAV	171 (23%)
Other	25 (3%)
<b>Proband Ethnicity</b>	
White	644 (85%)
Black	63 (8%)
Other	51 (7%)

proband: HLHS (8.33% versus 2.31%), CoA (5.73% versus 2.37%), and AS/BAV (6.43% versus 5.21%). For both brothers and sisters, risk varied based on the LSL phenotype of the proband. Similar to parents, brothers of proband with HLHS had the highest risk (8.33%, 95% CI: 4.38%-14.1%). In contrast, sisters of probands with AS/BAV had the highest risk (5.21%, 95% CI: 1.17%-11.74%).

Among avuncular relatives, the overall risk was 0.51% (95% CI: 0.30%-0.82%). Overall, and within categories defined by the proband's lesion, risks were generally higher for paternal as compared to maternal aunts/uncles, however they were equal for CoA (Table 6). For both maternal and paternal aunts/uncles the risk varied based on LSL phenotype of the proband with the highest risk observed for paternal aunts/uncles of probands with both HLHS (0.78%, 95% CI: 0.25%-1.8%) and AS/BAV (0.78%, 95% CI: 0.16%-2.27%). The highest risk for maternal aunts/uncles was observed for probands with AS/BAV (0.49%, 0.06-1.77).

Among first cousins, the overall risk was 0.74% (95% CI: 0.49%-1.06%) and was higher for paternal first cousins (0.90%, 0.54-1.42) than maternal first cousins (0.57%, 0.28-1.01). The risk to paternal cousins was also higher than maternal cousins for the three major lesion categories: HLHS (1.04% versus 0.70%), CoA (0.29% versus 0.27%), and AS/BAV (1.46% versus 0.86%). For both maternal and paternal cousins, risk varied based on LSL phenotype of the proband, with the highest risk observed for maternal and paternal cousins of probands with AS/BAV (0.86% and 1.46%, respectively).

**Table 6. Pre/recurrence risks by relationship and subdivided by proband lesion**

Relationship to Proband	Pre/Recurrence risk by proband lesion			
	n/Total % (95%CI)			
	HLHS n=299	CoA n=263	AS/BAV n=171	Total** N=758
<b>Parent</b>				
<b>Fam History only</b>	10/596 1.68 (0.81-306)	6/517 1.16 (0.43-2.51)	5/334 1.50 (0.49-3.46)	25/1496 1.67 (1.08-2.35)
<b>Fam Hx + echo</b>	19/596 3.19 (1.93-4.93)	14/517 2.71 (1.49-4.50)	8/334 2.40 (1.04-4.66)	43/1496 2.87 (2.09-3.85)
<b>Mother</b>				
<b>Fam History only</b>	4/295 1.36 (0.37-3.43)	2/256 0.78 (0.09-2.79)	2/168 1.19 (0.14-4.23)	9/744 1.21 (0.55-2.28)
<b>Fam Hx + echo</b>	5/295 1.69 (0.55-3.91)	7/256 2.73 (1.11-5.55)	3/168 1.79 (0.37-5.13)	16/744 2.15 (1.23-3.47)
<b>Father</b>				
<b>Fam History only</b>	6/301 1.99 (0.74-4.26)	4/261 1.53 (0.42-3.88)	3/166 1.81 (0.37-5.19)	14/752 1.86 (1.02-3.10)
<b>Fam Hx + echo</b>	14/301 4.65 (2.57-7.68)	7/261 2.68 (1.08-5.45)	5/166 3.01 (0.99-6.89)	27/752 3.59 (2.38-5.18)
<b>Sibling</b>	15/274 5.47 (3.10-8.87)	13/326 3.99 (2.14-9.72)	14/236 5.93 (3.28-9.75)	44/857 5.13 (3.76-6.83)
<b>Sister</b>	3/130 2.31 (0.48-6.60)	4/169 2.37 (0.65-5.95)	5/96 5.21 (1.17-11.74)	14/405 3.46 (1.90-5.73)
<b>Brother</b>	12/144 8.33 (4.38-14.1)	9/157 5.73 (2.65-10.6)	9/140 6.43 (2.98-11.85)	30/452 6.64 (4.52-9.34)
<b>Second Degree</b>	7/1211 0.58 (0.23-1.19)	4/1203 0.33 (0.09-0.85)	5/789 0.63 (0.21-1.47)	17/3302 0.51 (0.30-0.82)
<b>Mat. aunt/uncle</b>	2/566 0.35 (0.04-1.27)	2/599 0.33 (0.04-1.20)	2/405 0.49 (0.06-1.77)	6/1621 0.37 (0.14-0.80)
<b>Pat. aunt/uncle</b>	5/645 0.78 (0.25-1.8)	2/604 0.33 (0.04-1.19)	3/384 0.78 (0.16-2.27)	11/1681 0.65 (0.33-1.17)
<b>Third Degree</b>	12/1344 0.89 (0.46-1.55)	4/1421 0.28 (0.08-0.72)	12/1059 1.13 (0.59-1.97)	29/3934 0.74 (0.49-1.06)
<b>Mat. first cousin</b>	4/573 0.70 (0.19-1.78)	2/731 0.27 (0.03-0.98)	5/581 0.86 (0.28-2.0)	11/1940 0.57 (0.28-1.01)
<b>Pat. first cousin</b>	8/771 1.04 (0.45-2.03)	2/690 0.29 (0.04-1.04)	7/478 1.46 (0.59-2.99)	18/1994 0.90 (0.54-1.42)

\*\* N may be greater than the sum of three subtypes because other miscellaneous types of defects have not been subdivided.

Pre/recurrence risks were also estimated separately for the relatives of male and female probands for first degree relatives (Table 7) as well as second and third degree relatives (Table 8). Risks were not consistently associated with the sex of the proband (e.g. risks were higher for mothers of male probands as compared to female probands, whereas the opposite was true for fathers). The high risks to brothers of male probands (7.87%, 95% CI: 5.11%-11.48%) and in particular the brothers of male probands with HLHS (11.8%, 95% CI: 6.06%-20.18%) are, however, of note.

Table 9 summarizes the concordance rates for proband-affected relative pairs. Among affected parents, 65% (n=15) had a LSL and among these parents 47% (7/15) had the same lesion as their affected child. Concordance rates for AS/BAV were particularly high with all six of the affected parents of a proband with AS/BAV also having AS/BAV. Among affected sibs, 70% (n=31) had a LSL and among these sibs 55% (17/31) had the same lesion as the proband. Similar to parents, concordance rates for AS/BAV were particularly high with 10 of the 14 (71%) affected sibs of a proband with AS/BAV also having this condition. Concordance rates for second (24%) and third (21%) degree relatives were lower than those observed for first-degree relatives. The relatively small number of affected relative pairs in these categories precluded meaningful assessment of concordance for specific lesion types. The specific CHD observed in affected relatives who did not have a LSL are summarized in Table 10.

**Table 7. Pre/recurrence risks by relationship subdivided by both proband gender and proband lesion**

Relationship to Proband	Pre/Recurrence risk by proband gender and lesion, n/Total % (95%CI)						Total	
	HLHS		CoA		AS/BAV			
	Male (n=188)	Female (n=110)	Male (n=161)	Female (n=102)	Male (n=132)	Female (n=39)	Male (n=493)	Female (264)
<b>Parent</b>								
<b>Fam History only</b>	6/375 1.60 (0.59-3.45)	4/219 1.83 (0.50-4.61)	2/317 0.63 (0.08-2.26)	4/200 2.00 (0.55-5.04)	5/258 1.94 (0.63-4.46)	1/76 1.32 (0.03-7.11)	14/974 1.44 (0.79-2.40)	9/520 1.73 (0.79-3.26)
<b>Fam Hx + echo</b>	12/375 3.20 (1.66-5.52)	7/219 3.20 (1.29-6.47)	8/317 2.52 (1.10-4.91)	6/200 3.00 (1.11-6.41)	7/258 2.71 (1.10-5.51)	1/76 1.32 (0.03-7.11)	27/974 2.77 (1.83-4.01)	15/520 2.88 (1.62-4.71)
<b>Mother</b>								
<b>Fam History only</b>	2/185 1.08 (0.13-3.85)	2/109 1.83 (0.22-6.47)	1/157 0.64 (0.02-3.50)	1/99 1.01 (0.03-5.50)	2/130 1.54 (0.19-5.45)	1/38 2.63 (0.07-13.8)	6/484 1.24 (0.46-2.68)	3/259 1.16 (0.24-3.35)
<b>Fam Hx + echo</b>	3/185 1.62 (0.34-4.67)	2/109 1.83 (0.22-6.47)	5/157 3.18 (1.04-7.28)	2/99 2.02 (0.25-7.11)	2/130 1.54 (0.19-5.45)	1/38 2.63 (0.07-13.8)	11/484 2.27 (1.14-4.03)	5/259 1.93 (0.63-4.45)
<b>Father</b>								
<b>Fam History only</b>	4/190 2.11 (0.58-5.30)	2/110 1.82 (0.22-6.41)	1/160 0.63 (0.02-3.43)	3/101 2.97 (0.62-8.44)	3/128 2.34 (0.49-6.70)	0/38 0.0	8/490 1.63 (0.71-3.19)	6/261 2.30 (0.85-4.94)
<b>Fam Hx + echo</b>	9/190 4.74 (2.19-8.80)	5/110 4.55 (1.49-10.29)	3/160 1.88 (0.39-5.38)	4/101 3.96 (1.09-9.83)	5/128 3.91 (1.28-8.88)	0/38 0.0	17/490 3.47 (2.03-5.50)	10/261 3.83 (1.85-6.93)
<b>Sibling</b>	13/168 7.74 (4.18-12.87)	2/106 1.89 (0.23-6.65)	7/207 3.38 (1.37-6.84)	6/119 5.04 (1.87-10.65)	11/172 6.40 (3.24-11.15)	3/64 4.69 (0.98-13.1)	31/555 5.58 (3.83-7.83)	13/302 4.30 (2.31-7.25)
<b>Brother</b>	11/93 11.83 (6.06-20.18)	1/51 1.96 (0.05-10.45)	6/100 6.00 (2.23-12.60)	3/57 5.26 (1.10-14.62)	7/107 6.54 (2.67-13.02)	2/33 6.06 (0.74-20.2)	24/305 7.87 (5.11-11.48)	6/147 4.08 (1.51-8.67)
<b>Sister</b>	2/75 2.67 (0.32-9.30)	1/55 1.82 (0.05-9.72)	1/107 0.93 (0.02-5.10)	3/62 4.84 (1.01-13.5)	4/65 6.15 (1.70-15.01)	1/31 3.23 (0.08-16.7)	7/250 2.80 (1.13-5.68)	7/155 4.52 (1.83-9.08)

**Table 8. Pre/recurrence risks by relationship subdivided by proband gender**

Relationship to Proband	Pre/Recurrence risk by proband gender, n/Total % (95%CI)	
	Male	Female
<b>Second Degree*</b>	11/2120 0.52 (0.26-0.93)	6/1178 0.51 (0.19-1.11)
<b>Mat. aunt/uncle</b>	4/1033 0.39 (0.11-0.99)	2/587 0.34 (0.04-1.23)
<b>Pat. aunt/uncle</b>	7/1087 0.64 (0.26-1.32)	4/591 0.68 (0.18-1.72)
<b>Third Degree*</b>	21/2546 0.82 (0.51-1.26)	8/1385 0.58 (0.25-1.13)
<b>Mat. first cousin</b>	7/1239 0.56 (0.23-1.16)	4/698 0.57 (0.16-1.46)
<b>Pat. first cousin</b>	4/1307 0.31 (0.08-0.78)	4/687 0.58 (0.16-1.48)

\* Defect and sex specific risks were not calculated for second and third degree relatives due to small numbers within most categories

**Table 9. Concordance rates for affected relative pairs with the same LSL, different LSLs, or non-LSL defects**

<b>Affected Parent</b>			
Proband Diagnosis	Concordant – same lesion	Concordant – Different LSL	Discordant – not LSL
<b>HLHS (n=11)</b>	0	7 (64%)	4 (36%)
<b>CoA (n=6)</b>	1 (17%)	1 (17%)	4 (66%)
<b>AS/BAV (n=6)</b>	6 (100%)	0	0
<b>All (n=23)</b>	15 (65%)		8 (35%)
<b>Affected Sibling</b>			
Proband Diagnosis	Concordant – same lesion	Concordant – Different LSL	Discordant – not LSL
<b>HLHS (n=15)</b>	3 (20%)	5 (33%)	7 (47%)
<b>CoA (n=15)</b>	4 (27%)	7 (47%)	4 (27%)
<b>AS/BAV (n=14)</b>	10 (71%)	2 (14%)	2 (14%)
<b>All (n=44)</b>	31 (70%)		13 (30%)
<b>Affected aunt/uncle</b>			
Proband Diagnosis	Concordant – same lesion	Concordant – Different LSL	Discordant – not LSL
<b>HLHS (n=8)</b>	0	3 (37%)	5 (63%)
<b>CoA (n=4)</b>	1 (25%)	0	3 (75%)
<b>AS/BAV (n=5)</b>	0	0	5 (100%)
<b>All (n=17)</b>	4 (24%)		13 (76%)
<b>Affected cousin</b>			
Proband Diagnosis	Concordant – same lesion	Concordant – Different LSL	Discordant – not LSL
<b>HLHS (n=12)</b>	2 (17%)	2 (17%)	8 (66%)
<b>CoA (n=4)</b>	0	0	4 (100%)
<b>AS/BAV (n=12)</b>	0	2 (17%)	10 (83%)
<b>All (n=28)</b>	6 (21%)		22 (79%)

**Table 10. Diagnoses for discordant proband-affected relative pairs**

<b>Affected Parent – Discordant (n=8)</b>	
<b>Proband Diagnosis</b>	<b>Discordant Relative Diagnosis</b>
<b>HLHS (n=4)</b>	VSD; L-TGA; ASD; other
<b>CoA (n=4)</b>	PDA; TOF; unknown (2)
<b>AS/BAV (n=0)</b>	
<b>Affected Sibling – Discordant (n=13)</b>	
<b>Proband Diagnosis</b>	<b>Discordant Relative Diagnosis</b>
<b>HLHS (n=7)</b>	VSD (4); ASD; DORV; PS
<b>CoA (n=4)</b>	IAA; PDA; VSD; AVCD
<b>AS/BAV (n=2)</b>	other; unspecified hole in heart
<b>Affected aunt/uncle – Discordant (n=13)</b>	
<b>Proband Diagnosis</b>	<b>Discordant Relative Diagnosis</b>
<b>HLHS (n=5)</b>	ASD; unspecified hole in heart (4); unknown
<b>CoA (n=3)</b>	ASD; unspecified hole in heart; unknown
<b>AS/BAV (n=5)</b>	ASD; VSD; unspecified hole in heart (3)
<b>Affected cousin – Discordant (n=22)</b>	
<b>Proband Diagnosis</b>	<b>Discordant Relative Diagnosis</b>
<b>HLHS (n=8)</b>	PS; unspecified hole in heart (4); unknown (3)
<b>CoA (n=4)</b>	VSD (2); pulmonary artery anomaly; unknown
<b>AS/BAV (n=10)</b>	TGA; PS; AAA; unspecified hole in heart (4); unknown (2); other

[VSD: ventricular septal defect; ASD: atrial septal defect; PDA: patent ductus arteriosus; TOF: Tetralogy of Fallot; L-TGA: transposition of the great arteries (left); AVCD: atrioventricular canal defect; IAA: interrupted aortic arch; DORV: double outlet right ventricle; PS: pulmonary stenosis; AAA: abdominal aortic aneurysm]

**Table 11. Echocardiography findings among parents of probands with LSLs**

	<b>Total N (%)</b>	<b>Normal N % (95% CI)</b>	<b>Abnormal N % (95% CI)</b>
<b>Mother</b>	101 (50%)	94 93.1 (86.2-97.2)	7 6.9 (2.83-13.8)
<b>Father</b>	101 (50%)	88 87.1 (79.0-93.0)	13 12.9 (7.03-21.0)
<b>Total</b>	202	186 92.1 (87.5-95.4)	20 9.9 (6.15-14.9)
<b>Proband Diagnosis</b>			
<b>HLHS</b>	100 (50%)	91 91 (83.6-95.8)	9 9 (4.20-16.4)
<b>CoA</b>	62 (31%)	54 87 (76.2-94.3)	8 13 (5.74-23.9)
<b>AS/BAV</b>	26 (13%)	23 88.5 (69.9-97.6)	3 11.5 (2.45-30.2)
<b>Other</b>	5 (2%)	5 100	0
<b>Unknown</b>	9 (4%)	9 100	0

The apparently unaffected parents of 134 probands were evaluated by echocardiography. The distribution of LSLs among these probands (HLHS 50%, CoA 31%, AS/BAV 13%, Other/Unknown 6%) was similar to that of the full study sample (Table 5), although the proportion of probands with HLHS was somewhat higher (50% versus 39%). Both parents were evaluated in 68 (50%) families while the mother only was evaluated in 33 (25%) and the father only in 33 (25%) cases.

Among the parents evaluated by echocardiography, the overall prevalence of previously unrecognized CHD, such as BAV, was 9.9% (Table 11). The prevalence of previously unrecognized CHD was 12.9% (7.03-21.0) among fathers and 6.9% (2.83-13.8) among mothers. Fathers were nearly two times more likely to have previously unrecognized CHD compared to mothers (OR=1.98, 95% CI 0.76-5.20), although this result is not significant likely due to a smaller sample size. The prevalence of unrecognized CHD also varied as a function of the specific LSL phenotype of the proband, ranging from 0% for parents of probands with other/unknown lesions to 11.5% (2.45-30.2) among the parents of probands with AS/BAV.

When parents identified as having CHD by echo were included in the numerator for the precurrence risk estimates, risks increased in almost all categories (Tables 6 and 7). The inclusion of these data resulted in the greatest increase in the risk estimate for mothers of probands with CoA (0.78% to 2.73%) and fathers of probands with HLHS (1.99% to 4.65%).

## DISCUSSION

Currently, recurrence risks for left-sided cardiac lesions are based on combined data from multiple studies conducted 20 to 40 years ago (Nora, et al., 1991). These data support familial clustering of CHDs with a sibling recurrence of 2-3%. Offspring recurrence risks were also found to be elevated (4-18%) and to vary based on the sex of the affected parent. These recurrence risks are now the published numbers found in Harper's *Practical Genetic Counseling*, a reference book well known to those who practice genetic counseling (Harper, 2004). Given that many environmental (e.g. maternal consumption of folic acid) and maternal conditions (e.g. maternal diabetes and obesity), as well as diagnostic capabilities, have changed over the past twenty years, it is prudent to reassess the CHD recurrence risks currently used in clinical practice. Increased recognition of distinct subgroups of CHDs, which may each have different recurrence risks, makes reassessment of risk estimates for the individual groups sensible.

In this study, data collected from a clinic population at The Children's Hospital of Philadelphia were used to assess pre/recurrence risks for first (parents, siblings), second (aunts/uncles) and third (cousin) degree relatives of probands with LSLs (e.g. HLHS, CoA, and AS/BAV). Family history data were analyzed alone and with the inclusion of echocardiography data collected on parents of a subset of the probands. Concordance rates for proband-affected relative pairs were also examined.

*Practical Genetic Counseling* (Harper, 2004) provides risk estimates for overall congenital heart disease. These are the general numbers used when counseling regarding recurrence for CHD and when specific details of the proband's diagnosis are not available.

In a comparison of these estimates to those found in this study, the sibling risk estimate is higher in our LSL cohort than for all CHDs (Table 12). The risk estimates for second and third degree relatives found in this study and those for all CHDs are fairly comparable. However, in the present study, the risk to second degree relatives is lower than third degree relatives. While this may seem counterintuitive, it is likely a reflection of a number of factors including, advances in diagnostic capabilities and modern standards of care for evaluating murmurs in children, improved reporting of cousins as compared to older patients such as avuncular relatives, and older relatives who died in infancy may never have received a diagnosis. This finding was also reported by Loffredo, et al (2004) in a LSL cohort.

**Table 12.** Comparison of approximate recurrence risks for all CHD versus LSL only

<b>Relation to Proband</b>	<b>Risk, % Harper, 2004 All CHD</b>	<b>Risk, % (95% CI) Present study LSL only</b>
<b>Sibling</b>	2-3	5.0 (3.76-6.83)
<b>Second degree</b>	1-2	0.5 (0.30-0.82)
<b>Third degree</b>	<1*	0.7 (0.49-1.06)

\*According to Harper, data are inadequate.

In addition to providing overall CHD risk estimates for first, second and third degree relatives, *Practical Genetic Counseling* (Harper, 2004) also provides estimates of the risk to sibs of probands with specific LSLs. We found the pre/recurrence risks for any CHD when the proband is affected with HLHS, CoA or AS/BAV to be higher than risks quoted in Harper (Table 13). One possible explanation for this apparent increase in the risk to sibs of LSL probands is improvements in the diagnosis of CHD over time. Progress in echocardiography over the past 20 years has made diagnosis easier and more precise and so could account for part of the increase in risk estimates. Another possible explanation is the

increasing prevalence of potential maternal risk factors including pre-gestational diabetes and obesity, which would likely represent shared exposures for siblings. The incidence of these conditions has increased 37% and 60%, respectively since 1990 (Cowie et al., 2006; Flegal, Carroll, Kuczmarski, & Johnson, 1998; Ogden & Carroll, 2010; "Vital and health statistics. Current estimates from the National Health Interview Survey, 1988," 1989).

**Table 13.** Comparison of approximate recurrence risks for sibs of probands with left-sided cardiac lesions

<b>Proband Defect</b>	<b>Risk, % Harper, 2004*</b>	<b>Risk, % (95%CI) Present study</b>
<b>HLHS</b>	3	5.5 (3.1-8.9)
<b>CoA</b>	2	4.0 (2.1-6.7)
<b>AS/BAV</b>	2	6.0 (3.3-9.8)

\*Based on multiple studies collated by Nora JJ, Berg K, Nora AH (1991)

Among first-degree relatives in this study, the risk to male relatives (i.e. fathers and brothers) was increased relative to their female counterparts. This finding was also reported by Lewin, et al. (2004) in a LSL cohort. Estimates of risk to second and third degree relatives by sex of the relative were not determined, given the small number of affected relatives in these categories. When risk estimates were stratified based on the sex of the proband there was no clear pattern of increased risk for the relatives of either males or females. Among second and third degree relatives there was, however, a tendency for risks to be higher in paternal relatives as compared to their maternal counterparts. Previous studies have not commented on the maternal/paternal relative pre/recurrence risk.

Further subdividing the risk estimates, we found the pre/recurrence risk for brothers (7%) to be higher than sisters (3%) for all LSLs. In general, fathers of probands with LSLs were also more likely to be affected (4%) than mothers of probands (2%). However, the

precurrence risks were almost equal when the proband had CoA. The pre/recurrence risks for aunts/uncles and cousins were less than 1% for all LSLs and the division of these groups based on maternal or paternal lineage did not make a clear difference.

Subgroup analysis was also performed based on the gender of the proband and specific LSL. In general, the sex of the proband did not appear to influence the precurrence risks in parents. However, in siblings the pre/recurrence risk for brothers of male probands was almost double (~8%) that for brothers of female probands (4%). Much of this difference is comprised of the almost 12% pre/recurrence risk found for brothers of males with HLHS. Prior studies have not examined recurrence risk based on sex and lesion of the proband together. Of note, as the groups continued to be subdivided the numbers of affected relatives became quite small and therefore, these risk estimates are deemed imprecise.

This study also aimed to estimate the concordance rate among proband-affected relative pairs. Among affected parents and siblings the concordance rates were 65% and 70%, respectively. For each group, of those that were concordant, approximately half had the same lesion as the proband. The fact that there are no proband-affected parent pairs concordant for HLHS is not surprising given that individuals with HLHS have historically not survived to reach child-bearing age. In second and third degree relatives the concordance rate is lower than first degree relatives. Decreasing concordance with increasing degree of kinship is consistent with previous studies. Based on our findings, AS/BAV has the highest rate of concordance among the proband-affected first-degree relative pairs suggesting a largely genetic component, as found in previous studies (Cripe, et al., 2004). In those relatives that were discordant, the most common anomalies seen were VSD, ASD, and unspecified hole in heart. This is consistent with the general population

risks for CHD in which VSD and ASD are the most common CHDs, with a population prevalence of 0.5% and 0.1%, respectively (Drugan, 2006). Together, these findings suggest, based primarily on first-degree relatives, that LSLs are more closely related to each other than other CHDs.

Finally, this study aimed to estimate the proportion of parents of probands with a LSL who had a previously unrecognized aortic valve abnormality and were subsequently diagnosed by echocardiography. Approximately 10% of parents had abnormal echocardiograms showing bicuspid or thickened aortic valve or aortic regurgitation. This finding is similar to other studies that have identified aortic valve abnormalities by echocardiography in 7.5% - 12% of first degree relatives of LSL probands (Brenner, et al., 1989; Cripe, et al., 2004; Huntington, et al., 1997; Lewin, et al., 2004). Abnormal findings were more common in fathers (13%) than in mothers (7%). This may be accounted for by the male predominance of both CoA (M:F 2:1) and AS/BAV (M:F 2:1) in the general population (Nora, et al., 1991). The current study, comparable to Lewin, et al., uses a sample from the complete spectrum of LSLs, whereas others used a subset of proband LSLs (Brenner, et al., 1989; Huntington, et al., 1997; Lewin, et al., 2004; Loffredo, et al., 2004). Lewin, et al. performed echocardiograms on 282 first-degree relatives (parents and siblings) of probands with LSLs and found 21 (7.55%) individuals with aortic valve anomalies (2004). They found that the proportion of left heart anomalies for mothers, fathers, sisters, and brothers was not significantly different when compared by proband diagnosis or gender. We have confirmed the excess occurrence of BAV in first-degree relatives of probands with HLHS as well as other left-sided lesions.

BAV is often considered a benign lesion early in life, but complications of BAV including aortic stenosis, aortic regurgitation, and aortic dilation result in morbidity later in life (Ward, 2000). These complications should be monitored and interventions made as needed, making the early detection of BAV paramount and supporting previous recommendations to screen first degree relatives of probands with left-sided lesions (Huntington, et al., 1997).

Based on the 10% detection of valve abnormalities by echocardiography data, it is evident these studies increase risk estimates. However, our risk estimates are still an underestimate given that echocardiograms were only performed on a subset of parents.

As with all studies, this study had some limitations including the use of families ascertained through a single large referral center. Since such centers may serve a non-random subset of all LSL cases, this population may be enriched for more severe cardiac defects. Consequently, the pre/recurrence risks estimated from this population may not be generalizable to the broader population. In addition, the family history data were based on the report of the proband's parents. Hence, the diagnosis reported for affected relatives may not be accurate and some affected relatives may have not been identified. Further, given the timeframe of this study, some of the relatives would likely have been conceived prior to folic acid fortification. If such fortification is associated with a change in familial pre/recurrence risks, these estimates may not be reflective of the contemporary population. This study also had several strengths including a relatively large sample size, clinical confirmation of the diagnosis in probands and the classification of apparently unaffected parents by a single echocardiographer.

Convincing evidence exists for a substantial genetic component in the left-sided lesions, HLHS, CoA and AS/BAV. The results of this study are generally consistent with previous studies aiming to determine the prevalence of BAV in first-degree relatives of probands with left-sided lesions. Currently, no protocol exists for the examination of relatives of probands with LSLs; however, on the basis of these results and those of previous studies, echocardiographic screening of first-degree relatives of LSL probands is warranted. Pre/recurrence risks in this study were found to be overall higher than those used in current practice. Future population based studies can help to confirm or refute the findings of this study. While these numbers may not significantly change counseling methods, using these data combined with future population based studies can provide relevant updates to pre/recurrence risk estimates for left-sided cardiac lesions.

## REFERENCES

- Anderson, R. C. (1976). Fetal and infant death, twinning and cardiac malformations in families of 2,000 children with and 500 without cardiac defects. *Am J Cardiol*, 38(2), 218-224.
- Becerra, J. E., Khoury, M. J., Cordero, J. F., & Erickson, J. D. (1990). Diabetes mellitus during pregnancy and the risks for specific birth defects: a population-based case-control study. *Pediatrics*, 85(1), 1-9.
- Botto, L. D., Correa, A., & Erickson, J. D. (2001). Racial and temporal variations in the prevalence of heart defects. *Pediatrics*, 107(3), E32.
- Botto, L. D., Lin, A. E., Riehle-Colarusso, T., Malik, S., & Correa, A. (2007). Seeking causes: Classifying and evaluating congenital heart defects in etiologic studies. *Birth Defects Res A Clin Mol Teratol*, 79(10), 714-727. doi: 10.1002/bdra.20403
- Botto, L. D., Mulinare, J., & Erickson, J. D. (2000). Occurrence of congenital heart defects in relation to maternal multivitamin use. *Am J Epidemiol*, 151(9), 878-884.
- Boughman, J. A., Berg, K. A., Astemborski, J. A., Clark, E. B., McCarter, R. J., Rubin, J. D., & Ferencz, C. (1987). Familial risks of congenital heart defect assessed in a population-based epidemiologic study. *Am J Med Genet*, 26(4), 839-849. doi: 10.1002/ajmg.1320260411
- Boulet, S. L., Grosse, S. D., Riehle-Colarusso, T., & Correa-Villaseñor, A. (2010). Health Care Costs of Congenital Heart Defects. In D. F. Wyszynski, A. Correa-Villaseñor & T. P. Graham (Eds.), *Congenital heart defects : from origin to treatment* (pp. 493-501). Oxford ; New York: Oxford University Press.

- Brenner, J. I., Berg, K. A., Schneider, D. S., Clark, E. B., & Boughman, J. A. (1989). Cardiac malformations in relatives of infants with hypoplastic left-heart syndrome. *Am J Dis Child*, *143*(12), 1492-1494.
- Canfield, M. A., Collins, J. S., Botto, L. D., Williams, L. J., Mai, C. T., Kirby, R. S., Pearson, K., Devine, O., & Mulinare, J. (2005). Changes in the birth prevalence of selected birth defects after grain fortification with folic acid in the United States: findings from a multi-state population-based study. *Birth Defects Res A Clin Mol Teratol*, *73*(10), 679-689. doi: 10.1002/bdra.20210
- Cedergren, M. I., & Kallen, B. A. (2003). Maternal obesity and infant heart defects. *Obes Res*, *11*(9), 1065-1071. doi: 10.1038/oby.2003.146
- Christianson, A., Howson, C., & Modell, B. (2006). Global Report on Birth Defects: The Hidden Toll of Dying and Disabled Children. White Plains, NY: March of Dimes Birth Defects Foundation.
- Cowie, C. C., Rust, K. F., Byrd-Holt, D. D., Eberhardt, M. S., Flegal, K. M., Engelgau, M. M., Saydah, S. H., Williams, D. E., Geiss, L. S., & Gregg, E. W. (2006). Prevalence of diabetes and impaired fasting glucose in adults in the U.S. population: National Health And Nutrition Examination Survey 1999-2002. *Diabetes Care*, *29*(6), 1263-1268. doi: 29/6/1263 [pii]
- 10.2337/dc06-0062
- Cripe, L., Andelfinger, G., Martin, L. J., Shooner, K., & Benson, D. W. (2004). Bicuspid aortic valve is heritable. *J Am Coll Cardiol*, *44*(1), 138-143. doi: 10.1016/j.jacc.2004.03.050
- S0735109704007739 [pii]

- Drugan, A. (2006). Principles of "classic" genetics. In M. I. Evans (Ed.), *Prenatal diagnosis* (pp. 3-18). New York: McGraw-Hill Medical Pub. Division.
- Ferencz, C., Loffredo, C. A., Correa-Villasenor, A., & Wilson, P. D. (Eds.). (1997). *Genetic and Environmental Risk Factors of Major Cardiovascular Malformations* (Vol. 5). Armonk, NY: Futura Publishing Company.
- Ferencz, C., Rubin, J. D., Loffredo, C. A., & Magee, C. A. (Eds.). (1993). *Epidemiology of Congenital Heart Disease* (Vol. 4). Mount Kisco, NY: Futura Publishing Company.
- Ferencz, C., Rubin, J. D., McCarter, R. J., & Clark, E. B. (1990). Maternal diabetes and cardiovascular malformations: predominance of double outlet right ventricle and truncus arteriosus. *Teratology*, *41*(3), 319-326. doi: 10.1002/tera.1420410309
- Flegal, K. M., Carroll, M. D., Kuczmarski, R. J., & Johnson, C. L. (1998). Overweight and obesity in the United States: prevalence and trends, 1960-1994. *Int J Obes Relat Metab Disord*, *22*(1), 39-47.
- Fuhrmann, W. (1968). A family study in transposition of the great vessels and in tricuspid atresia. *Humangenetik*, *6*(2), 148-157.
- Fuhrmann, W., & Vogel, F. (1969). *Genetic counseling: a guide for the practicing physician*. New York: Longmans Springer Verlag.
- Gilboa, S. M., Correa, A., Botto, L. D., Rasmussen, S. A., Waller, D. K., Hobbs, C. A., Cleves, M. A., & Riehle-Colarusso, T. J. (2010). Association between prepregnancy body mass index and congenital heart defects. *Am J Obstet Gynecol*, *202*(1), 51 e51-51 e10. doi: S0002-9378(09)00903-X [pii] 10.1016/j.ajog.2009.08.005
- Harper, P. S. (2004). *Practical genetic counselling* (6th ed.). London ;: Arnold.

- Huntington, K., Hunter, A. G., & Chan, K. L. (1997). A prospective study to assess the frequency of familial clustering of congenital bicuspid aortic valve. *J Am Coll Cardiol*, 30(7), 1809-1812. doi: S0735-1097(97)00372-0 [pii]
- Ionescu-Ittu, R., Marelli, A. J., Mackie, A. S., & Pilote, L. (2009). Prevalence of severe congenital heart disease after folic acid fortification of grain products: time trend analysis in Quebec, Canada. *BMJ*, 338, b1673.
- Jenkins, K. J., Correa, A., Feinstein, J. A., Botto, L., Britt, A. E., Daniels, S. R., Elixson, M., Warnes, C. A., & Webb, C. L. (2007). Noninherited risk factors and congenital cardiovascular defects: current knowledge: a scientific statement from the American Heart Association Council on Cardiovascular Disease in the Young: endorsed by the American Academy of Pediatrics. *Circulation*, 115(23), 2995-3014. doi: CIRCULATIONAHA.106.183216 [pii] 10.1161/CIRCULATIONAHA.106.183216
- Jorgensen, G., Beuren, A. J., & Stoermer, J. (1971). [Genetic studies in different types of congenital heart defects]. *Monatsschr Kinderheilkd*, 119(7), 417-421.
- Food Labeling: Health Claims and Label Statements; Folate and Neural Tube Defects, FR 8752 C.F.R. (1996).
- Kitchiner, D., Jackson, M., Malaiya, N., Walsh, K., Peart, I., & Arnold, R. (1994). Incidence and prognosis of obstruction of the left ventricular outflow tract in Liverpool (1960-91): a study of 313 patients. *Br Heart J*, 71(6), 588-595.
- Koppel, R. I., & Mahle, W. T. (2010). Novel Methods to Screen for Heart Disease in Infants. In D. F. Wyszynski, A. Correa-Villaseñor & T. P. Graham (Eds.), *Congenital heart defects : from origin to treatment* (pp. 107-115). Oxford ; New York: Oxford University Press.

- Lewin, M. B., McBride, K. L., Pignatelli, R., Fernbach, S., Combes, A., Menesses, A., Lam, W., Bezold, L. I., Kaplan, N., Towbin, J. A., & Belmont, J. W. (2004). Echocardiographic evaluation of asymptomatic parental and sibling cardiovascular anomalies associated with congenital left ventricular outflow tract lesions. *Pediatrics, 114*(3), 691-696. doi: 10.1542/peds.2003-0782-L 114/3/691 [pii]
- Lin, A. E., & Garver, K. L. (1988). Genetic counseling for congenital heart defects. *J Pediatr, 113*(6), 1105-1109.
- Loffredo, C. A., Chokkalingam, A., Sill, A. M., Boughman, J. A., Clark, E. B., Scheel, J., & Brenner, J. I. (2004). 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, 124A*(3), 225-230. doi: 10.1002/ajmg.a.20366
- Mackie, A. S., Pilote, L., Ionescu-Ittu, R., Rahme, E., & Marelli, A. J. (2007). Health care resource utilization in adults with congenital heart disease. *Am J Cardiol, 99*(6), 839-843. doi: S0002-9149(06)02406-4 [pii]
- 10.1016/j.amjcard.2006.10.054
- Mills, J. L., Knopp, R. H., Simpson, J. L., Jovanovic-Peterson, L., Metzger, B. E., Holmes, L. B., Aarons, J. H., Brown, Z., Reed, G. F., & Bieber, F. R. (1988). Lack of relation of increased malformation rates in infants of diabetic mothers to glycemic control during organogenesis. *N Engl J Med, 318*(11), 671-676.
- Mitchell, S. C., Sellmann, A. H., Westphal, M. C., & Park, J. (1971). Etiologic correlates in a study of congenital heart disease in 56,109 births. *Am J Cardiol, 28*(6), 653-657.

- Mohan, U. R., Kleinman, C. S., & Kern, J. H. (2005). Fetal echocardiography and its evolving impact 1992 to 2002. *Am J Cardiol*, *96*(1), 134-136. doi: S0002-9149(05)00597-7 [pii]
- 10.1016/j.amjcard.2005.02.058
- Montana, E., Khoury, M. J., Cragan, J. D., Sharma, S., Dhar, P., & Fyfe, D. (1996). Trends and outcomes after prenatal diagnosis of congenital cardiac malformations by fetal echocardiography in a well defined birth population, Atlanta, Georgia, 1990-1994. *J Am Coll Cardiol*, *28*(7), 1805-1809. doi: S0735-1097(96)00381-6 [pii]
- 10.1016/S0735-1097(96)00381-6
- Moore, K. L., & Persaud, T. V. N. (2003). *The developing human : clinically oriented embryology* (7th ed.). Philadelphia, Pa.: Saunders.
- Mori, K., Ando, M., & Takao, A. (1973). Genetic aspects of congenital heart disease. *Jpn Circ J*, *37*(1), 35-39.
- Morris, C. D., Outcalt, J., & Menashe, V. D. (1990). Hypoplastic left heart syndrome: natural history in a geographically defined population. *Pediatrics*, *85*(6), 977-983.
- Nora, J. J. (1968). Multifactorial inheritance hypothesis for the etiology of congenital heart diseases. The genetic-environmental interaction. *Circulation*, *38*(3), 604-617.
- Nora, J. J., Berg, K., & Nora, A. H. (1991). *Cardiovascular diseases: genetics, epidemiology, and prevention*. New York, NY: Oxford University Press.
- Nora, J. J., & Nora, A. H. (1978). The evolution of specific genetic and environmental counseling in congenital heart diseases. *Circulation*, *57*(2), 205-213.
- Nora, J. J., & Nora, A. H. (1988). Familial risk of congenital heart defect. *Am J Med Genet*, *29*(1), 231, 233. doi: 10.1002/ajmg.1320290134

- O'Brien, S. E. (2010). Left Ventricular Outflow Tract Obstruction. In D. F. Wyszynski, A. Correa-Villaseñor & T. P. Graham (Eds.), *Congenital heart defects : from origin to treatment* (pp. 186-199). Oxford ; New York: Oxford University Press.
- Ogden, C. L., & Carroll, M. D. (2010). Prevalence of Overweight, Obesity, and Extreme Obesity Among Adults: United States, Trends 1976–1980 Through 2007–2008. *NCHS Health E-Stat*. Retrieved from
- Oyen, N., Poulsen, G., Boyd, H. A., Wohlfahrt, J., Jensen, P. K., & Melbye, M. (2009). Recurrence of congenital heart defects in families. *Circulation*, *120*(4), 295-301.  
doi: CIRCULATIONAHA.109.857987 [pii]  
10.1161/CIRCULATIONAHA.109.857987
- Pedersen, L. M., Tygstrup, I., & Pedersen, J. (1964). Congenital Malformations in Newborn Infants of Diabetic Women. Correlation with Maternal Diabetic Vascular Complications. *Lancet*, *1*(7343), 1124-1126.
- Phoon, C. K. L., Chun, A. J. L., & Srichai, M. B. (2010). Noninvasive Imaging of Congenital Heart Defects. In D. F. Wyszynski, A. Correa-Villaseñor & T. P. Graham (Eds.), *Congenital heart defects : from origin to treatment* (pp. 57-106). Oxford ; New York: Oxford University Press.
- Pierpont, M. E., Basson, C. T., Benson, D. W., Jr., Gelb, B. D., Giglia, T. M., Goldmuntz, E., McGee, G., Sable, C. A., Srivastava, D., & Webb, C. L. (2007). Genetic basis for congenital heart defects: current knowledge: a scientific statement from the American Heart Association Congenital Cardiac Defects Committee, Council on Cardiovascular Disease in the Young: endorsed by the American Academy of

Pediatrics. *Circulation*, 115(23), 3015-3038. doi: CIRCULATIONAHA.106.183056  
[pii] 10.1161/CIRCULATIONAHA.106.183056

Pierpont, M. E., Gobel, J. W., Moller, J. H., & Edwards, J. E. (1988). Cardiac malformations in relatives of children with truncus arteriosus or interruption of the aortic arch. *Am J Cardiol*, 61(6), 423-427. doi: 0002-9149(88)90298-6 [pii]

Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. (1992). In MMWR (Ed.), (Vol. 41, pp. 1-6): Centers for Disease Control.

Resta, R. G. (2006). Defining and redefining the scope and goals of genetic counseling. *Am J Med Genet C Semin Med Genet*, 142C(4), 269-275. doi: 10.1002/ajmg.c.30093

Roberts, W. C. (1970). The congenitally bicuspid aortic valve. A study of 85 autopsy cases. *Am J Cardiol*, 26(1), 72-83.

Rosano, A., Botto, L. D., Botting, B., & Mastroiacovo, P. (2000). Infant mortality and congenital anomalies from 1950 to 1994: an international perspective. *J Epidemiol Community Health*, 54(9), 660-666.

Rowland, T. W., Hubbell, J. P., Jr., & Nadas, A. S. (1973). Congenital heart disease in infants of diabetic mothers. *J Pediatr*, 83(5), 815-820.

Sanchez-Cascos, A. (1978). The recurrence risk in congenital heart disease. *Eur J Cardiol*, 7(2-3), 197-210.

Shah, D. J. (2010). Functional valve assessment: the emerging role of cardiovascular magnetic resonance. *Methodist Debaquey Cardiovasc J*, 6(1), 15-19. doi: 1947-6108-6-1-15 [pii]

- Shieh, J. T., & Srivastava, D. (2009). Heart malformation: what are the chances it could happen again? *Circulation*, *120*(4), 269-271. doi: CIRCULATIONAHA.109.878637 [pii] 10.1161/CIRCULATIONAHA.109.878637
- Studer, M. A., & Justino, H. (2010). Hypoplastic Left Ventricle. In D. F. Wyszynski, A. Correa-Villaseñor & T. P. Graham (Eds.), *Congenital Heart Defects: from origin to treatment* (pp. 241-260). Oxford; New York: Oxford University Press.
- Towbin, J. A., & Belmont, J. (2000). Molecular determinants of left and right outflow tract obstruction. *Am J Med Genet*, *97*(4), 297-303. doi: 10.1002/1096-8628(200024)97:4<297::AID-AJMG1280>3.0.CO;2-O [pii]
- van Beynum, I. M., Kapusta, L., Bakker, M. K., den Heijer, M., Blom, H. J., & de Walle, H. E. (2010). Protective effect of periconceptional folic acid supplements on the risk of congenital heart defects: a registry-based case-control study in the northern Netherlands. *Eur Heart J*, *31*(4), 464-471. doi: ehp479 [pii] 10.1093/eurheartj/ehp479
- Vital and health statistics. Current estimates from the National Health Interview Survey, 1988. (1989). *Vital Health Stat* *10*(173), 1-250.
- Ward, C. (2000). Clinical significance of the bicuspid aortic valve. *Heart*, *83*(1), 81-85.
- Watkins, M. L., Rasmussen, S. A., Honein, M. A., Botto, L. D., & Moore, C. A. (2003). Maternal obesity and risk for birth defects. *Pediatrics*, *111*(5 Part 2), 1152-1158.
- Williamson, E. M. (1969). A family study of atrial septal defect. *J Med Genet*, *6*(3), 255-265.
- Wren, C., Birrell, G., & Hawthorne, G. (2003). Cardiovascular malformations in infants of diabetic mothers. *Heart*, *89*(10), 1217-1220.

Wu, F. M., & Landzberg, M. J. (2010). Congenital Heart Defects in Adults. In D. F.

Wyszynski, A. Correa-Villaseñor & T. P. Graham (Eds.), *Congenital heart defects : from origin to treatment* (pp. 296-306). Oxford ; New York: Oxford University Press.

Wyszynski, D. F., Correa-Villaseñor, A., & Graham, T. P. (2010). *Congenital heart defects : from origin to treatment*. Oxford ; New York: Oxford University Press.

Zoethout, H. E., Carter, R. E., & Carter, C. O. (1964). A Family Study of Aortic Stenosis. *J Med Genet*, 55, 2-9.

## **VITA**

Sarah Elizabeth Swain was born in Wilmington, Delaware on November 15, 1985, daughter of Scott and Donna Swain. After completing her work at St. Mark's High School in 2003, she attended University of Delaware and was awarded the degree of Bachelor of Science in Biochemistry in 2007. After graduating, she worked for two years for A. I. duPont Hospital for Children in the molecular diagnostics laboratory. In 2009, she entered the University of Texas Health Science Center at Houston Graduate School of Biomedical Sciences in the program of Genetic Counseling.