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Non-Syndromic atrioventricular septal defects: a refined definition, associated risk factors, and prognostic factors for left atrioventricular valve replacement following primary repair

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NON-SYNDROMIC ATRIOVENTRICULAR SEPTAL DEFECTS: A REFINED DEFINITION, ASSOCIATED RISK FACTORS, AND PROGNOSTIC FACTORS FOR LEFT ATRIOVENTRICULAR VALVE REPLACEMENT FOLLOWING PRIMARY REPAIR

by

Sonali Subhashchandra Patel

An Abstract

Of a thesis submitted in partial fulfillment
of the requirements for the Doctor of
Philosophy degree in Epidemiology
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Thesis Supervisor: Professor Trudy L. Burns

ABSTRACT

Congenital heart defects (CHDs) constitute a major proportion of clinically significant birth defects and are an important component of pediatric cardiovascular disease. Atrioventricular septal defects (AVSDs) include a range of anomalies characterized by atrial, ventricular, and atrioventricular (AV) valve defects. AVSDs commonly occur in the presence of a syndrome, most frequently Down syndrome; they also occur in isolation and are referred to as non-syndromic AVSDs (NSAVSDs). These studies were performed to evaluate for presence of an intermediate phenotype in parents and siblings of a child with a NSAVSD, risk factors associated with NSAVSDs, and prognostic risk factors for left AV valve replacement following primary repair of an AVSD.

It was shown that the mean body surface area-standardized AV septal length (AVSL) was significantly shorter in the NSAVSD parents and siblings than in parents and siblings of syndromic AVSD case and control children. Using age- and gender-adjusted body surface area-standardized AVSL, it was determined that there was evidence for two component distributions in parents and siblings of NSAVSD children, suggesting the presence of an intermediate. Broadening the definition of AVSD to include those with a shortened AVSL may increase the power of genetic association and mapping studies to identify susceptibility genes.

Risk factors associated with NSAVSD were examined using the 1997-2005 National Birth Defects Prevention Study database. Mothers who actively smoked or were exposed to passive smoke anytime from one month prior to pregnancy through the end of the first trimester were more likely to have an infant with a NSAVSD. There was a suggestive association between AVSDs and use of antibacterial, antifungal, and antiviral medications. Additional investigations are warranted to investigate associations with specific medications as well as to

uncover possible gene-environment interaction effects that may modify these risks in order to develop improved primary prevention strategies.

Using the Pediatric Cardiac Care Consortium database, factors associated with time to first reoperation and time to replacement following primary AVSD repair were evaluated. Type of AVSD repair, closure of the mitral valve cleft, moderate to severe postoperative left AV valve regurgitation, and presence of postoperative complete heart block were associated with earlier time to reoperation after adjusting for age and weight at AVSD repair. Down syndrome and presence of postoperative mitral stenosis were associated with earlier time to replacement. Prognostic risk factors following left AV valve replacement in children who had previously undergone AVSD repair were also identified. A prosthetic valve size to body weight ratio of greater than 3 and the presence of Down syndrome were identified as predictors of in-hospital death following left AV valve replacement.

By adding to our knowledge of the AVSD familial and environmental risk factors from these studies, we will be able to (1) improve genetic counseling, (2) identify other family members for genetic testing, (3) begin to devise primary prevention strategies, and (4) improve treatment modalities. By recognizing prognostic factors which influence survival, optimal patient care can be devised which will not only improve treatment modalities, but also long-term survival.

Abstract Approved: _____
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PH.D. THESIS

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To Bill, my constant source of support and encouragement

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A	Atrium
AIC	Akaike’s information criterion
Ao	Aorta
aOR	Adjusted odds ratio
asAVSL	Age- and gender-adjusted, BSA-standardized AVSL
AS	Aortic sac
AoVS	Aortic valve stenosis
ASD	Atrial septal defect
AV	Atrioventricular
AVV	Atrioventricular valve segment
AVS	Atrioventricular septum
AVSD	Atrioventricular septal defect
AVSL	Atrioventricular septal length
BAV	Bicuspid aortic valve
BMP	Bone morphogenic protein
BSA	Body surface area
CC time	Cross-clamp time
CHD	Congenital heart disease
CI	Confidence interval
CNV	Copy number variant
CO	Carbon monoxide
CT	Conotruncal segment
DA	Ductus arteriosus
DNA	Deoxyribonucleic acid

DORV	Double outlet right ventricle
EDD	Estimated date of delivery
EMT	Epithelial mesenchymal transformation
FDA	Food and Drug Administration
FISH	Fluorescent in situ hybridization
GWA	Genome-side association
HIV	Human immunodeficiency virus
HLHS	Hypoplastic left heart syndrome
IAS	Interatrial septum
IVS	Interventricular septum
LA	Left atrium
LAVVR	Left atrioventricular valve regurgitation
LCC	Left common carotid
LSCA	Left subclavian artery
LV	Left ventricle
LVOTO	Left ventricular outflow tract obstruction
NA	Not applicable/available
NBDPS	National Birth Defects Prevention Study
NS	Not significant
NSAID	Non-steroidal anti-inflammatory drug
NSAVSD	Non-syndromic atrioventricular septal defect
PA	Pulmonary artery
PCCC	Pediatric Cardiac Care Consortium
PDA	Patent ductus arteriosus
PID	Pelvic inflammatory disease
Postop	Postoperative
PPS	Peripheral pulmonic stenosis

Preop	Preoperative
OR	Odds ratio
RA	Right atrium
RCC	Right common carotid
RMSE	Root mean squared error
ROC	Receiver operating characteristic
RR	Relative risk
RSCA	Right subclavian artery
RV	Right ventricle
RVOTO	Right ventricular outflow tract obstruction
SAVSD	Syndromic atrioventricular septal defect
sAVSL	BSA-standardized atrioventricular septal length
SD	Standard deviation
SE	Standard error
SLE	Systemic lupus erythematosus
SNP	Single nucleotide polymorphism
STRP	Short tandem repeat polymorphism
TAAD	Thoracic aortic aneurysm dissection
TAPVR	Total anomalous pulmonary venous return
TBX	T-box
TGA	Transposition of the great arteries
TOF	Tetralogy of Fallot
US	United States
UTI	Urinary tract infection
V	Ventricle
VSD	Ventricular septal defect
%ile	Percentile

CHAPTER 1
CARDIAC DEVELOPMENT AND TREATMENT OF CONGENITAL HEART DEFECTS

Introduction

This dissertation research focuses on congenital heart defects, specifically, atrioventricular septal defects. Three different databases are used to conduct the research – The Family Study of Endocardial Cushion Defects, The National Birth Defects Prevention Study, and The Pediatric Cardiac Care Consortium databases. The databases and the associated specific aims and hypotheses are presented first as an orientation to the research focus. The remainder of Chapter 1 provides background relevant to the three main research questions. To develop an understanding of congenital heart defects, the development of the cardiovascular system is described. With a better understanding of where in the developmental process various congenital heart defects arise, treatment options are discussed. Chapter 2 discusses genetic and non-genetic etiologies of congenital heart defects and atrioventricular septal defects.

Databases

Family Study of Endocardial Cushion Defects

The Family Study of Endocardial Cushion Defects, conducted between 1994 and 2004, evaluated children with a non-syndromic or syndromic atrioventricular septal defect (AVSD) and their parents (triads/trios). The patients were identified through cardiac catheterization, echocardiographic, and surgical records at the University of Iowa Hospitals and Clinics and recruited for the study. If the family agreed to participate, a three-generation pedigree was constructed and a health history questionnaire was administered over the phone.

The families, including siblings of the proband, were then scheduled for echocardiographic examinations and blood samples. One hundred fifty-five syndromic and non-syndromic AVSD trios were recruited and examined.

Children free of congenital heart defects and their parents and siblings from Muscatine, Iowa were recruited to serve as control families. Echocardiograms were obtained in a similar fashion from the families who agreed to participate. Echocardiograms were also obtained from available extended family members. Seventy-four control families were recruited and examined. The echocardiograms and the DNA samples that were obtained have been utilized for other investigations of congenital heart defects.

National Birth Defects Prevention Study

The National Birth Defects Prevention Study (NBDPS) was designed to identify infants with and without major birth defects and evaluate genetic and environmental factors associated with the occurrence of birth defects[1]. This ongoing case-control study includes case and control infants from birth defect surveillance registries in ten states (Arkansas, California, Georgia [Centers for Disease Control and Prevention], Iowa, Massachusetts, New Jersey, New York, North Carolina, Texas, and Utah). Cases have one or more of over 30 eligible birth defects. Information for potential cases is reviewed by clinical geneticists at each site to determine study eligibility. Infants with recognized or strongly suspected chromosomal abnormalities or single-gene conditions are excluded from the study. After inclusion in the study, all cases with one specific defect are then classified by clinical geneticists to establish consistency for the defect and to determine whether the defect pattern is isolated or multiple (>1 major malformation). Cases include all live born (all sites), still born (all sites except New Jersey), or induced abortions (all sites except Massachusetts and New

Jersey). Case infants include infants with isolated and multiple defects; infants with multiple eligible defects are included in each defect category.

Infants used as controls (100 per birth year per site) are randomly selected from birth certificates or birth hospital records. Controls are unmatched to cases; they are selected from the same base population as cases, with no major birth defects and an estimated date of delivery within the same year as cases. It has recently been shown that participating controls are similar to those not participating[1]. Mothers of case and control infants are interviewed. Parents are asked to collect buccal cell samples from themselves and their infants for deoxyribonucleic acid (DNA) testing. Information obtained from the interviews and the DNA specimens are being used to study genetic and environmental factors and gene-environment interaction effects for a broad range of birth defects. As of December 2005, 18,961 cases and 6,786 controls are included in the database.

Pediatric Cardiac Care Consortium

The Pediatric Cardiac Care Consortium (PCCC) consists of approximately 47 university-based hospitals in 20 states in the United States and two international centers. The PCCC Data Center, established in 1982, is a collaborative, voluntary effort of pediatric cardiologists and cardiothoracic surgeons from a variety of medical centers to gather and analyze data regarding operative results from procedures to diagnose or repair congenital heart defects. The major advantage of the PCCC is a collective pooling of data across cardiac centers that allows for statistical analysis and comparison not routinely possible at a single center because of inadequate sample size. The PCCC collects information on each child who undergoes a cardiac catheterization, an electrophysiologic study, and/or a cardiac operation, or dies with a cardiac

malformation in a prospective fashion. The data are summarized annually and individual reports are created for each center. Representatives from the centers meet annually, and data on the major operative procedures, including risk factors, patient profiles, and variations in adjusted mortality, are presented. Follow-up data are available if a patient had a subsequent procedure performed at a consortium institution. Follow-up data can also be ascertained by contacting member institutions for any given study.

Overview of Proposed Investigations

Paper 1 (presented in Chapter 3)

Aim

To define an intermediate phenotype for non-syndromic AVSDs using echocardiographic data obtained from child-parent trios.

Hypothesis

A subset of the "unaffected" parents and siblings of non-syndromic AVSD case children will have a shorter atrioventricular septal length (AVSL), after normalization to body surface area, than the parents and siblings of syndromic AVSD case children whose AVSL, in turn, will not be different from the parents and siblings of the control children.

Paper 2 (presented in Chapter 4)

Aim

To investigate the etiology of non-syndromic AVSDs by examining parental and environmental factors using affected and control children recruited for the NBDPS.

Hypothesis

Parental and environmental risk factors are associated with non-syndromic AVSDs.

Paper 3 (presented in Chapter 5)

Aim

To describe demographic, anatomic, operative, and/or outcome characteristics following left AV valve reoperation and to identify factors which optimize patient outcomes following left AV valve replacement in children with previously-repaired AVSDs.

Hypothesis

Prognostic factors, such as patient age at first repair, weight at first repair, AVSD morphology, length of time between surgeries, and/or presence of Down syndrome, are associated with a poor outcome of left AV valve replacement in patients with previously-repaired AVSDs.

Significance of Proposed Investigations

Description of an intermediate AVSD phenotype can result in improved ability for genetic counseling of at-risk families. Detailed definition of the AV septal length may also provide insight into defects of the formation of the AV septum, namely, perimembranous ventricular septal defects. Those genes known to be involved in the development AVSDs may also be involved in the formation of the septum, leading to identification of potential genetic etiologies for ventricular septal defects.

An improved understanding of the causes of AVSDs will permit insight into the pathobiological basis of the congenital heart disease problem and allow definition of disease risk, two critical elements for disease prevention. Knowledge of the underlying etiology for AVSDs will improve our ability to predict

disease course, counsel patients and families, and care for affected individuals. In other words, 1) there may be prognostic information for clinical outcomes; 2) there may be improved ability for genetic counseling; 3) there may be other family members for whom genetic testing is appropriate; and 4) there may be other important organ system involvement.

An improved understanding of the frequency of left AV valve replacement and its outcome will provide much needed insight into the prognosis of patients with AVSDs, will enable the best treatment options to be employed, and will allow better counseling of families. Increased awareness of the factors that influence the outcome following replacement reoperation in these patients will again allow for improved definition of disease risk. By recognizing those factors which promote improved outcomes, optimal patient care can be devised which will improve long-term results and may eventually circumvent the need for valve replacement, sparing patients from future reoperations.

Background of Congenital Heart Defects

Birth defects, which are defined as abnormalities of structure, function, or body metabolism, affect 33 in 1000 babies in the United States[2-4]. Congenital heart defects (CHDs) constitute a major proportion of clinically significant birth defects and are an important component of pediatric cardiovascular disease, with an estimated prevalence of six to nine per 1000 live births[5-7]. Neural tube defects and orofacial clefts, other common birth defects, each affect approximately one per 1000 live births[3, 8, 9]. CHDs are the leading cause of death from birth defects during the first year of life[10]. Over 91,000 life-years are lost each year in the United States (US) due to congenital heart defects[11].

Epidemiologic and pathological investigations have determined that the etiology of CHDs is heterogenous and includes chromosomal anomalies, single-

gene disorders, and genetic susceptibility to environmental exposures[12]. The cause of CHDs can be identified in approximately 10% of all cases. However, for a majority of cases the etiology is unknown.

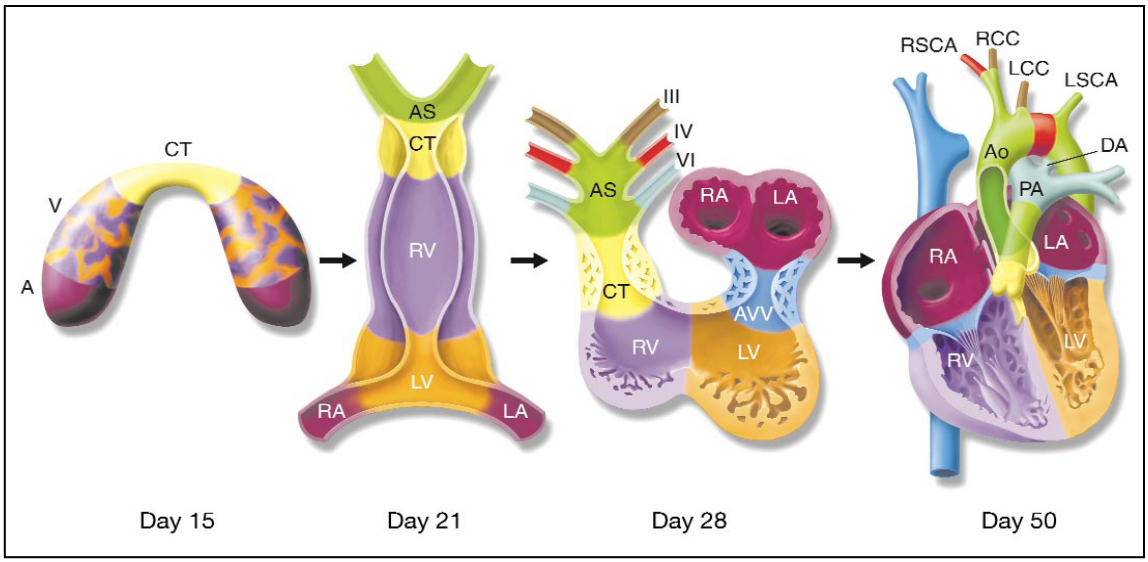
In 2000, the prevalence of CHDs in the US pediatric population was estimated at 623,000 and the number of adults living with CHDs was estimated at 787,000[13]. These estimates are likely to be low, due to loss to follow-up. The prevalence estimates represent the first time that the number of adults with CHDs has surpassed the number of children with CHDs. Affected individuals are now surviving well into their reproductive years. Thus, it is imperative that there is a better understanding of CHDs and their inheritance so that improved lifetime care can be provided to a growing patient population. In order to provide accurate genetic counseling for these families, it is of great importance to identify/define the genetic and environmental contributions to the etiology of CHDs.

Morphogenesis of the Cardiovascular System

The heart is the first organ to form in the developing embryo. Figure 1-1 depicts various stages during cardiac morphogenesis. A timeline of important stages occurring during the formation of the heart is depicted in Figure 1-2.

The earliest recognizable cardiac structure is evidenced at day 15 of gestation when the cardiac progenitor cells have been committed to a cardiogenic fate in response to an inducing signal and are organized into a crescent shape[14]. At 3 weeks of gestation, the bilaterally symmetric heart primordial cells migrate to the midline and fuse to form a single linear heart tube which is comprised of an inner endothelial lining and an outer myocardial cell layer separated by cardiac jelly, an extracellular matrix secreted by the myocardial precursor cells.

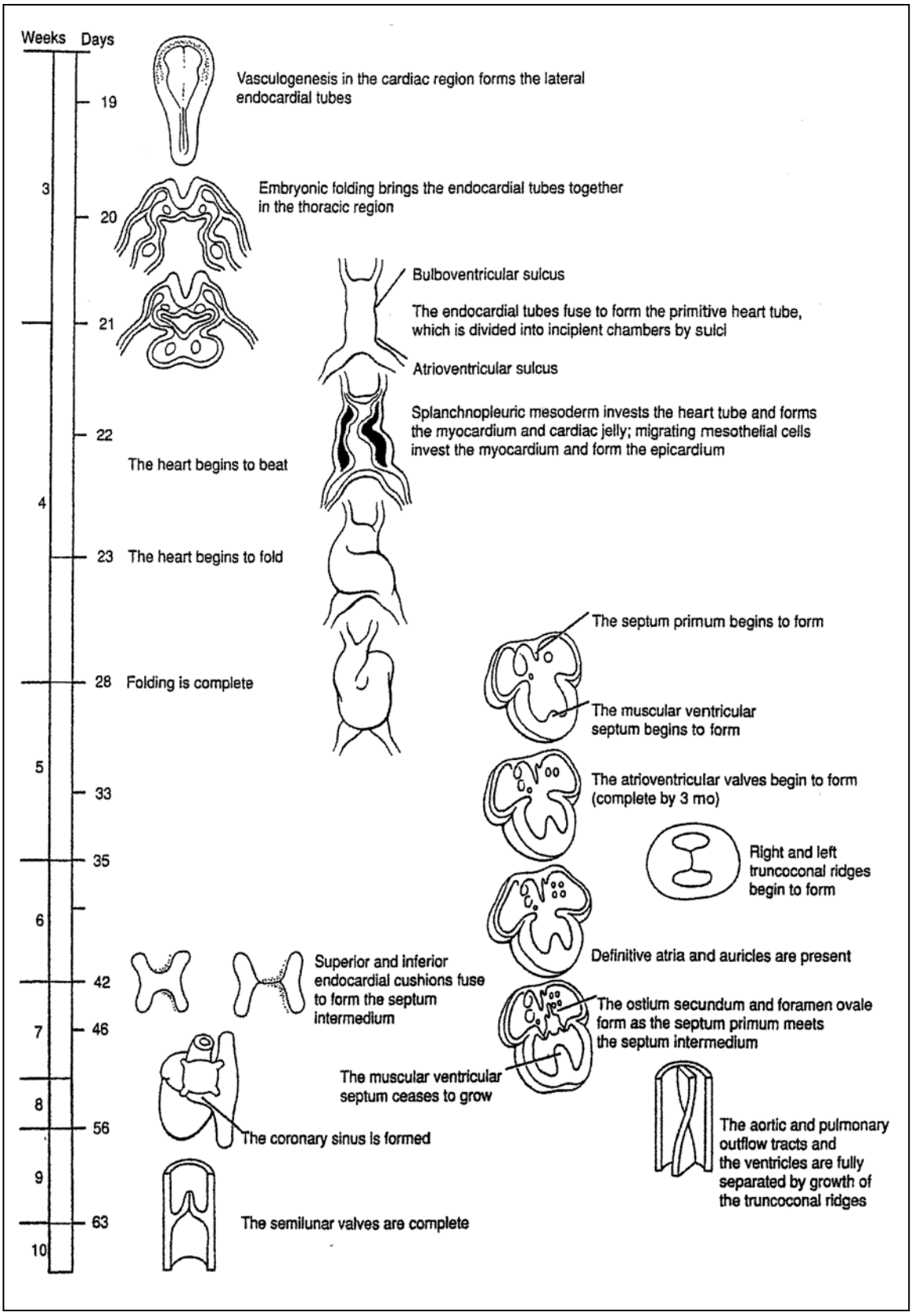
Figure 1-1: Schematic of Cardiac Morphogenesis[15]



Note: A=atrium, Ao=aorta, AVV=atrioventricular valve segment, AS=aortic sac, CT=conotruncal segment, DA=ductus arteriosus, LA=left atrium, LCC=left common carotid, LSCA=left subclavian artery, LV=left ventricle, PA=pulmonary artery, RA=right atrium, RSCA=right subclavian artery, RCC=right common carotid, RV=right ventricle, V=ventricle.

The tube structure initiates rhythmic contractions at approximately day 23[16]. The heart then undergoes rightward looping positioning the atria (inflow chambers) above the ventricles (outflow chambers) in response to the contractions. Rightward looping is essential for proper orientation of the ventricles and for alignment of the heart chambers with the vasculature[15]. Following cardiac looping, which is complete by day 28, the atrial and ventricular chambers of the heart become morphologically identifiable.

Figure 1-2: Timeline of Cardiac Morphogenesis[17]



During this time, blood begins to circulate through the embryo and is unidirectional by day 24[17]. Blood flow is considered in series at this stage, indicating that the blood flows in sequence from the first pump (pulmonary circulation) into the second pump (systemic circulation) and back around to the first.

Atrial septation is the first step in the separation of the systemic and pulmonary circulations. The septum primum is a wedge of tissue that grows caudally from the roof of the atrium towards the atrioventricular canal. Prior to the closure of the interatrial opening (ostium primum), programmed cell death, or apoptosis, occurs near the superior edge of the septum primum creating a new opening (ostium secundum), maintaining an interatrial communication[17]. To the right of the septum primum, a second septal structure (septum secundum) forms. The septum secundum does not close completely, leaving an interatrial channel (foramen ovale), which is the interatrial communication that is present throughout fetal life. Concurrently, the muscular interventricular septum is formed from the bulboventricular sulcus.

At about 5 weeks, the endocardial cushions of the atrioventricular canal appear. The endocardial cushions are derived from the cardiac jelly. Two major processes occur during the development of the atrioventricular canal[16]. The first is growth and fusion of the superior and inferior endocardial cushions which results in septation of the atrioventricular canal. Secondly, there is closure of the ostium primum and the ventricular septum by the superior and inferior endocardial cushions. The atrioventricular canal subsequently shifts to the right to align the atria with their respective ventricle. In addition to septation of the atrioventricular canal, the endocardial cushions also contribute to the formation of the tricuspid and mitral valves.

The outflow tract, also referred to as the conotruncus, undergoes

septation separating the aorta from the pulmonary trunk. Swellings from the right and left walls of the bulbus cordis fuse to form the conal septum[17]. As these swellings grow in a spiral fashion, the aorta and pulmonary trunk twist around each other. Part of the conotruncal swellings fuse with the inferior endocardial cushion and the muscular interventricular septum to form the membranous interventricular septum, separating the right and left ventricles. Extensive valvar remodeling and ventricular growth then takes places to ultimately form the developed heart[14].

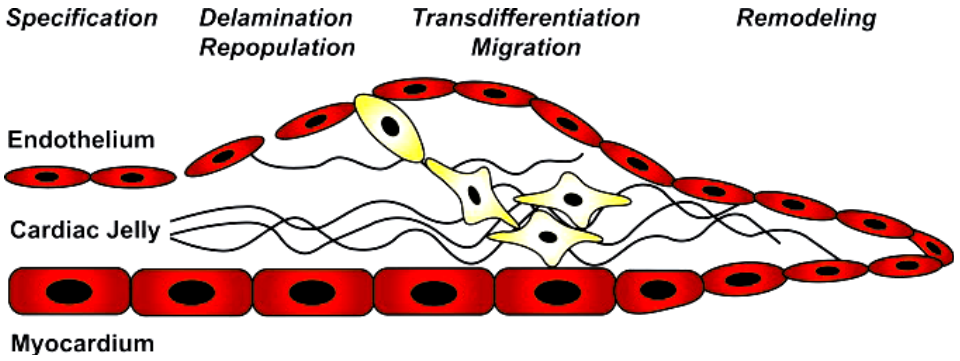
As has been described above, there are several developmental processes involved in the formation of the heart. The major categories of developmental processes involved in cardiac morphogenesis along with associated heart defects are summarized below:

1. *Cellular migration*: Early in development, neural crest cell migration contributes cells that participate in conotruncal septation. Disturbance of this process leads to conotruncal malformations, such as supracristal ventricular septal defects, tetralogy of Fallot, and transposition of the great arteries.
2. *Cardiac hemodynamics*: As blood flows through the developing heart, the differential pressure on the various areas of the chamber walls allows for changes in the chamber shape. Abnormal cardiac hemodynamics can lead to aberrant distention of the cardiac chambers and valves, which can alter their shape and function. Malformations linked to abnormal cardiac hemodynamics include hypoplastic left heart syndrome, coarctation of the aorta, and perimembranous ventricular septal defects.
3. *Cell death*: Apoptosis molds the developing heart by removing tissue, an important function in the formation of cardiac valves, the trabeculated ventricular wall, and the timely development of shunts between the developing right and left heart. Excessive cell death is associated with septal defects,

while insufficient cell death is associated with Ebstein's anomaly, a condition in which the tricuspid valve fails to separate from the ventricular wall.

4. *Extracellular matrix function*: Cardiac jelly forms the endocardial cushions at the atrioventricular orifice and in the outflow tract. The endocardial cushions act as anchors for the valves. Cardiac jelly also fills the space between the inner wall of the heart and the outer surface. Here, the cardiac jelly serves as the medium through which a subpopulation of endothelial cells lining the atrioventricular lumen detaches and migrates into the jelly, where they undergo transformation, forming myocardial cells that proliferate and give rise to the cardiac cells which contribute to cardiac valve formation. This process is known as epithelial-mesenchymal transformation (EMT). Figure 1-3 illustrates the EMT process. Atrioventricular septal defects can occur if the extracellular matrix does not form fully functional cardiac cushions.

Figure 1-3: Anatomy of Heart Valve Formation via Epithelial-Mesenchymal Transformation[18]



Note: A subset of endothelial cells overlying the future valve site is depicted undergoing delamination, differentiation, and migration into the cardiac jelly.

5. *Targeted growth*: Targeted growth processes are necessary for the proper formation of certain heart structures. For example, the direction of pulmonary

vein growth is determined by a particular growth signal from the left atrium. Abnormal targeted growth processes during development can lead to disorders, such as abnormal pulmonary venous return and cor triatriatum which stems from the faulty incorporation of the common pulmonary vein into the left atrium.

6. *Establishment of visceral situs and cardiac looping*: Visceral situs (establishment of right and left sides of the body) and looping defects result in ventricular inversion and reversed right or left position of organs.

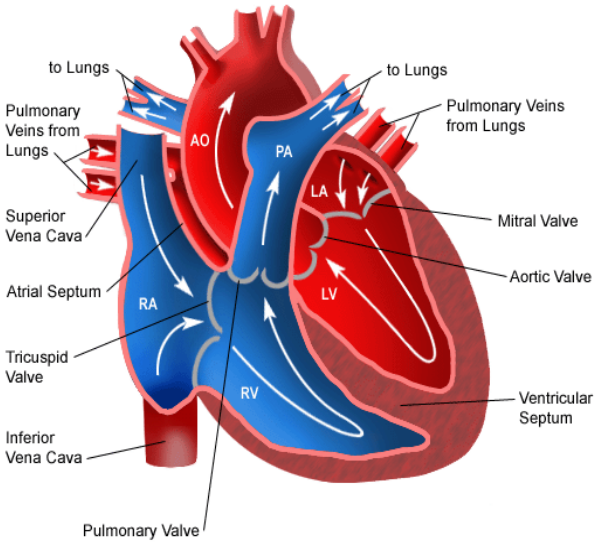
Atrioventricular Septal Defects

AVSDs, also known as atrioventricular canal defects or endocardial cushion defects, include a range of anomalies characterized by involvement of the atrial septum, the ventricular septum, and one or both of the atrioventricular valves. Figure 1-4 demonstrates defect components and mixing of blood. AVSDs account for approximately 7% of all congenital heart defects[19].

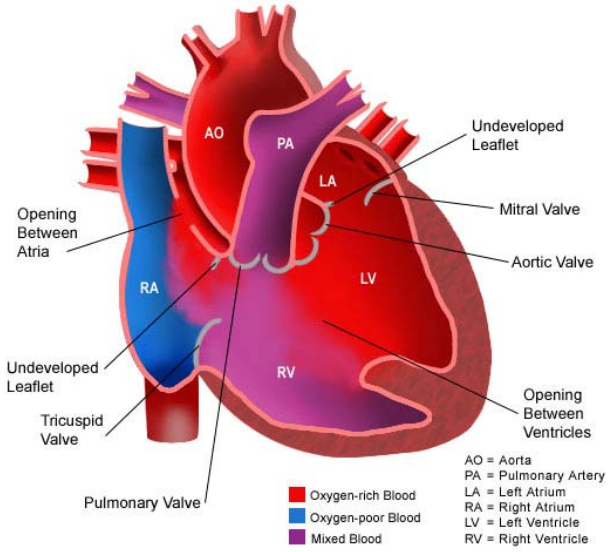
The AV septum and valves are formed from progenitor cardiac structures, endocardial cushions[20]. During morphogenesis, the endocardial cushions expand as they are infiltrated by extracellular matrix secreted from the surrounding myocardium. The cushions then fuse and undergo remodeling to form the AV valves and septa[21] . Formation of the AV valves occurs between estimated gestational ages of 52 and 56 days[22].

AVSDs arise from the abnormal development of the endocardial cushions where the superior and inferior cushions do not fuse completely. The degree of severity of the defect is dependent on the stage at which the developmental failure occurred[20]. As a consequence of non-fused cushions, the central portion of the heart or crux fails to form.

Figure 1-4: Normal Heart and Complete Atrioventricular Septal Defect Anatomy[23]



a. Normal Heart



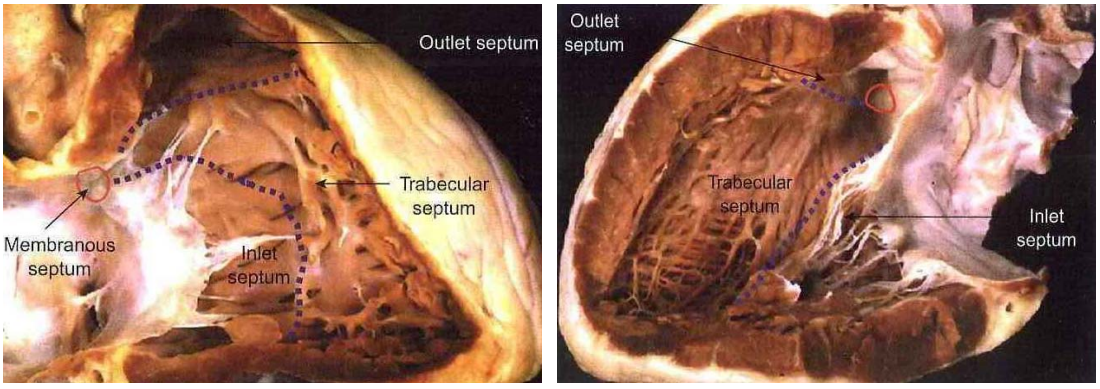
b. Complete Atrioventricular Septal Defect

There are two main types of AVSDs. The phenotype of the complete form consists of deficiency of the lower part of the atrial septum (atrial septal defect), the upper or inlet portion of the ventricular septum (ventricular septal defect), and a single common AV valve resulting in the free flow of blood or communication between the four cardiac chambers, thereby allowing the mixture of oxygen-rich

and oxygen-depleted blood. The partial form consists of a deficiency of the lower atrial septum (ostium primum), an inlet ventricular septal defect (VSD), but two separate AV valves. The left-sided valve usually is deficient in the central portion of the anterior leaflet which is referred to as a cleft[19]. Other forms of defects along the spectrum of AVSDs include cleft features of the mitral valve.

The ventricular septum can be divided into four zones – membranous, inlet, trabecular, and outlet septa (Figure 1-5). The membranous septum is the portion of the ventricular septum that is immediately beneath the aortic valve, while the remainder of the septum is referred to as the muscular septum. The membranous septum is comprised of two components – an atrioventricular portion and an interventricular portion.

Figure 1-5: Ventricular Septum Anatomy[24]

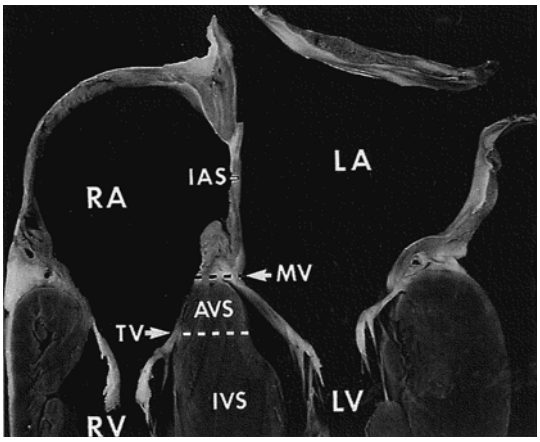


a. Right ventricular aspect **b. Left ventricular aspect**

The septal leaflet of the tricuspid valve normally inserts into the septum slightly closer to the apex than the septal leaflet of the mitral valve. There is a small portion of septal tissue superior to the tricuspid septal leaflet insertion that separates the right atrium from the left ventricle which is the atrioventricular septum (Figure 1-6). Absence of this AV septum contributes to the phenotype of

AVSDs. There are very few reports of the length of the AV septum in normal hearts. The majority of the literature referring to the length of the AV septum is in reference to Ebstein's anomaly, in which an abnormally formed tricuspid valve is positioned lower in the right ventricle. Body size and cardiac dimensions change dramatically during normal growth and development. Due to the rapid growth throughout childhood, interpretation of measurements of cardiovascular structures by echocardiography requires correction for body size, through the process of normalization[25, 26]. The distance between the mitral and tricuspid insertions, or the length of the AV septum, in normal hearts is less than 8 mm/m^2, normalized to body surface area[27, 28].

Figure 1-6: Anatomic Cross-Section Detailing the Atrioventricular Septum[27]



Note: RA=right atrium, LA=left atrium, RV=right ventricle, LV=left ventricle, IAS=interatrial septum, AVS=atrioventricular septum, IVS=interventricular septum.

A syndromic CHD is one that occurs in the presence of other clinically recognizable characteristics, which leads to the diagnosis of the condition. Most commonly, the condition is a chromosomal aberration. CHDs can also occur without other characteristics, which are referred to as a non-syndromic CHD.

Although cardiac malformations may occur in the setting of multiple birth defects as part of a syndrome, most are found as isolated non-syndromic defects. The Baltimore-Washington Infant Study, conducted between 1981 and 1989, analyzed a large cohort of children with CHDs and determined that approximately 30% of the cohort had a CHD in the presence of other anomalies while the other 70% had an isolated non-syndromic heart defect[29]. Familial cases have been described for nearly every type of cardiac malformation, suggesting primary genetic etiologies for a subset of non-syndromic CHDs. The majority of individuals with CHDs have no family history and are "sporadic" cases, but even for these sporadic cases of CHDs, epidemiologic studies have demonstrated an increased recurrence risk for cardiac malformations in subsequent pregnancies, supporting the existence of genetic predisposition[30].

Treatment of Congenital Heart Defects

The treatment of CHDs varies greatly depending on the specific defect and its severity. Broadly, treatment can involve observation, medical management, and interventional or surgical repair/palliation.

Treatment of Atrioventricular Septal Defects

AVSDs are typically surgically repaired between four and six months of age. The timing of the repair is optimally carried out prior to the development of pulmonary vascular disease. The results of surgical repair for complete AVSDs have improved markedly since the first repair which was performed in 1955[31-34]. Three main surgical techniques have been employed in the repair of AVSDs – a single patch method, a two-patch method, and a modified one-patch method. Prior to 1990, the single patch method and the two-patch method were standards of care. Since its description in 1997, the modified one-patch method has been

demonstrated to be superior to the other methods in terms of patient mortality and morbidity[35]. Early mortality following primary repair has decreased to less than 5% since the implementation of newer techniques[35].

Although the overall results of repair have improved, there continues to be development of hemodynamically significant left atrioventricular valve regurgitation following repair. As described above, AVSDs are characterized by a deficiency of the septum as well as an abnormal structure of the AV valve. This suggests that there is an inherent problem of the structural support that maintains the competency of the surgically repaired valve[34]. Left AV valve regurgitation following AVSD repair has been reported to occur at a rate of 6-14%[36-39]. Chronic left AV valve insufficiency worsens with time and results in considerable morbidity. The severity of left AV valve or mitral regurgitation has been demonstrated to increase by at least one grade over a three-year period in over 40% of patients following AVSD repair[40]. AV valve regurgitation leads to volume-overload. With increasing regurgitation, eccentric hypertrophy occurs, ultimately leading to left ventricular myocardial failure[41]. Although this is initially tolerated in the pediatric population, if persistent, ventricular dysfunction will occur[42].

Some risk factors for the development of left AV regurgitation following primary AVSD repair have been identified. Preoperative left AV valve regurgitation, dysplastic AV valves, and the absence of Down syndrome are significant risk factors for the development of severe postoperative AV valve regurgitation[33, 35, 43]. Preoperative left AV valve regurgitation can result in anatomic changes to the mitral valve annulus, which can affect surgical outcome of a primary AVSD repair. The regurgitant jet can exacerbate annular dilatation leading to degenerative changes and displacement of the mitral valve[33]. Severe left AV valve regurgitation can also lead to diminished contractility and eventually

cardiac decompensation[35]. Dysplastic AV valves lead to difficulty following repair as the dysplastic leaflets used to create two functional AV valves remain dysfunctional. Anatomical differences between Down syndrome and non-Down syndrome patients with AVSDs have been noted. These were described earlier. In addition, it has been noted that dysplastic valves are more commonly present in patients without chromosomal abnormalities[44].

Left AV valve regurgitation is initially medically managed, but when persistent, surgical management must be explored. Hemodynamically significant left AV valve regurgitation with the need for reoperation occurs in 4-15% of patients following primary repair of AVSDs[39, 45-47]. Left AV valve surgery can significantly improve clinical status, with a sustained improvement in ventricular chamber size but comes with inherent morbidity and mortality[35]. Surgical options for left AV valve regurgitation include repair via valvuloplasty, annuloplasty, or a combination versus left AV valve replacement. Investigations into repair versus replacement have been performed. These studies indicate that attempts at re-repair of the AV valve are usually successful[34, 35]. No significant difference in survival was found between patients undergoing valve repair or replacement[34]. The incidence of left AV valve reoperation reportedly has been reduced following introduction of the modified one-patch technique[48]. In a study examining three published reports of the one-patch repair, six published reports of the two-patch repair, and four published reports of the modified one-patch repair, it was shown that the frequency of left AV valve reoperation was 9.7%, 7.2%, and 2.0% respectively[48].

The management of left AV valve regurgitation is especially problematic as the effect of mechanical valve placement and its complications must be weighed judiciously. In patients with AVSDs, there is an unpredictable location of the atrioventricular node which renders the node vulnerable to injury during

reoperations on the left atrioventricular valve[34]. A serious complication related to the location of the AV node is the development of complete heart block requiring pacemaker implantation. The reported incidence of complete heart block after left AV valve replacement varies between 20 and 30%[49]. Other complications of valve replacement include thromboembolism, prosthetic valve infection, bleeding, paravalvular leak, need for multiple mechanical valve replacements due to growth with increasing age, and subsequent reoperation[35, 38]. Mortality rates following left AV valve replacement have been shown to range from 26 to 52%[37, 45]. A multi-institutional study examined multiple risk factors predictive of death following left AV valve replacement in children with congenital heart disease. The investigators identified the presence of complete AVSD morphology, the presence of Shone's syndrome, and an increased ratio of prosthetic valve size to body weight as significant prognostic factors associated with death[50]. A significant study limitation was that predicted left AV valve annulus dimensions based on weight were used for comparison as actual valve dimensions were not available[50]. It has been shown that survivors of left AV valve replacement had placement of a prosthetic valve within one Z-score of the echocardiographically measured left AV valve, agreeing with the prior findings of the multi-institutional study[51]. A follow-up study also showed that larger prosthetic valve size was a significant protective factor against a second left AV valve replacement[52].

Numerous accounts of the frequency of left AV valve replacement have been published. Table 1-1 lists recent descriptions of the frequencies of left AV valve regurgitation and subsequent replacement from a cohort of previously repaired AVSDs. Since 1990, a relatively stable frequency of AV valve replacement is reported, ranging between 0.5 and 2.0%. Prior to 1990, a small case series demonstrated an AV valve replacement frequency close to 15% in previously repaired AVSDs[53]. The increased frequency during these years is

likely related to technique of repair.

Table 1-1: Published Reports of Left Atrioventricular Valve Reoperations in Patients with Previously Repaired Atrioventricular Septal Defects

Reference	*N*	*LAVVR (%)*	*Reoperations (%)*	*Replaced (%)*
Bando et al.[54]	203	NA	8 (3.9)	2 (0.99)
Michielon et al.[43]	205	42 (20.5)	9 (4.4)	1 (0.48)
Alexi-Meskishvili et al.[46]	120	NA	7 (5.8)	1 (0.8)
Tweddell et al.[32]	115	NA	6 (5.2)	1 (0.9)
Gunther et al.[55]	320	NA	35 (10.9)	4 (1.3)
Suzuki et al.[33]	90	28 (3.1)	7 (7.8)	1 (1.1)
Ten Harkel et al.[35]	157	30 (19.1)	15 (9.6)	1 (0.64)
Malhotra et al.[34]	378	NA	23 (6.1)	8 (2.1)

Note: N=number of patients with previously repaired AVSDs, LAVVR=left atrioventricular valve regurgitation, NA=not available.

Data are expressed as frequency counts and percentages of total population.

Chapter 1 Summary

In conclusion, CHDs comprise a large proportion of clinically significant birth defects. When any birth defect is diagnosed, two of the first questions raised by the family are “What did we do wrong?” and “Will my child lead a normal life?” These, however, are difficult questions to answer especially in regards to CHDs.

Much about CHDs remains unknown. Research into cardiovascular embryology and development has identified multiple areas during cardiovascular morphogenesis as potential areas for the development of CHDs. It is hoped that further understanding of cardiac morphogenesis may led to potential preventive strategies and therapies.

Until research can provide methodology for the prevention of CHDs,

current treatment methods must be employed. The treatment of CHDs varies greatly depending on the specific defect and clinical condition of the infant. Treatment for AVSDs typically consists of surgical repair between four and six months of age. The results of surgical repair have been improving since the initial repair was performed in 1955. The major morbidity following surgical repair is the development of left AV valve regurgitation. The regurgitation can become severe enough to require additional repair of the valve or replacement of the valve. While investigations have identified risk factors for the development of left AV valve regurgitation, to date, no studies have identified early outcomes and prognostic factors following replacement of the left AV valve in patients with previously-repaired AVSDs.

The proposed investigations will enhance the current knowledge of the AVSD phenotype, risk factors, and outcomes and prognostic factors following valve replacement. Armed with this additional knowledge, clinicians may be better equipped to answer the difficult questions facing families of infants with AVSDs.

CHAPTER 2
GENETIC ETIOLOGIES OF AND NON-GENETIC RISK FACTORS FOR CONGENITAL HEART DEFECTS AND ATRIOVENTRICULAR SEPTAL DEFECTS

Introduction

This second introductory chapter provides background related to the genetic and non-genetic etiologies of congenital heart defects and in particular, atrioventricular septal defects. In addition to the genetic risk factors that are described, the concept of familial aggregation of cardiac structure measurements is discussed.

Genetic Etiologies of Congenital Heart Defects

The molecular basis of CHDs has been widely examined[13, 14, 56-62]. Cardiac genetic disorders can be classified into three categories: disorders which affect cardiogenesis, the conduction system, or cardiac muscle[60]. Numerous genetic and molecular associations with CHDs have been demonstrated. These associations are summarized in Tables 2-1 and 2-2. For ease of understanding, CHDs will be described based on broad defect categories, i.e., defects in cardiac septation, cardiac outflow and aortic arch defects, obstructive defects of the great vessels, and laterality defects.

Defects of cardiac septation are the most common type, accounting for nearly 50% of all CHDs [6]. These defects are categorized based on their location in the heart. Atrial and ventricular septal defects result in communications between the right and left collecting chambers (atria) or the right and left pumping chambers (ventricles). The genetic etiology of AVSDs, another type of septation defect, will be discussed in depth in the next section. If left unrepaired, cardiac septation defects can cause pulmonary overcirculation, leading to pulmonary vascular disease, atrial enlargement predisposing to atrial arrhythmias,

ventricular dilation, and ultimately a decreased life expectancy.

Table 2-1: Genes Associated with Syndromic Congenital Heart Defects

Syndrome	*Gene Association*	*Chromosome Location*
Alagille	*JAG1*	20p12
Char	*TFAP2B*	6p12
DiGeorge	*TBX1*	22q11
Ellis-van Creveld	*EVC* *EVC2*	4p16 4p15
Holt-Oram	*TBX5*	12q24
Leopard	*PTPN11*	12q24
Neurofibromatosis 1	*NF1*	17q11
Noonan	*PTPN11*	12q24
Marfan	*FBN1*	15q21
Williams	*ELN*	7q11

Genetic linkage analysis of large families with autosomal forms of CHDs has led to the identification of three transcription factors that play an important role in cardiac septation defects. *TBX5* encodes a transcription factor and is mutated in individuals with Holt-Oram syndrome, a syndrome characterized by atrial and ventricular septal defects along with upper limb anomalies[63, 64]. Transcription factors of the T-box (TBX) family are required for early cell-fate decisions, such as decisions necessary for differentiation and organogenesis[65]. T-box proteins tend to be expressed in specific organs or cell types, especially during development, and are generally required for the development of those tissues[65]. *TBX5* is highly expressed in the atrial and ventricular septum, and targeted deletion of *Tbx5* in mice results in septal defects in heterozygous embryos[66, 67].

Table 2-2: Genes Associated with Congenital Heart Defects

Defect	*Gene Association*	*Chromosome Location*
ASD	*NKX 2.5*	5q34-35
	GATA4	8p23
	TBX5	12q24
	MYH6	14q12
VSD	*NKX 2.5*	5q34-35
	GATA4	8p23
	TBX5	12q24
PDA	*MYHA11*	16p13
TOF	*NKX2.5*	5q34-35
	ZFPM2/FOG2	8q23
	JAG1	20p12
TGA	*CFC1*	2q21
	PROSIT240	12q24
	ZIC3	Xq26
DORV	*CFC1*	2q21
	NKX2.5	5q34-35
Supravalvular AoVS	*ELN*	7q11
BAV	*NOTCH1*	9q34
	KCNJ2	17q23-24
HLHS	*NKX2.5*	5q34-35
	HAND1	5q33
	HAND2	4q33
Heterotaxy	*LEFTYA*	1q42
	CFC1	2q21
	AVCR2B	3p21-22
	ZIC3	Xq26

Note: ASD=atrial septal defect, VSD=ventricular septal defect, PDA=patent ductus arteriosus, TOF=tetralogy of Fallot, TGA=dextro-transposition of the great arteries, DORV=double outlet right ventricle, AoVS=aortic valve stenosis, BAV=bicuspid aortic valve, HLHS=hypoplastic left heart syndrome.

As previously mentioned cardiac genetic disorders can be classified as defects affecting cardiogenesis, the conduction system, or cardiac muscle. Rarely, a cardiac genetic lesion affects more than one of these categories which is referred to as crossover[60]. Associations between mutations in the transcription factor NKX2.5 and cardiac septation defects were identified by studying several large families with autosomal dominant atrial septal defects and cardiac

conduction abnormalities in the form of complete atrioventricular heart block using a positional cloning approach[68]. Investigators have also identified *NKX2.5* mutations in individuals with other forms of CHDs such as tetralogy of Fallot and tricuspid valve abnormalities, supporting a role for this gene in diverse cardiac morphogenetic processes[69, 70]. *NKX2.5* encodes a transcription factor that is critical for cardiac development in mice, where targeted disruption results in embryonic lethality and cardiac failure at the heart looping stage[71, 72]. Atrial septal abnormalities have been identified in mice heterozygous for *Nkx2.5* consistent with the phenotype seen in humans[73]. Additional studies in genetically manipulated mice have demonstrated the importance of Nkx2.5 in the cardiac conduction system. *Nkx2.5* heterozygous and homozygous-null mice have hypoplastic or absent AV nodes, respectively, and examination of mice with a ventricular-restricted knockout of *Nkx2.5* demonstrated progressive loss of this AV nodal conduction tissue, leading to complete heart block. These studies demonstrated the dual role of *NKX2.5* in disease, both in cardiac formation and in the maintenance of the cardiac conduction system[74].

A known molecular partner of *NKX2.5*, *GATA4*, was identified as a genetic cause of atrial and ventricular septal defects without conduction disturbances by studying large pedigrees with familial CHDs [75-77]. One of the *GATA4* mutations was a missense mutation that disrupted a highly conserved glycine residue adjacent to the second zinc finger of *GATA4*, which is critical for protein-protein interactions[75]. Biochemical analysis of this mutation led to the discovery of a novel biochemical interaction between *Gata4* and *Tbx5* in an animal model[75]. This missense mutation in *Gata4* specifically disrupted the *Gata4-Tbx5* interaction while maintaining its ability to interact with *Nkx2.5*. In previous studies, *Tbx5* had been shown to interact with *Nkx2.5*, demonstrating that all three transcription factors could interact physically *in vitro*[78]. Mutations in *MYH6*,

a downstream transcriptional target of *GATA4* and *TBX5*, have also been implicated as a cause of atrial septal defects[79]. A mutation in any of these three genes, *TBX5, NKX2.5, GATA4,* can result in a defect in cardiac septation, which suggests that these genes may work to direct common molecular pathways that are critical for cardiac septa formation.

Ellis-van Creveld syndrome is an autosomal recessive condition which is characterized by atrial septal defects along with short stature, dysplastic nails and teeth, and skeletal abnormalities. Using several interrelated Amish families as well as other unrelated families, disease genes, *EVC1* and *EVC2*, were identified[80, 81]. The function of EVC remains unknown, however among the mutations discovered, six were truncating mutations, suggesting that the mechanism underlying the disorder is loss of the EVC protein[62].

Defects of the cardiac outflow tract and aortic arch account for 20-30% of all CHDs[6]. Malformations of the cardiac outflow tract are also referred to as conotruncal defects and include tetralogy of Fallot, transposition of the great arteries, truncus arteriosus, and double outlet right ventricle (DORV).

Tetralogy of Fallot (TOF), the most common type of cyanotic CHD, represents a type of septation defect. This defect occurs in the conotruncal septum and is characterized by a ventricular septal defect, overriding aorta, right ventricular hypertrophy, and pulmonary stenosis. As previously mentioned, *NKX2.5* mutations have been identified in patients with TOF[69]. ZFPM2/FOG2 is a zinc finger protein, which is expressed during early heart development, and acts as a coregulator of the transcription factor GATA4[82]. *Zfpm2/Fog2* knockout mice have demonstrated CHDs, including tricuspid atresia and TOF[82]. Point mutations have been identified in *ZFPM2/FOG2* in patients with TOF[83].

Transposition of the great arteries (TGA), the second most common type of cyanotic CHD, is another conotruncal septation defect. As a result of this

defect, the systemic and pulmonary circulations are separated with the deoxygenated blood directed to the aorta and the oxygenated blood directed to the pulmonary artery, this is not compatible with survival. The majority of patients have dextro-looped transposition of the great arteries (TGA), which is characterized by atrioventricular concordance and ventriculoarterial discordance. Nonsense mutations in *ZIC3* have been shown to segregate with TGA[84]. Point mutations have also been identified in *PROSIT240*, part of the thyroid hormone receptor–associated protein (TRAP) complex[85]. Several TRAP components have previously been shown to be important in early embryonic development in various organisms[86, 87]. ZIC3 is a member of the ZIC family of zinc finger proteins which are transcription factors involved in early stages of left-right body axis formation. Mutations in *ZIC3* have also been identified in patients with TGA[88]. The epidermal growth factor (*EGF*) gene family encodes extracellular proteins that participate in early embryogenesis[89]. *CFC*, which encodes for the protein CRYPTIC, is one of the four known family members of *EGF*. The developmental pathway involving *CFC1* has been demonstrated as being critical for conotruncal formation as point mutations have been identified in patients with TGA and double-outlet right ventricle, another conotruncal defect[89]. DORV has also been associated with mutations in *NKX2.5*, again demonstrating the heterogeneous effects of variation in this gene[90].

The 22q11 deletion (22q11del) syndrome, which is also known as velocardial facial syndrome or DiGeorge syndrome, is the most common genetic deletion syndrome and the second most common genetic cause of CHDs after trisomy 21[91]. The deletion spans ~3Mb of chromosome 22 and contains nearly 30 genes. Using knockout mouse models, it has been demonstrated that Tbx1, a T-box transcription factor that is expressed in the pharyngeal arches, is responsible for the predominant features of the 22q11del phenotype[92-94].

Heterozygous mice for a *Tbx1*-null allele had fourth aortic arch artery anomalies, including interrupted aortic arch and an anomalous right subclavian artery partially resembling the phenotype seen in humans of 22q11del, while homozygous mice demonstrated the human 22q11del phenotype with the entire spectrum of defects, including cleft palate, thymic aplasia, ear anomalies and cardiac defects[95-97]. This finding suggests that gene dosage is critical for phenotypic expression[95-97]. It has also been shown that there are *TBX1* mutations in individuals with the 22q11del phenotype who do not have a detectable 22q11 microdeletion by fluorescent in situ hybridization (FISH), implying that haploinsufficiency of *TBX1* results in the majority of the phenotypic features seen with 22q11del[98].

Another aortic arch abnormality is the patent ductus arteriosus. The ductus arteriosus is derived from the sixth aortic arch artery and is necessary for normal fetal life after ventricular and outflow tract septation. Soon after birth, the ductus normally closes. In some instances, however, the ductus remains patent or open. Pedigree analyses of individuals with Char syndrome, which is characterized by patent ductus arteriosus, dysmorphic facies, and digit anomalies, and with thoracic aortic aneurysm and/or aortic dissection (TAAD) syndrome, which is characterized by thoracic aortic aneurysm, aortic dissection, and patent ductus arteriosus, have identified mutations in the transcription factor *TFAP2β* and the myosin heavy chain 11 (*MYH11*) genes[99, 100].

Marfan syndrome is a common autosomal dominant disorder characterized by ectopia lentis, weakness of the aortic wall leading to valvular insufficiency, aneurysm formation, and dissection, and skeletal abnormalities. Mutations in *FBN1* have been identified in patients with Marfan syndrome[101, 102]. *FBN1* encodes a protein fibrillin, which is essential for the formation of elastic fibers found in connective tissue. Without the structural support provided by

fibrillin, many tissues are weakened and can lead to aneurysm formation and dissection.

Defects that obstruct the outflow tracts of the heart, either the aorta or pulmonary artery, can vary in their location and severity and in the most extreme instances lead to hypoplasia of the corresponding ventricle[14]. Hypoplastic left heart syndrome (HLHS) is a defect in which there is left-sided obstruction, mitral and aortic valve atresia, which leads to a hypoplastic left ventricle. Mutations in the *NKX2.5* gene have been identified in patients with HLHS[90, 103]. HLHS is believed to result from decreased flow to the left side of the heart during development, and this could occur as a consequence of haploinsufficiency for *NKX2.5* through the effects on the conduction system or left ventricular development[68, 104].

Additional genes implicated in hypoplastic chamber defects include heart and neural crest derivatives expressed 1 (*HAND1*) and 2 (*HAND2)*. These are basic helix-loop-helix transcription factors essential for heart development. HAND1 forms heterodimers with other proteins, such as HEY or HAND2, to regulate transcription of downstream target genes[105]. *HAND1* and *HAND2* were initially thought to regulate heart looping, as disruption of both genes results in arrested cardiac development at the looping stage[106, 107]. Deficiency of either protein leads to hypoplastic ventricles, suggesting a possible role in HLHS[107-109].

Williams syndrome is characterized by supravalvar aortic stenosis and peripheral pulmonary artery stenosis. Other syndromic features include elfin-like facial features, mental retardation, neonatal hypercalcemia, and a hypersocial personality. The genetic etiology was found to be a microdeletion on chromosome 7q11 where haploinsufficiency of the elastin gene, *ELN*, resulted in the cardiac defects[110]. Subsequent work identified point mutations in *ELN* in children with non-syndromic forms of supravalvar aortic stenosis[111, 112].

Thickened valve leaflets resulting in stenotic valves are a common form of CHDs. In mouse models, the absence of *Ptpn11*, which encodes the protein tyrosine phosphatase non-receptor type 11 Shp-2, results in dysplastic semilunar valves by its involvement in a Ras-signaling pathway mediated by epidermal growth factor receptor[113]. The importance of *PTPN11* in congenital heart disease was demonstrated by the identification of gain-of-function point mutations in patients with Noonan syndrome, whose phenotype commonly includes short stature, low-set ears, webbed neck, dysmorphic facies, and pulmonic stenosis often due to a bicuspid valve[114, 115]. Other mutations associated with Noonan syndrome include gain-of-function mutations in the *KRAS* and *SOS1* genes[116-118]. Mutations in *PTPN11* also lead to LEOPARD syndrome which is characterized by multiple lentigines, ECG conduction abnormalities, ocular hypertelorism, pulmonic stenosis, abnormal genitalia, growth retardation, and sensorineural deafness[117, 119].

The genetic etiology of neurofibromatosis type 1, characterized by pulmonic valve stenosis along with café-au-lait spots and fibromatous tumors of the skin, is loss-of-function mutations in the neurofibromin gene (*NF1*)[120, 121]. Reduced NF1 protein results in increased Ras-signaling and suggests a common pathway for pulmonary valve thickening. These findings implicate other members of this signaling pathway as candidate genes for human valvar disease[122].

Human genetic studies have identified the gene responsible for Alagille syndrome, which is characterized by intrahepatic bile duct paucity, vertebral anomalies, eye anomalies, and right-sided heart defects ranging from mild pulmonary stenosis to tetralogy of Fallot. Affected individuals were found to have mutations or chromosomal deletions encompassing *JAGGED-1*, a membrane-bound ligand[123, 124]. Subsequently, *JAGGED-1* mutations have been identified in

patients with apparently isolated pulmonary stenosis or tetralogy of Fallot who did not meet criteria for Alagille syndrome, suggesting that haploinsufficiency of this gene may contribute to presumed non-syndromic CHDs[125]. JAGGED-1 is a ligand for the Notch1–4 family of transmembrane receptors, which are involved in embryonic patterning and cellular differentiation[14].

Mutations in *NOTCH1* were identified as the etiology for aortic valve malformations in families with autosomal dominant aortic valve disease[126]. Linkage studies have identified a *NOTCH1* nonsense mutation in affected family members. This was supported by the discovery of a *NOTCH1* frameshift mutation in an unrelated family with similar aortic valve phenotype[126]. The predominant phenotype of the affected family members was a bicuspid aortic valve, the most common type of CHD with a prevalence of 1-2% in the population. An additional example of a crossover lesion involves Andersen syndrome. This syndrome is characterized by a bicuspid aortic valve with coarctation, ventricular arrhythmias, skeletal abnormalities, and facial dysmorphia. Linkage was demonstrated with the *KCNJ2* gene, which encodes the potassium channel Kir2.1 in a 95-member family with 32 affected individuals, suggesting that a mutation in this gene may also lead to bicuspid aortic valve with coarctation[127].

Other mediators of the NOTCH signaling pathway have been implicated in CHD formation. A target gene of the signaling pathway, *Hey2*, has been demonstrated to be involved in CHDs. *Hey2* knockout mice have been shown to have ventricular septal defects, tetralogy of Fallot, and tricuspid atresia[128-130]. In addition, the knockout mice demonstrated aortas and pulmonary arteries with thinner walls[131]. However, in a small study of patients with CHDs, no association between *HEY2* and CHDs was identified[132]. A separate study of patients with tricuspid atresia also failed to demonstrate an association with *HEY2*[133].

Laterality defects consist of defects stemming from abnormal cardiac looping. Proper folding of the straight heart tube aligns the atrial chambers with their appropriate ventricles and the right and left ventricles with the pulmonary artery and aorta, respectively[14]. During development, the heart is the first organ to disturb the bilateral symmetry of the early embryo.

Studies in several species led to the discovery of numerous signaling molecules that regulate left-right asymmetry and provide a framework in which to consider human left-right laterality defects. For example, in the chick embryo, asymmetric expression of Sonic hedgehog (Shh) leads to the expression of the transforming growth factor-β (TGF-β) members *Nodal* and *Lefty* in the left lateral plate mesoderm[134]. Nodal expression on the left-side of the developing embryo induces rightward looping of the straight heart tube[134]. In the right lateral mesoderm, an Activin receptor-mediated pathway inhibits *Shh* and *Nodal* expression. Conversely, the zinc finger transcription factor *Snail* is expressed in the right lateral mesoderm and is repressed by *Shh* on the left, resulting in unique gene expression profiles on the left and right of the embryo[135]. The Activin and Nodal-dependent pathways ultimately result in expression of the transcription factor Pitx2 on the left side of visceral organs, which is sufficient for the establishment of left-right asymmetry in the developing heart, lungs and gut[136].

Disruption of the signaling cascades on the left or right side of the embryo result in randomization of cardiac looping, and often lead to bilateral right (asplenia syndrome) or left (polysplenia syndrome) sidedness[14]. Point mutations of several genes involved in the left-right signaling cascade have been identified in patients with heterotaxy syndromes, including *ZIC3*, *ACVR2B* (activin A receptor IIB), *CFC1*, a cofactor of Nodal, and *LEFTYA*[84, 137-139].

Genetic Etiologies of Atrioventricular Septal Defects

The characteristic pattern of AVSDs is associated with Down syndrome (trisomy 21). Down syndrome is the most common genetic syndrome with a prevalence of one in 700 live births[140]. Approximately 70% of cases of complete AVSDs occur in individuals with Down syndrome[141].

Another observation among Down syndrome patients with CHDs is that certain types of CHDs, such as transposition of the great arteries, truncus arteriosus, and coarctation of the aorta, are rare, suggesting the possibility that the over-expression of the genes located on chromosome 21 may represent a protective factor for some types of CHDs[140]. As is seen in most cases of significant chromosomal aberration, AVSDs are also associated with other noncardiac congenital defects. Not all children with trisomy 21 have AVSDs, so environmental factors, genetic modifiers on chromosome 21 or other chromosomes, partial trisomy, or all of these must contribute to the manifestation of the phenotype[142].

AVSDs seen in children with Down syndrome are more commonly of the complete form. AVSDs in patients with and without Down syndrome differ not only in terms of the prevalence of partial or complete forms, but also in terms of the distribution of associated cardiac malformations. Left-sided obstructive lesions are rare in children with Down syndrome, and they are more commonly seen in AVSD patients without Down syndrome[140].

In order to fully characterize genes implicated in the pathogenesis of AVSDs, a better understanding of the pathways involved in endothelial cell proliferation and differentiation within the developing cardiac cushions is needed. Numerous studies have implicated a strict spatio-temporal expression pattern of the vascular endothelial growth factor (*VEGF)* gene in the control of endocardial cushion development[18]. VEGF is a pleiotropic factor that regulates cell

proliferation, vascular permeability, chemotaxis, and survival in endothelial cells and vasculogenesis and angiogenesis in the developing embryo[143]. Downstream mediators of VEGF signaling include NFATc1 in valve endothelial cells[144]. Transgenic mouse and in situ hybridization studies have suggested that VEGF is a specific mediator of heart valve development[145, 146]. It has also been suggested that the *VEGF*-expressing endothelial cells in the cushion-forming region may be the subpopulation of endothelial cells predetermined to undergo epithelial-mesenchymal transformation (EMT)[146]. Alternative hypotheses include VEGF-producing cells directly inducing the proliferation of adjacent endothelial cells or inducing neighboring endothelial cells to undergo EMT[18]. Additional studies have demonstrated that increases in *VEGF* expression result in inhibition of endothelial cell differentiation, resulting in negative regulation of EMT[145, 147].

In addition to its tight regulation during cardiogenesis, *VEGF* expression is also regulated by environmental exposures. For example, hypoxia induces *VEGF* gene expression and appears to contribute to the generation of major malformations in the atrioventricular canal and outflow tract in mice[145, 148]. V*egf* mutant mouse embryos, with overexpression of a specific VEGF isoform, have been shown to develop AVSDs, ventricular septal defects, pulmonary valve stenosis, and tetralogy of Fallot[149, 150]. Hyperglycemia reduces *VEGF* gene expression in the mouse model leading to inhibition of AV canal cushion EMT[151]. These studies suggest that *VEGF* expression must be tightly regulated, as over- or under-expression of *VEGF* leads to hypoplastic cardiac cushions. Using the mouse model of *VEGF* and AVSDs, a large case-control family study of affected child-parent triads (N=190) was conducted to investigate the possible association in humans[152]. The study demonstrated an association between the *VEGF* gene and AVSDs; in particular, *VEGF* alleles -2578C and -1154G were transmitted more frequently in children with AVSDs[152].

As previously mentioned, NFATc1 is a downstream mediator of VEGF signaling. The NFAT family of proteins has been shown to be crucial for cardiac valve development[153]. Mice deficient in expression of *Nfatc1* die secondary to a cardiac cushion defect. The exact defect differs between reports – failure of aortic and pulmonary valve development or defects in all four cardiac valves and septa[154, 155]. NFATc1 expression is limited to the endocardium overlying the developing cardiac cushion. Endocardial cells that have undergone EMT do not stain for NFATc1, suggesting that NFATc1 is downregulated during EMT or that the subpopulation of *NFATc1*-expressing cells does not undergo EMT[18]. Potential signaling partners of *NFATc1* include *VEGF* and *DSCR1* (Down syndrome critical region 1)[18, 144, 156].

The Notch family of transmembrane receptors, as discussed above, is involved in embryonic patterning and cellular differentiation.[14] Analysis of Notch mutations revealed an essential role for Notch in the control of endocardial cushion EMT[157]. It has also been demonstrated that knockout mice for *Notch1* have hypoplastic cardiac cushions, suggesting that the endocardium failed to undergo EMT in this region[157]. The mutants also demonstrated reduced *Tgfβ2* expression in the myocardium, leading to decreased expression of the transcription factor *snail*, which is discussed below[18].

Notch signaling pathway target genes, *Hey1* and *Hey2*, have been shown to be critically involved in restricting *BMP2* and *TBX2* expression to the AV canal[158, 159]. It has been suggested that *Hey1* and *Hey2* may prevent cells from expressing the AV canal-specific genes that lead to the precise formation of the AV canal boundary[159]. HEY2 has been demonstrated to provide an important myocardial signal to the endocardial cushion for proper septation and valve formation and function[131, 160]. Using a sample of formalin-fixed hearts with septation defects, the role of HEY2 was investigated[161]. Mutations in the binding

domains of HEY2 were identified in two patients with syndromic AVSDs[161].

Bone morphogenetic proteins (BMPs) are multi-potential proteins that regulate a plethora of cellular functions during development and adult life[162]. BMPs belong to the TGF-β family and comprise a subfamily of more than 20 members[162]. Bone morphogenetic protein-2 (BMP2) is uniquely expressed in the AV canal[163]. Deficiency of BMP2 results in AVSDs[164]. A downstream target of the BMP2 signaling pathway, *TBX2,* is also required for AV canal development[165]. *Tbx2* deficiency has been shown to result in the expression of chamber specific myocardial genes in the AV canal and deficient endocardial cushion formation, suggesting that *Tbx2* acts as a transcriptional repressor necessary for AV canal formation[166].

Bone morphogenetic protein-4 (*BMP4*) is also involved in numerous developmental events. BMP4 also regulates TBX2[162]. Expression of *BMP4* in the endocardial cushions and adjacent tissues is essential for early embryonic development of the mouse heart[167]. BMP4 has also been shown to be expressed in the outflow tract in mouse models. Using a transgenic mouse strain it has been shown that *Bmp4* deficient mice have a spectrum of AVSDs that correlates with the amount of *Bmp4* expression; the lower the level of *Bmp4*, the more severe the heart defect[168].

Compound mutants of other BMP ligands (BMP6/BMP7 and BMP5/BMP7) have been shown to result in defective endocardial cushion development in mice[164, 169, 170]. Along with BMP ligands, genetic disruption of BMP receptors also leads to dysregulated cardiac cushion formation. Knockout mice for *ALK3*, a BMP receptor gene, were observed to undergo normal cardiac cushion EMT, but the cushions were noted to be hypoplastic and failed to fuse properly[171]. It was also noted that the *ALK3* knockout mice also had decreased TGFβ2 expression, suggesting that the cardiac cushions may be hypoplastic due to decreased

TGFβ2[171]. TGFβ2 knockout mice have been previously shown to have abnormal tricuspid and mitral valve thickening, incomplete fusion of endocardial tissues and ventricular septal defects[172]. Other BMP receptors have been implicated in the pathogenesis of AVSDs. Utilizing a candidate screening approach, the coding regions of 32 candidate genes in patients with AVSDs (N=190) was sequenced[173]. Two variants in the BMP receptor gene, *ALK2*, were identified[173]. In vitro and in vivo functional analyses suggested that this mutation results in reduced BMP signaling capacity which will lead to deleterious AV canal formation[173].

Given the strong association of trisomy 21 and AVSDs, chromosome 21 was an obvious candidate chromosome to investigate for involvement in the development of Down syndrome related CHD or Down syndrome-CHD. Using molecular studies of rare individuals with CHDs and partial duplications of chromosome 21, a 10.5 Mb candidate critical region of chromosome 21, in the region of band 21q22.2-22.3 was identified[174, 175]. These studies suggested that subsets of the Down syndrome phenotype were associated with three copies of the critical region and that Down syndrome-CHD was caused by the overexpression of genes in the region[176, 177].

Through an exon cloning study, the gene *DSCR1*, Down syndrome critical region 1, was identified in this region[178]. It has been shown that *DSCR1* is highly expressed in the human heart and brain. *DSCR1* expression occurs in regions that correlate with areas of defective endocardial cushion development[18]. The *DSCR1* gene is a direct transcriptional target of NFATc1 proteins within the endocardium during the critical window of heart valve formation[156].

The critical region of chromosome 21 was further narrowed using 19 patients with partial trisomy 21 to a region measuring 5.5 Mb[141]. Based on eight patients with Down syndrome-CHD, it was suggested that trisomy of a gene in

the Down syndrome-CHD critical region is essential for production of Down syndrome-CHD, while trisomy for additional genes in the telomeric and other regions likely contributes to the phenotypic variability of Down syndrome-CHD[141]. An additional candidate gene, Down syndrome cell adhesion molecule (*DSCAM*), was identified for Down syndrome-CHD[141]. *DSCAM* has been shown to be expressed in the mouse fetal heart during development prior to endocardial cushion fusion and in the human fetal heart at 12 weeks of development and to mediate cell-cell adhesion[141, 179]. Additional support for a potential role for *DSCAM* in Down syndrome-CHD includes its identity as a cell adhesion molecule of the immunoglobulin superfamily. It has been speculated that overexpression of *DSCAM* may have the potential to disturb transformation, migration, and/or proliferation of mesenchymal cells, possibly contributing to the increased intercellular adhesion and the abnormal cushion development seen in Down syndrome-CHD[141].

Additional support for the involvement of chromosome 21 as the possible genetic location of AVSD candidate genes is based on the trisomy 16 mouse model[180]. Mouse chromosome 16 contains several genes located on the same chromosome or homologous to genes on human chromosome 21[181]. In addition to the syntenic relationship between these chromosomes, trisomy 16 mice share some of the phenotypic features found in Down syndrome, including a high incidence of AVSDs[182].

The trisomy 16 mouse model has also provided additional insights into the tissues that contribute to AV septation and valve formation. It has been demonstrated that mouse models with AVSDs due to trisomy 16 lack discrete mesenchymal structures that have origins outside the AV cushion[183]. These structures which are associated with the dorsal mesocardium are referred to as the dorsal mesenchymal protrusion or mesenchymal tissue that protrudes into

the dorsal aspect of the atrial cavity[184]. This mesenchymal structure has been shown to fill the gap between the mesenchymal atrial septum and the endocardial cushions[184, 185]. Loss of crucial hedgehog-dependent signals results in AVSDs due to improper differentiation and migration of the dorsal mesenchymal protrusion, suggesting that this tissue is crucial for AV septation[186]. Sonic hedgehog homolog (*SHH*) is a gene which plays a key role in the regulation of organogenesis. It has also been demonstrated that a primary defect in the dorsal mesocardium can result in AVSDs, implying that AVSDs may be the result of multiple defects during cardiac development[186]. Two genes, *EVC* and *LBN* (formerly known as *EVC2)*, have been shown to be present in the dorsal mesenchymal protrusion[187]. It has been suggested that *EVC* and *LBN* have coordinate functions in a pathway related to the hedgehog signaling pathway during cardiac development and a mutation in either *EVC* or *LBN* could interfere with the signaling function and or prevent dorsal mesenchymal contribution to AV septation during cardiac development[187].

AVSDs are classified as defects of cardiac septation. Based on the previous identification of individuals with other types of septal defects and mutations in *NKX2.5,* this gene was investigated for its role in AVSDs. Direct sequencing revealed 53 *NKX2.5* mutations, including nonsynonymous substitutions in the homeodomain of *NKX2.5*, in formalin-fixed heart tissues of patients with ASDs, VSDs, and AVSDs[188]. Mutations of somatic origin in the binding domains of *NKX2.5* were associated specifically with AVSD and resulted in loss of protein function[189]. No difference in the mutation spectrum was noted in syndromic and non-syndromic AVSDs[188]. Additional investigations using the same sample also identified somatic mutations in the molecular partners of *NKX2.5*, *TBX5* and *GATA4*[190, 191]. As the mutations in the *NKX2.5* gene were identified in samples that had been fixed in formalin for over 22 years, a

replication study was performed. In this study, tissue from a cohort of 28 patients with septal defects was sequenced for mutations[192]. No evidence of somatic mutations in *NKX2.5* was found suggesting that the previous findings may have been due to fixation artifacts or a low DNA yield from the archival samples[192].

GATA4 is a transcription factor known to be essential for cardiac development. A study of two unrelated pedigrees of septal defects identified linkage to the *GATA4* locus. All affected members in both families had secundum atrial septal defects, and a subset of these had additional cardiac defects, including AVSDs. Mutations were identified in both families, demonstrating a functional variant[75]. AVSDs can be part of the phenotypic spectrum of *GATA4* mutations.

As discussed above, *HAND1* and *HAND2* were initially thought to contribute to cardiac looping. Absence of Hand1 in mice results in embryonal lethality, as well as in a wide spectrum of cardiac abnormalities including failed cardiac looping, defective chamber septation and impaired ventricular development[193, 194]. Mutations in *HAND1* have been identified in the formalin-fixed collection of hearts with septation defects discussed above[195]. The mutations were more frequently detected in the N-terminal region, which is essential for DNA binding and protein-protein interactions of HAND1[195]. While these mutations suggest a broader role of *HAND1*, the mutations were not linked to a specific disease phenotype[195]. This study was conducted using heart tissue that has been found to have multiple genetic abnormalities, such as mutations in *NKX2.5, GATA4*, *TBX5*, and *HEY2* in addition to mutations in *HAND1*. The multiple mutations in this population suggest that there may be combinatorial interactions of mutated cardiac transcription factors affecting heart development.

Due to the association between 3p deletion (3p-) syndrome and AVSDs, chromosome 3 has been evaluated for an association with the development of

AVSDs. Using a combination of fluorescent in-situ hybridization and polymorphic markers, the critical region for the development of AVSDs seen in 3p- syndrome was defined as the interval between *D3S1263* and *D3S3594* on chromosome 3[196]. This locus has been renamed *AVSD2*. A candidate gene, *CRELD1*, near the *AVSD2* locus is expressed in cardiac tissues during development[20]. The *CRELD1* locus is distal to the *AVSD2* locus. Analysis of the *CRELD1* gene from patients with non-syndromic AVSDs revealed heterozygous missense mutations in 6% of the study population[20]. If there is incomplete penetrance for AVSDs associated with 3p- syndrome, then the critical region would extend telomerically and may include *CRELD1*[197]. The presence of unaffected family members carrying a *CRELD1* mutation showed that *CRELD1* mutations are neither necessary nor sufficient to cause AVSDs[142]. *CRELD1* mutations are thought to confer susceptibility for AVSDs[198].

Identification of *CRELD1* as a susceptibility gene for AVSDs has led to consideration of *CRELD2*, another human CRELD gene homolog, as a candidate gene for AVSDs[142]. There is a high degree of similarity between their two protein products, suggesting that *CRELD1* and *CRELD2* are functionally related. Additional support for *CRELD2* as a susceptibility gene is the overlap in temporal and spatial patterns of expression of both genes.

It has been demonstrated that collagen VI is expressed within the developing endocardial cushions in a pattern that accompanies cell migration and valve remodeling during mouse embryogenesis[199]. It is thought to act as a bridge between the cell surface and the surrounding extracellular matrix[200]. Genetic variations in the alpha region of the collagen VI gene are associated with Down syndrome related AVSDs[201]. Collagen VI is more densely organized in the nuchal skin of fetuses with Down syndrome[202]. In addition, the collagen VI content of the gingival extracellular matrix is increased in Down syndrome[203]. A

study examining fetal hearts with AVSDs revealed increased staining of collagen VI in hearts with Down syndrome, suggesting overexpression of collagen VI plays a role in the pathogenesis of Down syndrome-related AVSDs[203].

The search for a genetic etiology has also been ongoing for non-syndromic AVSDs. Non-syndromic AVSDs do not have any associated characteristics or chromosomal abnormality. Non-syndromic AVSDs are estimated to occur in approximately one per 10,000 live births[204]. Most non-syndromic AVSDs have been considered to be sporadic or the result of multifactorial inheritance[205]. However, there are numerous reports of non-syndromic AVSDs transmitted within families.

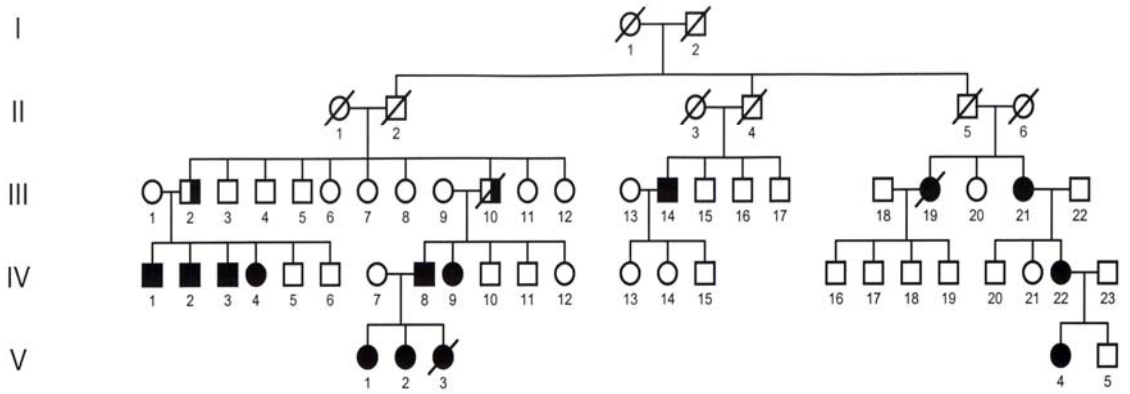
Table 2-3 lists reported multiplex pedigrees with non-syndromic AVSDs where at least three affected individuals were described[198, 205-216]. A multiplex pedigree is defined as a pedigree with at least two affected individuals. The existence of several reports of families with multiple affected individuals suggests that some cases of non-syndromic AVSDs may result from a major susceptibility gene[205]. The reports of familial non-syndromic cases suggest that the defect segregates with a Mendelian pattern. The pattern of recurrence indicates an autosomal dominant mechanism with monogenic or oligogenic inheritance in these pedigrees[29]. However, even in these pedigrees, the development of AVSDs has a multifactorial appearance given the observation of non-penetrance and variable expressivity[205].

Table 2-3: Summary of Reported Multiplex Pedigrees with Non-Syndromic Atrioventricular Septal Defects

Reference	*Year Reported*	*Number Affected*	*Number of Generations*
Yao et al.[206]	1968	4	1
Nora et al.[207]	1971	4	1
O'Nuallain et al.[208]	1977	6	3
Tennant et al.[209]	1984	3	2
DiSegni et al.[210]	1985	4	2
Wilson et al.[211]	1993	11	3
Digilio et al.[216]	1993	6 2 2 2 6	2 2 2 2 3
Digilio et al.[212]	1994	4	2
Kumar et al.[213]	1994	3	2
Gennarelli et al.[214]	1994	7 6	3 2
Cousineau et al.[215]	1994	12	4
Amati et al.[198]	1995	6 7	2 3
Sheffield et al.[205]	1997	14	4

The Minnesota Atrioventricular Septal Defect Pedigree was first reported by investigators at the University of Minnesota in 1985. Pedigree analysis demonstrated AVSDs with an autosomal dominant pattern of inheritance and incomplete penetrance. The pedigree is shown in Figure 2-1 and illustrates the 14 affected individuals as well as obligate heterozygotes. Individual III-2 is a confirmed unaffected obligate heterozygote. Individual IV-2 had an isolated cleft mitral valve and significant mitral insufficiency requiring surgical repair. Individuals IV-4, V-3 and V-4 had complete AVSDs. All other affected individuals had ostium primum atrial septal defects and cleft mitral valves. The unaffected first-degree relatives and spouses of affected individuals are also included.

Figure 2-1: Minnesota Atrioventricular Septal Defect Pedigree[205]



Note: Affected individuals with confirmed AVSDs are represented by solid symbols, obligate heterozygotes by half-filled symbols, and unaffected individuals by open symbols.

Initial studies at the University of Iowa to investigate a possible genetic etiology for non-syndromic AVSDs in this pedigree focused on chromosome 21, given the association with trisomy 21. A detailed linkage analysis of the Minnesota pedigree using highly informative short tandem repeat polymorphisms (STRPs) from chromosome 21 was performed[215]. Many of the polymorphisms evaluated were within or near the critical region for Down syndrome-CHD. Twelve STRPs were chosen for linkage analysis. The average heterozygosity of the STRPs analyzed was greater than 0.70. Using two-point analysis and assuming 90% penetrance, the entire long arm of chromosome 21 could be excluded. However, assuming 50% penetrance, the critical region for Down syndrome (near 21q22) could not be entirely excluded. Multipoint analysis assuming 50% penetrance excluded the entire long arm of chromosome 21, including the critical region. These results were confirmed by multipoint analysis using only affected individuals. Therefore, regardless of the true penetrance, chromosome 21 was excluded as the location of a gene linked to AVSDs in this

pedigree[215].

A genome-wide screen using STRPs was then performed in an attempt to identify a genetic locus involved in the development of AVSDs in this pedigree, which by then included two additional affected members[205]. The major hypothesis of this study was that the existence of a major locus was necessary (but not sufficient) for the development of AVSDs. A few individuals were genotyped with densely spaced STRPs across the genome. These individuals included the proband (IV-3) and three affected relatives (IV-4, V-1, and V-4). In addition, a pool of DNA from 13 of the 14 affected individuals and a DNA pool from 40 unrelated normal individuals were also genotyped for each marker. This would be expected to yield numerous markers for which an allele is identical by state in a few distantly related affected individuals, but few markers for which alleles are identical by descent in the affected individuals. STRPs which were the shared allele in at least three of four AVSD affected individuals and in which the shared allele was a predominant allele in the affected DNA pool were considered to be of interest. Approximately 60 STRPs showed evidence of a shared allele in all four affected individuals. For only a third of these STRPs was the shared allele a predominant allele in the affected DNA pool, compared with the control pool. It was observed that a cluster of four contiguous markers which displayed a shared allele in the four AVSD subjects and predominance in the affected DNA pool was located on chromosome 1. Linkage analysis was performed which identified a locus on chromosome 1. No other region of the genome was noted to include more than two contiguous markers with similar features. Genotyping of these markers in all members of the pedigree established phase for each marker, and indicated that 13 of the 14 individuals shared the same haplotype. The genotype of affected individual III-9 was determined by genotyping her spouse and four children. Genotyping of

additional markers between and outside these STRPs identified a region shared by all 14 affected individuals. Linkage using only affected individuals was supported by a LOD score of 4.01 at θ = 0, and by the common haplotype across the interval shared by all affected individuals. Based on the narrowest shared interval in affected individuals, the AVSD susceptibility locus mapped in this study lies between flanking markers *D1S3471* and *D1S1587*, an interval of ~12 cM. This locus (1p21-31) has been renamed *AVSD1.* More dense genotyping subsequently further narrowed the candidate interval to less than 5 cM.

To date, three linkage analyses have been conducted of non-syndromic AVSDs and markers on chromosome 21 in four pedigrees[211, 214, 215]. Each of these studies excluded the involvement of the Down syndrome critical region, suggesting that at least in these four multiplex pedigrees familial non-syndromic AVSDs are not likely due to genes located on chromosome 21. Genes on chromosome 21 could however modify the effect of the mutation that is likely segregating in each pedigree. The anatomical differences and the absence of linkage in the molecular analysis of chromosome 21 in families with AVSDs suggest that the genetic basis of AVSDs in non-syndromic patients may be different from that in patients with Down syndrome[213].

Another focus has been on chromosome 8. AVSDs are associated with 8p- or 8p deletion syndrome[142]. Similar to the work done on chromosome 21, it was hypothesized that a gene(s) involved in the etiology of non-syndromic AVSDs may be located on chromosome 8. Previous analysis defined a critical region related to the development of AVSDs in the 8p23.1→pter region[217]. Two families were identified with non-syndromic AVSDs assumed to be inherited in an autosomal dominant pattern. However, linkage analysis of seven highly polymorphic markers on chromosome 8, as well as multipoint analysis in two pedigrees excluded the involvement of chromosome 8 in these families[198].

CCN1 is a protein associated with the extracellular matrix which promotes cell adhesion, migration, proliferation, differentiation, and survival or death[218]. CCN1 is not required for EMT or cell proliferation or differentiation in the endocardial cushion tissue. However, it was demonstrated that deficiency of CCN1 results in accelerated apoptosis in the endocardial cushion tissue, suggesting that CCN1 is essential for heart septation and valvuloseptal morphogenesis[218]. *Ccn1* knockout mice displayed severe AVSDs and embryonic death, while heterozygous mice were observed to have ostium primum atrial septal defects[218]. These findings implicate *CCN1* as a candidate gene for AVSDs.

Genetic Techniques

Newer genotyping techniques are now available than were available at the time of the identification of a majority of genes associated with AVSDs. Two widely used marker types are short tandem repeat polymorphisms (STRPs) or microsatellites and single nucleotide polymorphisms (SNPs). STRPs are short sequences of tandemly repeated DNA sequence, usually 2-5 base pairs in length. The estimated density of microsatellites is one every 6,000 base pairs[219]. SNPs are any polymorphic variant of a single nucleotide at a specific locus, usually consisting of two alleles (where the rare allele frequency is ≥ 1%). SNP frequency throughout the genome is every 100-300 base pairs. Therefore, genomes contain many more SNPs than STRPs. An advantage of the STRPs is that they are highly polymorphic, producing greater levels of variation and providing more information per marker, much more than the diallelic SNPs. STRPs mutate rapidly (typically 10^{-3} – 10^{-5} per locus per generation) in contrast to SNPs, which arise at a low rate (10^{-8} – 10^{-9} per locus per generation)[220]. STRPs mutate by addition or subtraction of repeats, while SNPs arise through

changes in single base-pairs. A major advantage of SNPs is that they are much more plentiful, and the hope is that their greater density will compensate for the smaller amount of information per SNP. SNPs are also easier to genotype. Microsatellites were the genetic markers of choice during the late 1980s. Since the late 1990s, SNPs have become more prominent.

In high density oligonucleotide SNP arrays, thousands of probes are arrayed on a small chip, allowing for a large number of SNPs to be interrogated simultaneously. Because SNP alleles only differ in one nucleotide and because it is difficult to achieve optimal hybridization conditions for all probes on the array, the target DNA has the potential to hybridize to mismatched probes. This is addressed somewhat by using several redundant probes to interrogate each SNP. Probes are designed to have the SNP site in several different locations as well as containing mismatches to the SNP allele. By comparing the differential amount of hybridization of the target DNA to each of these redundant probes, it is possible to determine specific homozygous and heterozygous genotypes. Although, oligonucleotide microarrays have a comparatively lower specificity and sensitivity, the scale of SNPs that can be interrogated is a major benefit.

Analytical approaches also differ based on marker type utilized. Microsatellites lend themselves to multipoint linkage analysis, which evaluates linkage of a disease to multiple markers in a small region, while SNPs can be used in whole genome association or linkage studies. Numerous genome-wide association (GWA) studies have been performed, with many identifying associations between specific genetic variations and diseases. However, as successful as these studies have been in the identification of these associations, the genetic variations identified only explain a small fraction of the disease burden in the population[221].

The “Common Disease Rare Variant” hypothesis refers to the idea that a

significant proportion of the inherited susceptibility to relatively common human disease may be due to the additive effects of a series of low frequency variants in a variety of different genes, each responsible for a moderate but detectable increase in relative risk[221, 222]. Most GWA studies have not been able to detect the effects of polymorphisms with minor allele frequencies of less than 5% (i.e., rare variants) for numerous reasons. Many polymorphisms have not been identified and have not been included in the genotyping platforms. In theory, very large GWA studies could detect the effects of some variants; however, study power decreases exponentially with the allele frequency limiting its utility[222]. In order to identify these rare variants, DNA sequencing technologies must be employed[221]. Once identified, these variants can be prioritized based on variant frequency, function of gene targeted, predicted function of the variant, as well as the presence of disease-associated SNPs in the same region[222].

Full genome sequencing, also referred to as whole genome sequencing, complete genome sequencing, or entire genome sequencing, is a laboratory process that determines the complete DNA sequence of an organism's genome. Resequencing of candidate genes or other genomic regions of interest in patients and controls is a key step in the detection of mutations associated with disease[223, 224]. Resequencing techniques can be divided into those which test for known mutations (genotyping) and those which scan for any mutation in a given target region (variation analysis). Typical mutations that can be identified are substitution, insertion, and deletion mutations.

An alternative approach to full genome sequencing involves the targeted resequencing of all protein-coding subsequences, the exome, which requires ~5% as much sequencing as a whole human genome[225-227]. Sequencing of the exome, rather than the entire human genome, is well justified as an efficient strategy to search for alleles underlying rare Mendelian disorders. First,

positional cloning studies focused on protein-coding sequences have, when adequately powered, proven highly successful at identification of variants underlying monogenic diseases[223, 224, 228]. Second, the clear majority of allelic variants known to underlie Mendelian disorders disrupt protein-coding sequences[229]. Splice acceptor and donor sites represent an additional class of sequences that are enriched for highly functional variation and are therefore targeted as well. Third, a large fraction of rare nonsynonymous variants in the human genome are predicted to be deleterious[230]. This contrasts with non-coding sequences, where variants are more likely to have neutral or weak effects on phenotypes, even in well-conserved non-coding sequences[231, 232]. The exome therefore represents a highly enriched subset of the genome in which to search for variants with large effect sizes.

Another technique employed in the identification of genetic variation involves copy number variation. A copy number variant (CNV) is a segment of DNA in which copy number differences, which may range from one kilobase to several megabases in size, have been found by comparison of two or more genomes[233]. Deletions, duplications, triplications, insertions, and translocations are thought to result in CNVs. CNVs may either be inherited or caused by *de novo* mutations. CNVs can be caused by genomic rearrangements such as deletions, duplications, inversions, and translocations[234]. Segmental duplications which are greater then 10 kb can lead to genomic instability and are susceptible to genomic rearrangements resulting in CNVs[235]. Copy number variation can be identified by cytogenetic techniques such as fluorescent in situ hybridization, comparative genomic hybridization, array comparative genomic hybridization, and by virtual karyotyping with SNP arrays.

The fact that DNA copy number variation is a widespread and common phenomenon among humans was first realized following the completion of the

human genome project[236, 237]. It is estimated that approximately 0.4% of the genomes of unrelated people typically differ with respect to copy number[238]. *De novo* CNVs have been observed between identical twins who otherwise have identical genomes.

Like other types of genetic variation, some CNVs have been associated with susceptibility or resistance to disease. Copy number variation has been associated with autism, schizophrenia, idiopathic learning disability, and non-small cell lung cancer[233, 238-241]. In addition, a higher copy number has been associated with lower susceptibility to human HIV infection, and a low copy number of FCGR3B (the CD16 cell surface immunoglobulin receptor) can increase susceptibility to systemic lupus erythematosus and similar inflammatory autoimmune disorders[242, 243]. It has recently been demonstrated that patients with non-syndromic tetralogy of Fallot result from *de novo* CNVs suggesting that mutation within those loci may be etiologic for other cases of TOF[244].

CNVs encompass more DNA than SNPs. CNVs can be limited to a single gene or include a contiguous set of genes. CNVs can result in having either too many or too few of the dosage sensitive genes, which may be responsible for a substantial amount of human phenotypic variability, complex behavioral traits, and disease susceptibility[245, 246].

Summary of Genetic Findings

Numerous genetic associations have been described for various types of CHDs. These associations are detailed in Table 2-2. A majority of these genes have roles in different subtypes of CHDs, suggesting that individual genes are not defect specific adding to the complexity of identifying genetic etiologies of CHDs.

In addition, numerous genes have been investigated for their role in the

development of syndromic and non-syndromic AVSDs. These candidate genes have been identified based on their role in endocardial cushion differentiation, formation, and/or function. Additional candidate genes have been proposed based on their association with other forms of septation defects. Table 2-4 lists the possible genetic associations with AVSDs and their chromosomal locations.

While these genes have been proposed as associated with AVSDs, no causal variant(s) within these genes have been identified. The next step in the identification of genetic etiologies of AVSDs is determination of the causal variant within these candidate genes/loci employing technologies as previously described.

Familial Aggregation of Cardiac Structure Measurements

For a quantitative trait, familial aggregation (the tendency for a trait to cluster in families) can be examined by estimating correlation coefficients between pairs of relatives, e.g., between parents and children, or in extended families using variance components analysis[247]. For a qualitative trait, familial risk can be quantified using the λ statistic, which represents a ratio of the risk of the trait in family members, e.g., siblings, compared to the risk in the general population. This risk is usually representative of the risk for a specific type of family member, e.g., the sibling relative risk λ_S estimated for siblings of affected individuals. The risk ratio is increased with greater genetic contribution. Significant evidence for familial aggregation suggests that genetic and/or environmental factors are likely to be involved in the etiology of the trait. The heritability of a trait reflects the magnitude of these correlation coefficients and is the portion of the trait variance that can attributed to additive genetic factors[248]. Heritability has been estimated for a number of quantitative echocardiographic measurements.

Table 2-4: Genes with Possible Associations with Atrioventricular Septal Defects

Gene Association	Chromosome Location
AVSD1	1p21-31 *
CCN1	1p21-31 *
ALK2	2q22
AVSD2	3p25
CRELD1	3p25
LBN	4p15
EVC	4p16
HAND2	4q33
NXK2.5	5q34-35
HAND1	5p33
VEGF	6p12
HEY2	6q21
SHH	7q36
GATA4	8p23
HEY1	8q21
NOTCH	9q34
ALK3	10q22
BMP4	14q22-23
TBX2	17q23
NFATc1	18q23
BMP2	20p12
COL6A1	21q22
DSCR1	21q22
DSCAM	21q22
CRELD2	22q13

Note: * denotes those loci/genes associated with non-syndromic atrioventricular septal defects.

An example of familial aggregation analysis is seen with left ventricular mass. Left ventricular hypertrophy, i.e., thickening of the left ventricular muscle, is a major and independent risk factor for cardiovascular morbidity and mortality in adults[249]. Increased left ventricular mass results from the complex interaction between genetic, environmental, and lifestyle factors[250-255]. In a study examining seven monozygotic and 15 dizygotic African-American twin pairs, the heritability of left ventricular mass was estimated to be 0.90 in the monozygotic twins and

0.33 in the dizygotic twins, suggesting left ventricular mass has strong genetic influences[252]. Another study involving 110 twin pairs estimated heritability of left ventricular mass to be 0.69[256]. In a random sample of 159 Polish and Russian families, it was determined that the mother-offspring correlation coefficient was 0.28[250]. This was significantly different from the father-offspring correlation coefficient of 0.04, suggesting that maternal factors may have more impact on left ventricular mass of offspring than do paternal factors[250].

A search for evidence of familial aggregation has also been conducted for congenital left ventricular outflow tract obstruction malformations which include an anatomically varied set of defects with a wide spectrum of clinical severity. Defects within this spectrum include aortic valve stenosis, bicuspid aortic valve, coarctation of the aorta, hypoplastic left heart syndrome, and interrupted aortic arch. It was postulated that classification of CHDs into groups based on the suspected developmental mechanism may aid in uncovering the genetic components[257]. This concept was employed in an investigation of the inheritance of left ventricular outflow tract malformations. First-degree relatives of cases with bicuspid aortic valve have been demonstrated to have an increased risk for the same defect when compared to relatives of individuals without a congenital heart defect (Relative Risk (RR)=5.05, 95% confidence interval (CI) 2.2-11.7)[258]. The broad sense heritability was estimated to be 0.49, based on a general population frequency of 0.9%[258]. A separate investigation comprised of 309 participants estimated the heritability of bicuspid aortic valve to be 0.89, based on a general population frequency of 1%[259]. In addition, the same investigators estimated the heritability of hypoplastic left heart syndrome to be 0.99, suggesting that the defect is determined largely by genetic factors[260].

The heritability of several aortic arch and aortic valve measurements was found to be significant using quantitative trait heritability analysis in a large study

of 190 first-degree relatives of children with a left ventricular outflow tract obstruction malformation[261]. For example, the heritability was estimated to be 0.96 for aortic root measurements, 0.72 for sinotubular junction measurements, and 0.57 for aortic valve annulus measurements in first-degree relatives of children with a left ventricular outflow tract obstructive malformation[261]. As these traits have demonstrated high heritability, it has been suggested that investigations to identify genetic variants be conducted to explain some of the variability in the traits[261]. In other words, genetic variants or quantitative trait loci that may be shared by relatives likely explain a substantial portion of the high heritability.

Non-Genetic Risk Factors for Congenital Heart Defects

Little is known regarding non-genetic risk factors for the development of congenital heart defects. Defect prevention has been hampered by a lack of information about modifiable risk factors for abnormalities in cardiac development[262]. The proportion of cases of CHDs that are potentially preventable through changes in the fetal environment is unknown, although it has been suggested that the fraction of cases attributable to identifiable and potentially modifiable factors may be as high as 30% for some defects[263]. The lack of information regarding modifiable risk factors has made it difficult to develop population-based strategies to reduce the burden of illness from CHDs and for couples to make lifestyle choices to reduce the likelihood of having a child with a CHD[262].

The ideal study design to identify possible risk factors for the development of CHDs would be a prospective cohort study. In this design, two groups of individuals are assembled with respect to the presence or absence of a risk factor and then followed forward in time to see if the incidence of disease is

different in the exposed compared to the unexposed. The rarity of cardiovascular malformations as a whole in the general population and the extreme rarity of each subtype precludes the prospective approach, owing to the immense financial cost[264]. There are no published reports of large prospective cohort studies examining risk factors associated with CHDs.

The majority of known information regarding risk factors comes from large population-based case-control studies. As the critical period for cardiac development is between two and seven weeks of gestational age, the risk factors that are discussed below are limited to parental exposures during the periconceptional period, which is defined as the three months prior to pregnancy through the third month (first trimester) of pregnancy[262, 265].

Table 2-5 summarizes risk factors and exposures that have been shown to be associated with CHDs. Table 2-6 lists risk factors/exposures that are not associated with CHDs. For the purposes of this discussion, risk factors associated with CHDs will be classified into maternal conditions, medications, non-therapeutic medications (maternal and paternal), occupational and other environmental exposures (maternal and paternal), and sociodemographic characteristics (maternal and paternal). Although the term “environmental exposure” conjures up the image of smog or a toxic waste dump, it refers more broadly to any factor which is not genetic and more specifically, to the fetal-placental-maternal environment[266].

Table 2-5: Risk Factors and Exposures Associated with Congenital Heart Defects

Category	*Risk Factor/Exposures*
Maternal illness	Obesity Diabetes Mellitus Phenylketonuria Rubella Febrile Illness Thyroid
Medications	Isotretinoin Thalidomide Lithium Zidovudine Antifungal Tetracycline Trimethoprim-Sulfonamide Non-steroidal anti-inflammatory drugs Indomethacin Oral contraceptive pill Clomiphene Angiotensin-converting enzyme inhibitors β-blocker Aspirin
Non-therapeutic drugs (maternal)	Alcohol Cocaine Marijuana Cigarette Smoking Vitamin A Spermicide
Occupational and/or Environmental Exposures	Organic solvents Paint/Varnish Lead/metals Mineral Oil Trichloroethylene Agricultural Exposures
Sociodemographic characteristics (maternal)	Maternal age Low socioeconomic status Maternal stress
Non-therapeutic drugs (paternal)	Alcohol Cocaine Marijuana Cigarette Smoking Paternal Age Low socioeconomic status

Table 2-6: Non-Genetic Risk Factors with No Evidence of Association with Congenital Heart Defects

Category	*Risk Factor/Exposure*
Maternal Illness	Systemic Lupus Erythematosus Human Immunodeficiency Virus
Medications	Metronidazole Ampicillin Benzodiazepines
Non-therapeutic Drugs	Caffeine

Maternal Conditions

Obesity

The prevalence of obesity is increasing in the United States at an alarming rate[267]. Multiple studies have examined the association between maternal pre-pregnancy obesity and CHDs. Findings from these studies have been inconsistent. Using a case-control design, one study examined the association of maternal obesity and multiple categories of birth defects in 1370 infants from California and Illinois; an association of maternal obesity with defects of the great vessels was identified (OR=6.2, 95% CI 1.4-27.4)[268].

Using a large European registry, an increased risk of conotruncal defects, specifically truncus arteriosus (OR=6.3, 95% CI 1.6-24.8) and transposition of the great arteries (Odds Ratio (OR)=4.4, 95% CI 1.1-17.7), in relation to maternal obesity was described[269, 270]. In addition, a study conducted using the Metropolitan Atlanta Congenital Defects Program database found a two-fold risk elevation for CHDs among obese women[271]. A six-fold risk elevation for CHDs among obese black women from a large university-based institution with over 38,000 deliveries has also been described[270]. Additional case-control studies did not find statistically significant increased risk for any heart defects in relation to maternal obesity[272-274].

The most recent study, however, again identified an association between

maternal pre-pregnancy obesity and CHDs. Using data from the National Birth Defects Prevention Study, an association between maternal obesity and CHDs was observed (OR=1.18, 95% CI 1.08-1.29)[275]. The risk remained elevated when stratified by defect, including conotruncal defects (OR=1.16, 95% CI 1.0-1.36), total anomalous pulmonary venous return (OR=1.53, 95% CI 1.03-2.28), hypoplastic left heart syndrome (OR=1.32, 95% CI 1.02-1.72), pulmonary valve stenosis (OR=1.36, 95% CI 1.12-1.66), Ebstein's anomaly (OR=1.78, 95% CI 1.02-3.13), and atrial septal defects (OR=1.29, 9%% CI 1.07-1.55)[275]. The inconsistent findings among these investigations suggest the possibility of confounding by other factors associated with nutrition, such as intake of micronutrients or use of vitamin supplements, or associated with obesity, such as type 2 diabetes.

Diabetes Mellitus

Multiple studies have demonstrated an association between CHDs and maternal pregestational diabetes, and less consistently, with gestational diabetes[274, 276-286]. For example, in the Baltimore-Washington Infant Study, a significant association was observed between maternal diabetes and CHDs (OR=2.98, 95% CI 1.85-4.81)[287]. Maternal diabetes has also been demonstrated to be an independent risk factor for CHDs [288]. The associations with gestational diabetes have been hypothesized to be due to inclusion of a subgroup of women with previously undetected type 2 diabetes among women classified as having gestational diabetes[280, 284, 289]. It has been shown that there is a greater risk of CHDs with maternal pregestational diabetes than with maternal gestational diabetes (OR=4.64, 95% CI 2.87-7.51 vs. OR=1.59, 95% CI 1.27-1.99)[290]. CHDs associated with maternal pregestational diabetes include laterality and looping defects, transposition of the great arteries, ventricular septal defects, hypoplastic left heart syndrome, conotruncal defects, outflow tract defects, cardiomyopathy,

patent ductus arteriosus, and non-syndromic AVSDs[274, 285, 291, 292]. Diabetes appears to induce malformation before the seventh week of gestation, during the critical period of organogenesis[293].

Although the mechanisms underlying the association between diabetes and CHDs are not well-understood, it appears that hyperglycemia plays a critical role[290]. There is a positive association between hyperglycemia during embryogenesis and risk for congenital malformations among infants of diabetic mothers[294-296]. Among diabetic women with good glycemic control, the prevalence of birth defects is similar to that of the general population[297]. Although glycemic control has been shown to reduce risk of birth defects, achieving and maintaining euglycemia early in pregnancy remains a challenge since many women with diabetes do not plan their pregnancies and do not achieve adequate glycemic control prior to conception[296, 298]. Glycemic control has been proposed as a prevention strategy for birth defects, however, as prevalence of diabetes and risk factors for diabetes continue to rise, it appears that this may not be solely effective[267, 299].

Even though congenital malformations associated with maternal diabetes are presumed to be related to abnormalities in maternal metabolic fuels essential for embryogenesis, precise mechanisms are unclear[300]. One hypothesis is that abnormal glucose levels disrupt expression of a regulatory gene in the embryo leading to embryotoxic apoptotic cellular changes[301]. In animal studies, it has been demonstrated that diabetic embryopathy can be prevented by antioxidants suggesting that oxidative stress resulting from metabolic abnormalities and generation of free radicals may be another mechanism[302-305]. It has also been suggested that the down-regulation of genes involved in the development of the cardiac neural crest could contribute to the pathogenesis of maternal diabetes-induced CHDs[306].

Epilepsy

Infants born to mothers with epilepsy are at increased risk for birth defects, including CHDs [307-309]. It remains undetermined whether maternal seizures are independently associated with CHDs as several therapy-related factors could account for the increased risk. These factors include direct teratogenic effects of the anticonvulsant medications and an indirect effect of the drugs by interfering with folate metabolism.

Phenylketonuria

Untreated maternal phenylketonuria is associated with a greater than six-fold increased risk of CHDs[310, 311]. The most frequent defects seen are tetralogy of Fallot, ventricular septal defect, patent ductus arteriosus, and single ventricle. With strict diet control prior to conception and during pregnancy, the increased risk of a CHD can be reduced[310, 312].

Systemic Lupus Erythematosus

Although a large proportion of infants with congenital complete heart block are born to women with systemic lupus erythematosus, no associations have been identified between maternal connective tissue disease and an increased risk of CHDs[313-315].

Rubella

An association between maternal rubella infection and birth defects was first noted in the early 1940s[316]. Maternal rubella infection during pregnancy can result in patent ductus arteriosus, pulmonary valve abnormalities, peripheral pulmonic stenosis, and ventricular septal defects[317-319]. It has also been demonstrated that the risk of rubella embryopathy can be eliminated by ensuring that every woman of childbearing age is immunized against rubella[320, 321].

Febrile Illnesses

There have been reports suggesting that maternal febrile illness during the first trimester of pregnancy may be associated with an increased risk of CHDs [274, 322-324]. Maternal febrile illness during the first trimester has a two-fold elevated risk of an infant with a CHD [274, 322]. Some studies have demonstrated that the association between maternal febrile illness and CHDs is defect specific, including moderate pulmonic stenosis (OR=2.5, 95% CI 1.3-4.6, right-sided obstructive defects (OR=2.2, 95% CI 1.2-4.2), tricuspid atresia (OR=5.2, 95% CI 1.3-20.2), left-sided obstructive defects (OR=2.7, 95% CI 1.5-4.7), coarctation of the aorta (OR=2.7, 95% CI 1.2-6.0), and ventricular septal defects (OR=1.8, 95% CI 1.1-2.9)[274, 322].

In a few of these studies, the febrile illness was defined as an influenza-associated fever. These studies reported an increased risk of CHDs (OR=1.8, 95% CI 1.4-2.2)[325]. However, in an animal study, it was demonstrated that the influenza virus does not cross the placenta until late in gestation, beyond the critical period of cardiac development[326].

It has also been reported that there is an elevated risk of CHDs in mothers with acute pelvic inflammatory disease during the second and/or third month of pregnancy (OR=2.6, 95% CI 1.2-5.4)[327]. Fever may be a confounder in this situation, as it is a hallmark for the diagnosis of pelvic inflammatory disease.

Another common etiology of maternal fever is maternal urinary tract infection. It has been reported that there is a risk elevation for certain types of CHDs associated with maternal urinary tract infection, although a characteristic of a urinary tract infection is fever[328].

The mechanism underlying the association between febrile illnesses and CHDs is unclear. One hypothesis is altered apoptosis. Apoptosis, which is involved in cardiac morphogenesis, can be altered by fever and infection[329-331]. *In*

vivo and *in vitro* studies have also reported that folate depletion can increase cell apoptosis whereas folate restoration can rescue cells from apoptosis, suggesting a role for multivitamins[332-334]. An alternate possibility is that CHDs are a direct result of the infection. It also remains unclear if the association is confounded by maternal fever, maternal infection, or the use of medications for the fever and infection.

Human Immunodeficiency Virus (HIV)

Maternal infection with HIV can transmit the infection to an infant in a vertical fashion. Children infected with HIV in utero have an increased risk of dilated cardiomyopathy and left ventricular hypertrophy[335, 336]. These children are also more likely to have a decreased left ventricular shortening fraction, although their left ventricular function remains normal to slightly decreased[335]. While these changes have been noted in infants born to HIV-infected mothers, maternal HIV has not been associated with an increased risk of congenital cardiac malformations[337].

Thyroid Disorders

Maternal thyroid disorders have been investigated for an association with congenital malformations. The results, however, have been mixed. A majority of the studies have not identified an association between maternal thyroid dysfunction and congenital malformations[274, 338, 339]. In a study using the National Birth Defects Prevention Study database, an association was observed between maternal thyroid disease and left-sided obstructive defects (OR=1.5, 95% CI 1.0-2.3)[340].

Medications*

Folic Acid

Multivitamin supplements containing folic acid may reduce the risk of some types of CHDs, similar to the known risk reduction for neural tube defects seen with folic acid[341]. In a Hungarian randomized trial, the use of multivitamins containing folate was associated with an approximately 60% overall reduction in risk of CHDs (RR=0.42; 95% CI 0.19-0.98)[341]. Using data from the Atlanta Birth Defects Case-Control Study (N=158), a 43% lower risk of conotruncal defects with the use of folate containing multivitamins (OR=0.57, 95% CI 0.33-1.0) was identified[342]. Among anatomic subgroups, transposition of the great arteries had the greatest reduction in risk (OR=0.36, 95% CI 0.15-0.89)[342]. A similar approach was taken in California with a population-based case-control study which found a 30% lower risk of conotruncal defects in mothers who used folate containing multivitamins (OR=0.7, 95% CI 0.46-1.1)[343]. Among anatomic subgroups, tetralogy of Fallot had the greatest risk reduction (OR=0.54, 95% CI 0.30-0.98)[343]. Another study performed using the Baltimore-Washington Infant Study population, which examined the relationship between maternal folate consumption prior to pregnancy and outflow tract defects, did not find a protective effect of folic acid[344].

As these studies were focused on specific types of CHDs, additional studies were performed to determine if a similar finding existed with other types of CHDs. An extension of the study using the data from the Atlanta Birth Defects Case-Control Study (N=1049) identified a 24% risk reduction for CHDs with periconceptional multivitamin use[345]. The risk reduction was strongest for outflow tract defects (OR=0.46, 95% CI 0.24-0.86) and ventricular septal defects (OR-

* Food and Drug Administration (FDA) Categories of Risk for Birth Defects defined in Table 2-7.

0.61, 95% CI 0.38-0.99)[345]. A hospital-based case-control study did not reveal a decreased risk of outflow tract or ventricular septal defects with multivitamin use[346].

Table 2-7: Food and Drug Administration Categories of Risk for Birth Defects

Category	*Description of Risk*
A	No fetal risk shown in controlled human studies
B	No human data available. Animal studies show no fetal risk or animal show a risk but not a fetal risk
C	No controlled studies on fetal risk available (benefit of drug use must clearly justify potential fetal risk).
D	Studies show fetal risk in human beings (use of drug may be acceptable even with risks).
X	Risk to fetus clearly outweighs any benefit from use of drug.

The timing of multivitamin initiation was also found to be critical. Reduction in risk was present when the multivitamin supplementation was used around the time of conception or early in the first month of pregnancy, but not when use started during the second or third months of pregnancy[345]. This finding implies that the underlying mechanism of folate is most effective during the periconceptional period, a critical period for cardiac development.

In addition to testing the association between multivitamin use and CHDs, other studies have focused on folate antagonists, such as trimethoprim, triamterene, carbamazepine, phenytoin, phenobarbital, and primidone. Use of sulfasalazine (FDA category B) or other dihydrofolate reductase inhibitors was associated with an increased risk of having an infant with a CHD (RR=3.4; 95% CI 1.8-6.4)[347]. The risk following use of a dihydrofolate reductase inhibitor was similarly increased for each type of defect (i.e., conotruncal, ventricular septal defect, other CHD)[347]. A similar finding was replicated in a large population-

based case-control study in Hungary[348]. It was determined that infants of pregnant women who used trimethoprim-sulfonamide (FDA category C) medications during the second and third months of pregnancy were at increased risk of CHDs (OR=2.1, 95% CI 1.4-3.3)[348]. Collectively examining the folate studies reveals that the results are mixed and therefore, the findings are inconclusive.

Retinoid Medications

Maternal ingestion of isotretinoin (FDA category X), typically used to treat acne, has been demonstrated to lead to congenital malformations, including CHDs. Features of isotretinoin embryopathy include central nervous system malformations, cleft palate, eye/ear abnormalities, and cardiac defects, specifically conotruncal defects, ventricular septal defects, and pulmonary stenosis[349]. The risk does not appear to be increased if the medication is discontinued prior to conception[349].

Thalidomide

Thalidomide (FDA category X) was originally marketed as an analgesic and antiemetic drug, in the 1950s. As safety studies during pregnancy were typically not performed, there was no knowledge of the drug crossing the placenta and entering fetal circulation. Numerous pregnant mothers used this medication for relief of morning sickness leading to many infants born with birth defects. The medication was subsequently removed from the market; however, there has been a renewed interest in its use. Thalidomide is currently being used for treatment of multiple myeloma and other cancers. It is a known cardiac teratogen and is contraindicated during pregnancy. Cardiovascular malformations associated with thalidomide include atrial and ventricular septal defects and complex conotruncal defects[350]. No safe dose of thalidomide has been established during the critical period of gestation. Cases of thalidomide

embryopathy have been described following maternal ingestion of as little as one 50 mg capsule during this time period[262].

Lithium

An association between maternal treatment with lithium (FDA category D) during pregnancy and the occurrence of Ebstein's anomaly, a CHD in which an abnormally formed tricuspid valve is positioned lower in the right ventricle than normal, has been observed[351-355]. In a voluntary reporting registry, CHDs were observed in 18 of 225 (8%) infants born to mothers who had taken lithium during the first trimester of pregnancy[262]. Ebstein's anomaly accounted for one third of the CHDs[262]. Contradicting these reports, a prospective multicenter study did not find an association between lithium and CHDs, suggesting that lithium is not a cardiac teratogen[356].

Anticonvulsant Medications

As mentioned above, it has been difficult to determine whether the cardiovascular malformations seen in infants born to mothers with epilepsy are due to the disease or the anticonvulsant therapy (FDA category D). The results from these studies are also difficult to interpret as the effects of the anticonvulsant may be confounded by multiple factors[357, 358]. Women with epilepsy are treated with multiple therapies, either serially or simultaneously, and most are treated with an anticonvulsant medication, leaving no control group for reference.

Antiviral/Antiretroviral Medications

A Medicaid record linkage study observed an association between congenital anomalies and maternal use of zidovudine (FDA category C)[359]. An elevated risk of CHD, specifically septation defects, was noted (OR=2.24, 95% CI 1.19-4.21)[359]. When separated by trimester, however, the risk elevation remained significant during the second trimester, outside of the critical period for

organogenesis, suggesting that maternal use of zidovudine is not associated with development of CHDs[359].

Antifungal Medications

Two studies have reported no increase in the frequency of congenital anomalies, including CHDs, in infants born to mothers who ingested a single oral dose of fluconazole during pregnancy (FDA category C)[360, 361]. However, there are multiple case reports of mothers who were treated with high-dose fluconazole during most of the first trimester for fungal meningitis and bore infants with an unusual pattern of malformations, including CHD[362-364].

Two meta-analyses have described no increased risk between maternal metronidazole use and congenital anomalies[365, 366]. One of the studies which was included in these meta-analyses specifically examined a large group of infants with CHDs (N=984)[367]. A separate investigation utilizing data from the Baltimore-Washington Infant Study also evaluated the relationship between the use of metronidazole during pregnancy and CHDs. An association was observed with CHDs, specifically outflow tract anomalies with normally related great vessels (OR=6.0, 95% CI 1.8-20.7) and membranous ventricular septal defects (OR=12.2, 95% CI 3.0-50.2)[274].

A large case-control study (N=11,821) was recently conducted using the National Birth Defects Prevention Study database to investigate the association between maternal use of antifungal medications during the first trimester of pregnancy and birth defects[368]. An elevated risk for hypoplastic left heart syndrome was observed in infants whose mothers used any antifungal medication during the first trimester of pregnancy (OR=2.30, 95% CI 1.04-5.06)[368].

Antibiotics

A case-control study performed in Massachusetts (N=1644) reported an association between maternal ampicillin (FDA category B) treatment and CHDs (OR=3.3, 95% CI 1.3-8.1), specifically TGA (OR=7.7, 95% CI 1.3-38.0)[369]. Subsequent studies have not observed a similar association between maternal ampicillin use and CHD[370-372]. Multiple large studies have not observed an association between maternal treatment with penicillin and congenital anomalies, including CHDs[373-375].

Tetracycline, an antibiotic commonly used to treat acne, is known to lead to deciduous tooth staining in the infant if ingested by the mother during pregnancy. A case-control study performed in Massachusetts observed an association between maternal tetracycline use and CHDs (OR=3.3, 95% CI 1.4-7.6), specifically aortic valve stenosis (OR=14.0, 95% CI 2.5-63)[369]. To date, no other studies have investigated this association.

As mentioned above, maternal use of trimethoprim-sulfonamide (FDA category C) during the second or third month of pregnancy was found to be associated with CHDs (OR=4.8, 95% CI 1.5-16.1)[347]. Similar findings were reported from a case-control study conducted in Hungary (OR=2.1, 95% CI 1.4-3.3)[348]. These risks were reduced if the mother concomitantly took folic acid supplementation[347, 348].

Benzodiazepines

Maternal use of diazepam (FDA category D) or other benzodiazepines during the first trimester of pregnancy was found to be associated with CHDs[369, 376]. Reanalysis of the data from these studies and a follow-up study failed to confirm a significant association[370, 372]. In addition, a case-control study examining maternal risk factors associated with ventricular septal defects conducted in Finland did not observe an association with maternal ingestion of

diazepam during the first trimester of pregnancy[377]. To date, no other studies have investigated this relationship.

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

Maternal use of NSAIDs during pregnancy has been investigated for its potential role in development of birth defects. In a large registry-based study (N=2557), a significant association between maternal use of NSAIDs and CHDs (OR=1.86, 95% CI 1.32-2.62) was observed[378]. Associations have also been reported between maternal use of ibuprofen during pregnancy and TGA (OR=2.5, 95%CI 1.2-4.9), ventricular septal defects (OR=1.9, 95% CI 1.0-3.5), and bicuspid aortic valve (OR=4.1, 95% CI 1.8-9.3)[263]. Using the National Birth Defects Prevention Study database, the relationship between maternal use of NSAIDs and ventricular septal defects was investigated; no significant association was identified[379].

Two studies have observed an association between indomethacin tocolysis and persistent patent ductus arteriosus[380-382]. There have also been case reports of persistent pulmonary hypertension and premature closure of the ductus arteriosus in infants whose mothers took other forms of NSAIDs during pregnancy, including naproxen, diclofenac, ketoprofen, indomethacin, and sulindac[383-393].

Hormonal Medications

Associations between oral contraceptive use during pregnancy and CHDs have been suggested since the pill was first marketed in the 1960s[394]. Multiple studies have been conducted in an attempt to elucidate the role of oral contraceptives in the development of CHDs. A potential association between oral contraceptive use and CHDs was identified in a large case-control study involving over 50,000 pregnancies (OR=2.3, 95% CI 1.4-3.7)[394, 395]. Following restriction of exposure to the first trimester for affected infants and a random

review to confirm exposure classification of unaffected infants, there was no evidence for increased risk in a reanalysis investigation[396]. With the conflicting information, the authors of the original study re-examined and reanalyzed their entire dataset demonstrating an increased risk than was previously reported (OR=2.5, 95% CI 1.1-4.9); this difference was due to disease and exposure misclassification[397]. In a separate case-control study using data from the Baltimore-Washington Infant Study (N=110), oral contraceptive use during pregnancy was not found to be associated with CHDs[398]. A meta-analysis of prospective studies has also failed to detect an association[399].

Clomiphene was first introduced in 1967 for the treatment of infertility. Given the possible association between oral contraceptives and CHDs, clomiphene was investigated soon after its introduction. The Baltimore-Washington Infant Study observed an association between maternal use of clomiphene and coarctation of the aorta (OR=4.5, 95% CI 1.0-19.9) and tetralogy of Fallot (OR=3.2, 95% CI 1.6-6.3)[274]. In a study examining infants with conotruncal defects, no association between clomiphene use and conotruncal defects was identified [291].

Antihypertensive Medications

A cohort study with 29,507 infants utilizing vital records and hospitalization claims from a database of Medicaid patients was designed to evaluate the risk of congenital malformations following maternal treatment with angiotensin-converting enzyme inhibitors for maternal hypertension[400]. An increased risk of CHDs was identified in infants of mothers exposed during the first trimester of pregnancy (OR=3.72, 95% CI 1.89-7.30)[400]. No significant association was identified between use of other antihypertensive medications and CHDs, suggesting that the malformation may be attributed to the angiotensin-converting enzyme inhibitor medication as opposed to maternal hypertension[400]. In contrast,

a study from the Swedish Medical Birth Registry identified an association between maternal use of antihypertensive medications and CHDs that was not specific for angiotensin-converting enzyme inhibitors (OR=2.59, 95% CI 1.92-3.51)[401]. A large case-control study using data from the National Birth Defects Prevention Study, confirmed an increased risk of CHDs in infants whose mothers used antihypertensive medications during the first trimester of pregnancy (OR=1.8, 95% CI 1.1-2.7)[402]. Increased risks were observed for all classes of antihypertensive medications, except for calcium channel blockers. A significant association, however, was only seen with β-blockers[402]. Risk elevation was identified for specific defects, including pulmonary valve stenosis (OR=2.6, 95% CI 1.3-5.4), Ebstein's anomaly (OR=11.4, 95% CI 2.8-34.1), coarctation of the aorta (OR=3.0, 95% CI 1.3-6.6), and secundum atrial septal defects (OR=2.4, 95% CI 1.3-4.4)[402]. Women with untreated hypertension were also found to have an increased risk for Ebstein's anomaly (OR=2.1, 95% CI 1.0-4.3) and secundum atrial septal defects (OR=1.3, 95% CI 1.0-1.6), suggesting that the underlying hypertension may play a role in these malformations[402].

Aspirin

A case-control study conducted in Massachusetts reported an association between maternal aspirin use during the first trimester and CHDs (OR=1.3, 95% CI 1.0-1.7)[369]. When this association was further investigated after stratifying by specific defect, an elevated risk continued to be observed between maternal aspirin use and TGA (OR=3.3, 95% CI 1.7-6.6)[369]. Additionally, another case-control study identified an increased risk of truncus arteriosus in infants whose mothers used aspirin during the first trimester of pregnancy (OR=2.1, 90% CI 1.1-3.9)[370]. However, a larger case-control (N=1381) study was conducted which refuted these findings[403]. The larger study also failed to demonstrate a dose-effect pattern[403].

Non-therapeutic Drugs (Maternal)

Caffeine

Caffeine is known to cross the placenta, and concern that maternal ingestion of caffeine may lead to birth defects prompted the FDA to caution pregnant women to limit their caffeine intake[262]. A large case-control study (N=2030) evaluated risk for cardiac malformation associated with caffeine ingestion from consumption of coffee, tea, and soda[404]. No risk was identified for any of the three types of caffeine-containing products[404]. In a population-based cohort study (N=850) of mothers who drank greater than eight cups of coffee a day, the frequency of CHDs was not increased over expected[405]. Another cohort study (N=595) of mothers who drank greater than four cups of coffee daily, again demonstrated that the frequency of CHDs was not increased from expected[406]. Using data from the National Births Defects Prevention Study, the consumption of coffee, tea, soda, and chocolate was examined for an association with CHDs[407]. No evidence for a teratogenic effect of caffeine was identified[407]. Multiple other studies have failed to identify an association between caffeine consumption and CHD risk[274, 408].

Alcohol

Following the initial description of fetal alcohol syndrome in 1973, several studies have documented the wide range of teratogenic effects of alcohol consumption during pregnancy on birth outcomes, including CHDs[409]. There are several possible mechanisms for an association between maternal alcohol consumption and abnormal development of the fetal heart. Alcohol may impact heart development through its contribution to impaired conversion of retinol to retinoic acid, antagonism of the NMDA receptor, compromised nutritional status, or vascular disruptive events[410-412].

Multiple studies have failed to identify an increased risk of CHDs and

maternal alcohol consumption during pregnancy[291, 413]. A case-control study conducted in Finland limited to infants with ventricular septal defects did not find a significant association with maternal alcohol use[377]. A case-control study that examined the risk of congenital malformations with different doses of alcohol consumption in Spain identified an increased risk of CHDs only with the highest level of maternal consumption of alcohol per day, categorized as >92 g/day (OR=11.93, 95% CI 1.62-246.00)[414].

Other studies have specifically examined the risk of conotruncal malformations in relation to maternal alcohol consumption. A case-control study conducted in Finland did not observe a significant association between maternal alcohol consumption and conotruncal defects[408]. A population-based case-control study estimated that relative to non-consumers, women who consumed alcohol less than once a week had a 1.3-fold increased risk of an infant with a conotruncal defect (95% CI 1.0-1.9) and women who consumed alcohol once a week or more had a 1.9-fold increased risk (95% CI 1.0-3.4)[415]. An extension of the previous study using the same population-based registry continued to observe similar associations[416]. The study demonstrated an increased risk of conotruncal defects in infants whose mothers consumed alcohol less than once a week (OR=1.5, 95% CI 1.0-2.2); specifically TGA defects (OR=1.9, 95% CI 1.1-3.2)[416].

In a study of maternal self-report of alcohol use during pregnancy, an elevated risk of ventricular septal defects was identified among mothers who reported heavy drinking (≥10 drinks per week) using the Atlanta Birth Defects Case-Control Study (OR=3.13, 95% CI 1.19-8.22)[417]. A case-control study using the Finnish Register of Congenital Malformations, observed an increased risk of atrial septal defects in infants whose mothers consumed alcohol during the first trimester of pregnancy (OR=1.9, 95% CI 1.0-3.4)[418].

Illicit Drugs

The pregnancy outcomes of maternal use of illicit drugs have been the subject of multiple studies. A case report suggested that maternal cocaine ingestion may result in single ventricular defects (e.g., HLHS, tricuspid atresia) by inducing coronary occlusion in the developing fetal heart[419]. Using the Atlanta Birth Defects Case-Control Study data, the role of maternal cocaine ingestion in the induction of single ventricles was investigated[420]. None of the 27 case infants were exposed to cocaine during early pregnancy[420]. In a study of 214 infants with positive toxicology screens for cocaine, there was an increased frequency of CHDs[421]. Peripheral pulmonary stenosis was the most frequent defect[421]. A meta-analysis did not reveal a significant association between CHDs and maternal cocaine use during pregnancy[422]. In a study using data from the National Birth Defects Prevention Study, no significant associations were identified between maternal cocaine use and CHDs[423]. However, small case-control studies have observed associations of maternal cocaine use with an increased risk of heterotaxy (OR=3.7, 95% CI 1.3-10.7) and ventricular septal defects (OR=2.4, 95% CI 1.3-4.4)[274, 424].

Maternal marijuana use has also been investigated for an association with CHDs. An elevated risk of ventricular septal defects in relation to maternal self-report of marijuana use was observed using the Atlanta Birth Defects Case-Control Study data (OR=2.35, 95% CI 1.43-3.86)[417]. The risk remained elevated when separated by frequency of marijuana use (≤2 times per week, ≥3 times per week)[417]. In a study using The National Birth Defects Prevention Study data, no significant association with CHDs and maternal marijuana use during pregnancy was identified[423].

Cigarette Smoking

Cigarette smoking is another exposure investigated for a possible role in CHDs. A meta-analysis of studies published between 1971 and 1999 did not find a significant association between maternal smoking and CHDs overall, but did observe significant associations between maternal smoking and truncus arteriosus (OR=1.23, 95% CI 1.02-1.49), atrial septal defects (OR=1.63, 95% CI 1.04-2.57), and patent ductus arteriosus (OR=1.3, 95% CI 1.05-1.62)[425]. In a study using the California Birth Defects Monitoring Program data, an increased risk of CHDs overall was observed in infants with Down syndrome whose mothers smoked during pregnancy (OR=2.1, 95% CI 1.2-3.5)[426]. In addition, increased risks were observed between maternal smoking and Down syndrome infants with tetralogy of Fallot (OR=4.6, 95% CI 1.2-17), atrial septal defects (OR=2.2, 95% CI 1.1-4.3) and AVSDs (OR=2.3, 95% CI 1.2-4.5)[426]. An increased risk of atrial septal defects was also seen in a Swedish study evaluating risk of CHDs in infants of mothers who smoked cigarettes during pregnancy[425]. A retrospective cohort study of 18,016 infants also observed a significant association between maternal smoking and CHDs (OR=1.56, 95% CI 1.12-1.82)[427]. Using data from the National Birth Defects Prevention Study an increased risk of septation defects in infants whose mothers smoked during the periconceptional period was reported (OR=1.44, 95% CI 1.18-1.76)[428]. This risk increased with the number of cigarettes smoked[428]. An association was also seen between mothers who were described as heavy smokers (≥25 cigarettes a day) and infants with pulmonary valve stenosis[428].

Vitamin A

Vitamin A is essential for life occurring naturally as retinol, a preformed vitamin A compound, and as provitamin A carotenoids. Carotenoids are generally considered to be safe, but as mentioned above retinol metabolites are

teratogenic in both animals and humans[429]. Animal models that have been exposed to retinol and retinoic acid have demonstrated outflow tract defects, suggesting a specific outcome of this exposure[430-432]. The Baltimore-Washington Infant Study revealed a nine-fold increased risk for TGA in infants whose mothers had an increased intake of supplemental vitamin A (OR=9.2, 95% CI 4.0-21.2)[429]. A cohort study also confirmed these findings (RR=4.8, 95% CI 2.2-10.5)[433]. Multiple other studies, however, have not identified an increased risk of conotruncal defects[434-436].

Spermicide

Spermicides and male condoms are two of the most common reversible methods of contraception used by women of childbearing age in the United States[437]. Women may become pregnant while using contraceptives as a result of incorrect use, inconsistent use, or contraceptive failure. In a case-control study of 4665 infants, no association was observed between spermicide use and CHDs[438]. Additional case-control studies failed to identify a significant association between spermicide and CHDs[439, 440]. The National Birth Defects Prevention Study database was utilized to examine the potential role of spermicide and male condoms on CHDs[437]. Maternal spermicide use, including use of diaphragms, was associated with a significant increase in the occurrence of ventricular septal defects (OR=2.4, 95% CI 1.25-4.62)[437]. No significant associations were noted between male condom use and CHDs.

Occupational and/or Environmental Exposures

Organic Solvents

Organic solvents are carbon-containing compounds that are used routinely in commercial industries. In the Baltimore-Washington Infant Study, the role of organic solvents was investigated with respect to CHDs. In individual

diagnostic group analyses, associations between maternal exposure to solvents during the first trimester of pregnancy was observed for hypoplastic left heart syndrome (OR=3.4, 95% CI 1.6-6.9), coarctation of the aorta (OR=3.2, 95% CI 1.3-7.9), pulmonary valve stenosis (OR=5.0, 95% CI 1.3-8.7), and TGA (OR=3.4, 95% CI 1.5-7.5)[274]. A case-control study performed in Finland also observed an increased risk of ventricular septal defects in infants whose mothers were exposed to organic solvents during the first trimester of pregnancy (OR=1.5, 95% CI 1.0-3.7)[441].

Maternal exposure to paint and varnishes was also observed to be associated with specific defects, including Ebstein's anomaly (OR=3.6, 95% CI 1.4-9.3)[274]. In a case-control study using the Finnish Register of Congenital Malformations database, maternal exposure to dyes, lacquers, or paints was associated with conotruncal defects (OR=2.9, 95% CI 1.2-7.5)[408, 418]. A case-control study conducted in California, observed a similar association between conotruncal defects and maternal exposure to organic dyes (OR=5.0, 95% CI 1.3-16.7)[442]. Significant associations were also observed between maternal exposure to lead and other metals and total anomalous pulmonary venous return (OR=6.8, 95% CI 1.5-31.5), outflow tract defects (OR=3.5, 95% CI 1.1-12.9), and ventricular septal defects (OR=4.7, 95% CI 1.2-19.3)[274]. Maternal exposure to mineral oil was also observed to be significantly associated with coarctation of the aorta (OR=5.9, 95% CI 1.8-19.2)[443].

Water Contamination

Multiple studies examined the relationship between maternal exposure to contaminated water and CHDs. Trichloroethylene is a hydrocarbon solvent used as a metal degreasing agent and an intermediate product in the production of polyvinyl chloride[444]. It also has uses as an anesthetic, antiseptic, and a solvent for dry cleaning and coffee decaffeination. Trichloroethylene is volatile, and

therefore, a majority of the chemical released into the environment will evaporate. However, in some groundwater environments, it can persist for years causing contamination of water supplies. Animal studies of trichloroethylene have demonstrated conflicting results. In small mammals, trichloroethylene inhalation has not resulted in teratogenesis; however, studies of chick embryos have demonstrated significant cardiac teratogenesis, particularly if the trichloroethylene exposure was early in gestation[445, 446]. Studies conducted in Arizona, New Jersey, Massachusetts, North Carolina, and Wisconsin did not identify an association between maternal exposure to contaminated water and CHDs[444, 447-450]. Numerous studies were conducted in Santa Clara, California following contamination of the groundwater with trichloroethylene by an electronics manufacturing plant. Using a case-control study design, a three-fold increased risk for congenital malformations was noted in infants whose mothers were exposed to the contaminated water during pregnancy (OR=3.1, 95% CI 1.1-10.4)[451]. In a case-control study which reviewed medical records of infants whose maternal residence was in a contaminated versus a non-contaminated area, a significant association between exposure to contaminated water and CHDs was observed (OR=2.2, 95% CI 1.2-4.0)[452]. Another case-control study (N=145) using the California Birth Defects Monitoring Program database demonstrated an increased risk for CHDs in infants whose mothers consumed tap water as opposed to bottled water following water contamination (OR=2.0, 95% CI 1.0-4.0)[453]. However, two large reviews have been performed which have reported no association between maternal exposure to trichloroethylene-contaminated water and CHDs[444, 454].

Herbicides, Pesticides, and Rodenticides

In a study using the Metropolitan Atlanta Congenital Defects Program, an increased risk of conotruncal defects was observed in infants whose mothers

were employed in the agricultural industry (OR=16.1, 95% CI 3.1-85.5), suggesting that exposure to herbicides and pesticides may contribute to the formation of CHDs[291]. The Baltimore-Washington Infant Study identified associations between maternal pesticide exposure and ventricular septal defects (OR=1.3, 95% CI 1.0-1.5) and total anomalous pulmonary venous return (OR=9.0, 95% CI 2.0-41.1)[274]. Further analysis also demonstrated an association between TGA and herbicide (OR=2.8, 95% CI 1.2-6.9) and rodenticide (OR=4.7, 95% CI 1.5-14.2) exposure[264]. A case-control study using data from the California Birth Defects Monitoring Program identified an increased risk of conotruncal defects in infants whose mothers had been exposed to insecticides during the first trimester of pregnancy (OR=2.2, 95% CI 1.3-3.9)[455].

Air Quality

Observational studies have reported associations between maternal exposure to environmental pollution and congenital malformations[456]. Higher risks have been reported among mothers residing within close proximity to municipal solid waste incinerators, landfill sites, and hazardous waste sites[457-462]. Five studies have focused on investigating a possible association between ambient air pollution and CHDs. The first was conducted in Southern California using data from the California Birth Defects Monitoring Program where high levels of ambient carbon monoxide (CO) during the second month of gestation was associated with an increased risk of ventricular septal defects (OR=2.95, 95% CI 1.44-6.05)[463]. A similar case–control study in Texas examined exposures during weeks 3–8 of gestation and reported associations between increased levels of ambient CO and conotruncal defects (OR=1.46, 95% CI 1.03-2.08) and tetralogy of Fallot (OR=2.04, 95% CI 1.26-3.29)[464]. Other findings from this study include associations between maternal exposure to increased particulate matter and non-syndromic atrial septal defects (OR=2.27, 95% CI 1.43-3.60), and

maternal exposure to increased levels of sulfur dioxide and non-syndromic ventricular septal defects (OR=2.16, 95% CI 1.51-3.09)[464]. Using the Metropolitan Atlanta Congenital Defects Program database, a study examined exposures during weeks three through seven of gestation and the risks of CHDs[465]. A significant association between maternal exposure to increased particulate matter and patent ductus arteriosus was observed (OR=1.60, 95% CI 1.11-2.31)[465]. Another recent study evaluated ambient air quality and risks of CHDs in Brisbane, Australia[466]. Findings from this study include an increased risk of pulmonary valve defects in relation to maternal exposure of increased levels of ozone (OR=2.96, 95% CI 1.34-7.52)[466]. The study also observed an increased risk of aortic valve defects in relation to maternal exposure to increased levels of sulfur dioxide (OR=10.76, 95% CI 1.50-179.80)[466].

Sociodemographic Characteristics (Maternal)

Maternal Age

In the Baltimore-Washington Infant Study, maternal age was not associated with non-syndromic CHDs overall[274]. However, when the analysis was stratified by defect, maternal age greater than 30 years was found to be associated with an increased risk of TGA (OR=1.7, 95% CI 1.1-2.7) and Ebstein's anomaly (OR=2.6, 95% CI 1.4-4.8)[274]. Maternal age greater than 34 years was associated with an increased risk of atrial septal defects (OR=1.6, 95% CI 1.0-2.5) and bicuspid aortic valves (OR=2.5, 95% CI 1.3-4.8), while maternal age less than 20 years was associated with an elevated risk of tricuspid atresia (OR=2.8, 95% CI 1.3-6.4)[274]. In a study using data from the Metropolitan Atlanta Congenital Defects Program, advanced maternal age, defined as ages 35-40 years, was associated with all CHDs (OR=1.12, 95% CI 1.03-1.22)[467]. When stratified by defect, a significant association was observed for advanced

maternal age and risk of right ventricular outflow tract defects (OR=1.28, 95% CI 1.10-1.49) and tricuspid atresia (OR=1.24, 95% CI 1.02-1.50)[467].

Maternal Socioeconomic Status/Education

In a study using the Danish National Birth Cohort database, an association was reported between maternal and paternal low socioeconomic status and CHDs (OR=1.6, 95% CI 1.3-2.0)[468]. A large case-control study (N=344,214) using data from the California Birth Defects Monitoring Program, examined maternal socioeconomic status and education level for their association with CHDs[469]. No significant associations were identified, although there appeared to be an increased risk for conotruncal defects in infants whose mothers were of a lower socioeconomic status[469]. The possible association between maternal socioeconomic status and conotruncal defects was also investigated using the National Birth Defects Prevention Study database[470]. Again, increased risks were identified for conotruncal defects, specifically TGA, in relation to low maternal education[470].

Maternal Stress

There have been studies examining the relationship between maternal stress and birth defects. One mechanism by which maternal stressors may cause birth defects is through increased production of corticosteroids[471]. Corticosteroids are teratogenic for various organ systems in animal models[472, 473]. Stressful life events have been shown to be associated with elevated maternal corticotrophin-releasing hormone and corticosteroid levels during pregnancy[474]. Another potential mechanism by which stress may cause birth defects is negative coping behaviors which lead to deleterious exposures, such as cigarette or alcohol use or poor nutritional intake.

A case-control study conducted using the Metropolitan Atlanta Congenital Defects Program database, observed an increased risk of conotruncal defects in

infants whose mothers described stress related to job loss, divorce, separation or death of a close friend or relative (OR=2.4, 95% CI 1.4-4.2)[291]. Using the California Birth Defects Monitoring Program database, a similar association between maternal stress and conotruncal defects was described (OR=1.4, 95% CI 1.0-2.1), with a larger effect seen in mothers who had not completed high school (OR=2.4, 95% CI 1.3-4.8)[475]. An extension of the previous study was performed with an in-depth maternal stress interview and resulted in a similar association between maternal stress and conotruncal defects[471].

Non-therapeutic Drugs (Paternal)

Alcohol

Infants of fathers who self-reported moderate drinking, defined as 5-9 drinks per week, were found to have an increased risk of ventricular septal defects (OR=3.98, 95% CI 1.60-9.91)[417].

Illicit Drugs

Paternal cocaine and marijuana use was also investigated for an association with CHDs. The Baltimore-Washington Infant Study reported an assocation of paternal cocaine use and ventricular septal defects (OR=1.9, 95% CI 1.3-2.9), atrial septal defects (OR=2.3, 95% CI 1.3-4.2), and tricuspid atresia (OR=4.8, 95% CI 1.6-14.0)[274]. A study focused on non-syndromic ventricular septal defects from the Balitmore-Washington Infant Study, identified an association between the defect and paternal marijuana use (OR=1.36, 95% CI 1.05-1.76)[476]. A significant association was also reported between ventricular septal defects and cocaine use among older fathers (OR=3.92, 95% CI 1.30-11.86)[476]. An elevated risk of ventricular septal defects was observed in infants whose fathers self-reported marijuana use during the periconceptional period in a study using the Atlanta Birth Defects Case-Control Study database (OR=2.21,

95% CI 1.11-4.38)[417].

Cigarette Smoking

The Baltimore-Washington Infant Study observed an association between heavy paternal smoking (>20 cigarettes per day) and atrial septal defects (OR=1.7, 95% CI 1.1-2.7)[274]. A case-control study in California observed an increased risk of conotruncal defects in infants whose mothers and fathers smoked (OR=1.9, 95% CI 1.2-3.1); specifically TGA (OR=2.5, 95% CI 1.3-4.8)[477]. The association did not remain significant if only one parent smoked[477]. An association between fathers who were heavy smokers (>20 cigarettes per day) and laterality defects was also observed in the Baltimore-Washington Infant Study (OR=5.6, 95% CI 2.5-12.9)[274].

Sociodemographic Characteristics (Paternal)

Paternal Age

Some conditions, such as Marfan syndrome, are the result of new dominant mutations. New dominant mutations are more common in older fathers[478]. The occurence of Marfan syndrome due to new mutations has been shown to be greater in older fathers[479].

As increasing paternal age has been demonstrated to be associated with autosomal dominant conditions, its role in the occurrence of CHDs was investigated. In a study conducted using data from the Metropolitan Atlanta Congenital Defects Program, an association between increasing paternal age and atrial septal defects (OR=1.95) and ventricular septal defects (OR=1.69) was observed after controlling for maternal age and race[478]. An increased risk of pulmonary valve stenosis was also observed with increasing paternal age in study conducted among 14,685 members of the Kaiser Foundation Health Plan[480]. This study, however, did not demonstrate an association between

increased paternal age and ventricular septal defects, atrial septal defects, or patent ductus arteriosus[480]. A population-based retrospective cohort study conducted using the US national linked birth/infant mortality database provided by the National Center for Health Statistics identified an association between advanced paternal age and ventricular septal defects (OR=1.23, 95% CI 1.11-1.36)[481]. A study limited to non-syndromic ventricular septal defects from the Baltimore-Washington Infant Study did not identify an association with increasing paternal age[476].

In contrast, a study conducted in China did not identify a relationship between advancing paternal age and CHDs[482]. Of interest, an increased risk of an infant with a CHD was noted for fathers younger than 25 years (OR=2.27, 95% CI 1.85-2.79)[482]. The risks remained elevated for specific defects, including ventricular septal defect, patent ductus arteriosus, and tetralogy of Fallot[482]. A case-control study using data from the British Columbia Health Surveillence Registry identified an association between younger paternal age (less than 20 years) and ventricular septal defects (OR=2.0, 95% CI 1.1-3.6)[483]. Atrial septal defects, tetralogy of Fallot, coarctation of the aorta, and pulmonary valve abnormalities also showed an increased risk, but their confidence intervals included the null value 1.0[483]. A hypothesis for the observation of risk elevation with younger paternal age is the occurrence of new dominant mutations due to environmental or liftstyle risk factors[483].

Paternal Socioeconomic Status/Education

Using the National Birth Defects Prevention Study database, the role of paternal socioeconomic status was investigated in relation to conotruncal defects[470]. An elevated risk of conotruncal defects was observed in infants whose fathers had a low level of education, defined as completion of high school or less[470]. An increased risk for TGA was also observed for fathers who were

unemployed during the periconceptional period[470]. While the observed risks were elevated, they were not significant, possibly due to small sample sizes when stratified by education or employment status.

Non-Genetic Risk Factors for Atrioventricular Septal Defects

While numerous risk factors have been examined for their relationship with CHDs, very few risk factors have shown a significant association with AVSDs. Table 2-8 provides a summary of risk factors and exposures associated with AVSDs.

Table 2-8: Risk Factors and Exposures Associated With Atrioventricular Septal Defects

Condition	*Risk Factor/Exposure*
Maternal Illness	Diabetes Urinary tract infections
Medications	Non-steroidal anti-inflammatory drugs (Ibuprofen) Antitussive medications Antibiotic medications
Non-therapeutic Drugs	Cigarette smoking (maternal) Cocaine
Occupational	Paint/Varnishes (maternal) Frequent fireplace use Welding (paternal) Ionizing radiation (paternal)

As mentioned above, very few studies have actually investigated the roles of potential risk factors in the development of CHDs and more specifically, AVSDs. One of the largest investigations of risk factors of CHDs was the Baltimore-Washington Infant Study. This large case-control study, performed between 1981 and 1989, analyzed mulitple potential risk factors for their role in an association with CHDs. This study not only examined the role of potential risk

factors on CHDs overall, but also performed individual diagnostic analyses. There were 363 infants placed in the AVSD category. Of these infants, 210 were also diagnosed with Down syndrome.

Maternal Conditions

Diabetes Mellitus

A significant association was observed in the Baltimore-Washington Infant Study between maternal diabetes and infants with CHDs. Further analysis demonstrated an increased risk between maternal diabetes and complete AVSDs (OR=22.8, 95% CI 7.4-70.5)[288]. When the cases were analyzed separately based on presence or absence of Down syndrome (N=155, N=30), a significant association was also identified for non-syndromic complete AVSDs, (OR=20.6, 95% CI 5.6-76.4)[484].

Urinary Tract Infections

In a study conducted using the National Birth Defects Prevention Study database, an association was observed between mothers who had an urinary tract infection during the first trimester of pregnancy and AVSDs (OR=2.29, 95% CI 1.11-4.73)[328].

Medications

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

An association between maternal use of ibuprofen during the first trimester of pregnancy and syndromic AVSDs was reported from the Baltimore-Washington Infant Study (OR=2.49, 95% CI 1.42-4.34)[274].

Antitussive Medications

The Baltimore-Washington Infant Study evaluated maternal use of antitussive medication during the periconceptional period for its role in the

development of CHDs. When stratified by individual diagnosis, an association between maternal antitussive use and non-syndromic AVSDs was observed (OR=6.3, 95% CI 1.9-21.6)[274]. Further analysis performed using this dataset demonstrated a significant association between maternal antitussive medication use and complete non-syndromic AVSDs (OR=8.8, 95% CI 1.2-48.2)[484]. While this association was significant, the small sample size of 30 case infants, along with a wide confidence interval, should be noted. Given the public concern regarding use of antitussive medications during pregnancy, a larger case-control study (N=3616) was conducted using data from the Spanish Collaborative Study of Congenital Malformations. No significant association between maternal antitussive use and CHDs was identified[485].

Antibiotics

Using data from the National Birth Defects Prevention Study, an association between AVSDs (N=128) and maternal antibacterial medication use was reported (OR=1.7, 95% CI 1.1-2.6)[486]. The association did not remain significant when stratified by type of antibiotic medication.

Non-therapeutic Drugs (Maternal)

Cigarette Smoking

The Baltimore-Washington Infant Study examined the role of maternal cigarette use during the periconeptional period and CHDs. A significant association between heavy maternal cigarette use (mothers who smoked >20 cigarettes per day) and non-syndromic AVSDs was reported (OR=2.50, 95% CI 1.21-5.19)[274]. Using the National Birth Defects Prevention Study database, the infants of mothers who were classified as medium smokers (mothers who smoked 15- 24 cigarettes per day) were also observed to have an increased risk of AVSDs, (OR=2.18, 95% CI 1.04-4.55)[428].

Illicit Drugs

Maternal cocaine use was significantly associated with non-syndromic AVSDs in a study conducted using the Baltimore-Washington Infant Study database (OR=3.45, 95% CI 1.05-11.40)[274]. No other maternal illicit drug use was found to be significantly assciated with AVSDs.

Occupational and/or Environmental Exposures (Maternal)

Organic Solvents

Maternal exposure to paint during the first trimester of pregnancy was reported to be associated with syndromic AVSDs (OR=1.77, 95% CI 1.19-2.63)[274]. A significant association between non-syndromic AVSDs and maternal exposure to varnishes was also observed (OR=4.54, 95% CI 1.36-15.18)[274]. This association was only significant, however, if there was concomitant paternal exposure to varnishes.

One interesting environmental exposure examined by the Baltimore-Washington Infant Study was frequent fireplace use. Maternal report of frequent use of the fireplace during the periconceptual period was reported to be associated with syndromic AVSDs (OR=1.76, 95% CI 1.21-2.56)[274].

Occupational and/or Environmental Exposures (Paternal)

Occupational Exposures

A significant association between paternal exposure to welding and syndromic AVSDs was noted in the Baltimore-Washington Infant Study (OR=1.82, 95% CI 1.14-2.92)[274]. In addition, the study also identified an association between paternal occupational exposure to ionizing radiation and non-syndromic AVSDs (OR=4.54, 95% CI 1.36-15.18)[274].

Summary of Non-Genetic Risk Factor Findings

Little is known regarding risk factors for CHDs; numerous factors and exposures have been examined for their role in the development of CHDs. As is summarized in Table 2-9, significant associations between multiple risk factors and CHDs, including AVSDs, have been identified.

A majority of these identified risk factors have conflicting study results, suggesting that additional investigations need to be performed. Recall bias is of concern, as most mothers of infants with CHDs have a more detailed recollection of exposures in comparison to mothers of healthy children. As most exposures of interest are those during the periconceptional period, recall of exposures may be difficult due to intervening time.

In addition, most of the studies conducted have been with small sample sizes due to the rarity of specific types of CHDs. It is also difficult to perform precise measurements of some exposures, such as occupational and environmental exposures.

It is interesting to note that a large proportion of the risk factors were observed to be associated with a variety of CHDs, suggesting that chance associations may have been observed as opposed to true associations. Mechanisms for these associations are difficult to define, as multiple categories of defects were found to be associated with a specific risk factor, further implying that these associations were by chance.

Table 2-9: Non-Genetic Risk Factors Associated with Specific CHDs

Defect	*Risk Factor/Exposure*
Aortic valve abnormalities	Air pollution Tetracycline
Atrioventricular septal defects	Diabetes UTI NSAIDs (Ibuprofen) Antitussive medications Antibiotic medications Cigarette smoking (maternal) Cocaine Paint/Varnishes (maternal) Frequent fireplace use Welding (paternal) Ionizing radiation (paternal)
Bicuspid aortic valve	NSAIDs
Cardiomyopathy	Diabetes mellitus
Coarctation of the aorta	Febrile illness Clomiphene β-blockers Organic solvents (mineral oil)
Conotruncal defects	Diabetes mellitus Obesity Isotretinoin Thalidomide Agricultural exposures Air pollution Organic solvents (paint/varnish) Maternal stress
Ebstein's anomaly	Obesity Lithium β-blockers Organic solvents (paint/varnish) Maternal age
HLHS	Diabetes mellitus Obesity Antifungal medications Organic solvents
Laterality/Looping Defects	Diabetes mellitus Cocaine (maternal) Cigarette smoking (paternal)
LVOTO defects	Febrile illness Thyroid disorder
Outflow tract defects	Diabetes mellitus Lead/metal

Table 2-9 Continued

Defect	*Risk Factor/Exposure*
Patent ductus arteriosus	Diabetes mellitus
	Rubella
	Indomethacin
	Cigarette smoking (maternal)
	Air pollution
PPS	Rubella
RVOTO defects	Febrile illness
	Maternal age
Tetralogy of Fallot	Clomiphene
	Cigarette smoking (maternal)
	Air pollution
Pulmonary valve abnormalities	Obesity Rubella Febrile illness Isotretinoin β-blocker Organic solvent Air pollution
Septal defects	Diabetes mellitus Obesity Rubella Febrile illness Zidovudine Isotretinoin Thalidomide Advanced age (maternal and paternal) Cigarette smoking (maternal and paternal) Cocaine (maternal and paternal) Alcohol (maternal and paternal) Marijuana (maternal and paternal) Spermicide Air pollution
TAPVR	Obesity Lead/metals Pesticide
TGA	NSAIDs Aspirin Alcohol (maternal) Vitamin A Advanced age (maternal) Organic solvents Agricultural exposures Low SES (paternal)

Table 2-9 Continued

Defect	*Risk Factor/Exposure*
Tricuspid atresia	Febrile illness Aspirin Cigarette smoking (maternal) Advanced age (maternal) Cocaine (paternal)
Truncus Arteriosus	Cigarette smoking (maternal)

Note: NSAIDs=non-steroidal anti-inflammatory drugs, HLHS=hypoplastic left heart syndrome, LVOTO=left ventricular outflow tract obstructive, PPS=peripheral pulmonic stenosis, RVOTO=right ventricular outflow tract obstructive, TAPVR=total anomalous pulmonary venous return, TGA=transposition of the great arteries, SES=socioeconomic status.

Chapter 2 Summary

Research into the genetic etiology of CHDs has determined that not only are there genes that confer susceptibility to specific heart defects, but also that individual susceptibility genes are not defect specific. In addition, potential candidate genes have been identified for AVSDs. A majority of these potential genetic associations with AVSDs have been identified through further characterization of the cardiovascular system developmental pathway. Although numerous candidate genes have been identified, continued genetic research is necessary to identify additional genetic mechanisms and specific genetic variants and interaction effects leading to AVSDs.

Risk factors for specific heart defects have been identified, but many of these associations have not been replicated, suggesting that additional investigations need to be conducted. A majority of the risk factors for CHDs, and more specifically AVSDs, were identified from one large case-control study, again pointing to the need for further investigation.

Given the complexity of CHDs, it has been theorized that the etiology is multifactorial, suggesting that there is an interaction between the genetic etiologies and parental and/or environmental exposures. The multifactorial

model is characterized by four principles. These principles include the following: (1) Several loci (although this is not an unlimited number) are involved in the expression of the trait. (2) There is no dominance or recessivity at these loci. (3) The loci act in concert in an additive fashion, each adding or detracting a small amount from the phenotype. (4) The environment interacts with the genotype to produce the final phenotype. Conceptualizing this model relative to CHDs, and more specifically AVSDs, has been easy, but putting the model into practice has proven difficult. As has been mentioned previously, many types of CHDs are rare and finding large pedigrees with affected families is difficult.

Identification of genetic etiologies of and risk factors for AVSDs may provide additional prognostic information for clinical outcomes, an improved ability for genetic counseling, and additional information for identification of other family members for whom genetic testing is appropriate.

CHAPTER 3
IS A SHORTER ATRIOVENTRICULAR SEPTAL LENGTH AN INTERMEDIATE PHENOTYPE IN THE SPECTRUM OF NON-SYNDROMIC ATRIOVENTRICULAR SEPTAL DEFECTS?

Abstract

Background

Atrioventricular septal defects (AVSDs) include a range of anomalies characterized by the involvement of the atrial and/or ventricular septa and the abnormal development of the atrioventricular (AV) valves. The atrioventricular septum (AVS) is the portion of the septal tissue that separates the right atrium from the left ventricle. Deficiency of the AVS contributes to the AVSD phenotype. Shortening of the atrioventricular septum in relatives of children with non-syndromic AVSD might reflect the threshold model of disease, where the liability for these individuals who inherit fewer AVS shortening alleles is below the threshold for a recognizable AVSD to occur.

Methods

The AVS length (AVSL) was measured in three apical four-chamber views in echocardiograms of clinically unaffected parents (N=118) from families that were identified through a child with non-syndromic AVSD (N=67), in parents (N=149) identified through a child with Down syndrome in conjunction with an AVSD (N=83), and in parents (N=109) of families with no history of congenital heart disease (N=73). Similar measurements were made in unaffected siblings (N=92) of non-syndromic AVSD cases, siblings (N=117) of syndromic AVSD cases, and siblings (N=90) of controls. Ten percent of the entire sample was re-measured by an independent investigator and a paired t-test was performed to evaluate interrater reliability. Group differences were evaluated with analysis of variance with Student Newman-Keuls post-hoc comparisons and chi-square

testing. Univariable and multivariable analyses were performed to evaluate for an association between body surface area-standardized AVSL (sAVSL) and age or gender. The distribution of the age- and gender-adjusted, body surface area-standardized AVSL (asAVSL) within each subgroup was evaluated for evidence of admixture using a likelihood ratio test. The heritability of asAVSL in non-syndromic AVSD families, syndromic AVSD families, and control families was estimated based on measurements from siblings and parents in each group.

Results

No significant differences were seen between case and control families in terms of % male, age, weight, and height. Interrater reliability of AVSL measurements was tested with a paired t-test ($p=0.57$). Age and gender were associated with sAVSL in the non-syndromic AVSD and syndromic AVSD case parent and case sibling groups; however only age was associated with sAVSL in the control parents and siblings. The sAVSL was significantly shorter in non-syndromic AVSD case parents and syndromic case parents ($p<0.0001$) when compared to control parents. There was no significant difference between the sAVSL of parents of non-syndromic AVSD cases and parents of syndromic AVSD cases, although the non-syndromic AVSD case parent group had a shorter mean sAVSL. The sAVSL was significantly shorter in non-syndromic AVSD case siblings when compared to syndromic AVSD case siblings and control siblings ($p<0.0001$). There was significant evidence for two asAVSL components in the non-syndromic AVSD case parent, non-syndromic AVSD case sibling, and control sibling groups ($p=0.0177$, $p=0.0080$, and $p=0.0109$); no evidence of multiple components was noted in the other three groups. Heritability of asAVSL in non-syndromic families was 0.44, in syndromic families was 0.43, and in control families was 0.68. The high heritability in the control

families suggests that there may be polygenic involvement in the determination of AVS length.

Conclusions

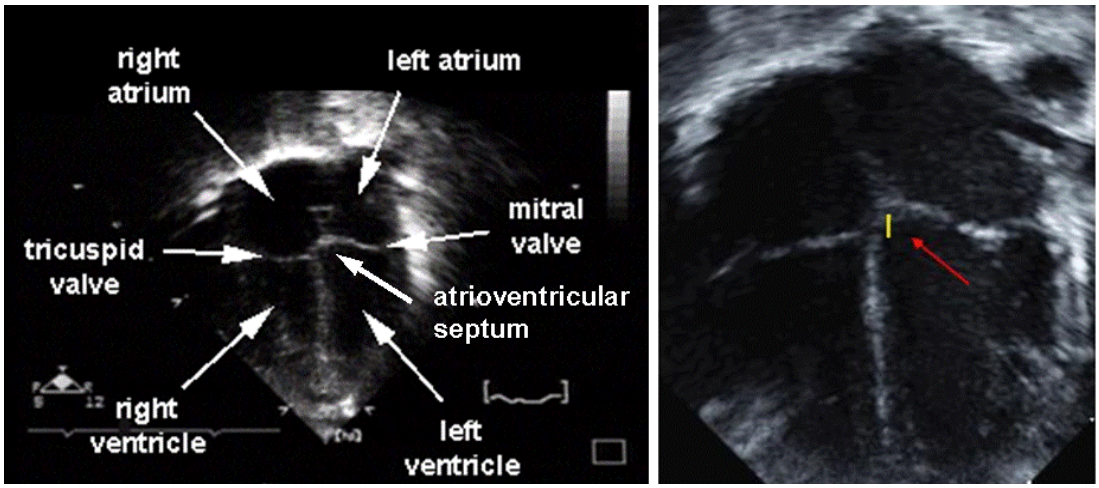
Evidence for two component distributions from the analysis of asAVSL for case parents and case siblings suggests the presence of an intermediate phenotype for non-syndromic AVSD. Broadening the definition of AVSD to include those with a shortened AVSL may increase the power of genetic association and mapping studies to identify susceptibility genes for AVSD.

Introduction

Congenital heart defects (CHDs) constitute a major proportion of clinically significant birth defects and are an important component of pediatric cardiovascular disease, with an estimated prevalence of six to nine per 1000 live births[5-7]. Atrioventricular septal defects (AVSDs), also known as atrioventricular canal defects or endocardial cushion defects, include a range of anomalies characterized by involvement of the atrial septum, the ventricular septum, and one or both of the atrioventricular (AV) valves; they account for approximately 7% of all CHDs[19].

With normal cardiac development, the septal leaflet of the tricuspid valve inserts into the septum slightly closer to the apex than the septal leaflet of the mitral valve (Figure 3-1). There is a small portion of septal tissue superior to the tricuspid septal leaflet insertion that separates the right atrium from the left ventricle; this is the atrioventricular septum (AVS) (Figure 3-1).

Figure 3-1: Echocardiographic View of Cardiac Anatomy



a. **b.**

Note: The hinge points of the tricuspid valve sit slightly lower than the hinge points of the mitral valve as seen in figure 3-1a. The red arrow in figure 3-1b points to a yellow line which depicts the measurement of the atrioventricular septum (from the hinge point of the mitral valve to the hinge point of the tricuspid valve).

AV septal length (AVSL) was measured in a single study of 41 patients with Ebstein's anomaly, 20 patients with secundum atrial septal defects, and 20 patients with severe tricuspid valve regurgitation without a congenital defect, and 20 normal controls[487]. In this investigation, the AVSL ranged from 7-50 mm in the patients with Ebstein's malformation, 0-10 mm in those with structurally normal hearts, 2-14 mm in the patients with atrial septal defects, and 2-15 mm in those with severe tricuspid valve regurgitation without a cardiac defect. Considerable overlap was noted between the AVSL distributions of the control patients (i.e., those with a normal tricuspid valve structure) and the Ebstein's anomaly patients. Once standardized by body surface area (BSA), it was noted that all of the patients with normally structured tricuspid valves had a BSA-standardized AVSL (sAVSL) less than 8 mm/m^2, while those with Ebstein's anomaly had a sAVSL greater than 8 mm/m^2, providing the basis for the displacement index which can

assist in diagnosing Ebstein's malformation[27, 28, 487]. Absence of the AVS results in the AVSD phenotype, implying that those patients with a complete AVSD have an AVS that measures 0 mm.

Details regarding the normal development of the AVS are relatively unknown. AVSDs are due to a defect in the endocardial cushion formation and fusion, suggesting a possible role of the endocardial cushions in the development of the AVS.

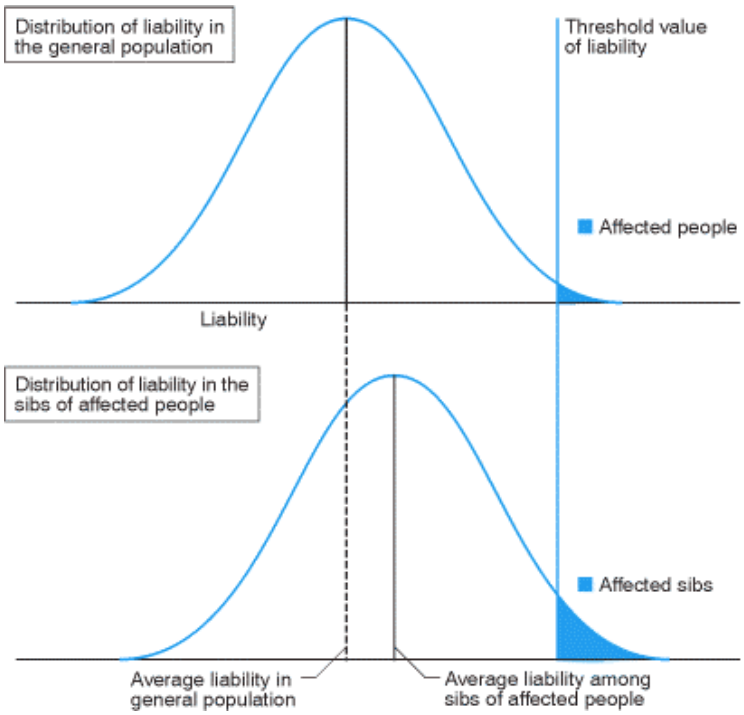
Although AVSDs commonly occur in the setting of Down syndrome, they also occur in infants without a diagnosed syndrome. Non-syndromic AVSDs are estimated to occur in approximately one per 10,000 live births[204].

Most non-syndromic AVSDs are considered to be sporadic or the result of multifactorial inheritance[205]. However, there are numerous reports of non-syndromic AVSDs transmitted within families, suggesting that the defect segregates with a Mendelian pattern[198, 205-216]. The pattern of recurrence has most often suggested an autosomal dominant model with monogenic or oligogenic inheritance[29]. Although AVSDs appear to be transmitted in an autosomal dominant fashion, there are parents in these pedigrees who do not demonstrate the phenotype of an AVSD or a defect along its spectrum, and yet they may have multiple affected offspring. These parents may have an intermediate phenotype, e.g., a shortened AVSL; however, no intermediate phenotype has yet been sought.

There are a few potential mechanisms for a shortened AVS in the parents and siblings of non-syndromic AVSD children. The most logical is demonstrated by Falconer's polygenic threshold model for discontinuous traits. Every individual is assumed to have an underlying liability (vulnerability, susceptibility, or predisposition) for the trait, which is assumed to be normally distributed and represents the sum of all the multifactorial effects, both genetic and

environmental that are relevant to the trait for that individual (Figure 3-2).

Figure 3-2: Threshold Model for Multifactorial Traits[488]



Note: The individuals whose liability is above the threshold value are affected. The relatives of affected individuals have a higher average liability than the general population mean and a greater proportion of them have liability exceeding the threshold. Therefore, the condition tends to aggregate in families.

The liability is impossible to directly measure as most of the causal components are still unknown and therefore can not be measured. The threshold model states that as the number of polygenes (i.e., any of a group of genes, each having a small quantitative effect, that together produce a wide range of phenotypic variation) and environmental exposures for a trait increases, the liability increases. When the liability reaches a threshold, a recognizable disease occurs. In this case, the phenotype of AVSD represents the disease and

an individual with a liability to the left of the threshold may have a shortened AVSL which is not currently recognized as being part of the AVSD spectrum. As the number of AVSL shortening alleles an individual inherits increases, their liability increases and at higher liability values they are likely to manifest the clinically recognizable disease of non-syndromic AVSD.

Biologic plausibility for the shortening of the AVS in parents and siblings of AVSD cases might also be reflected in the genetic principle of anticipation, which describes the tendency for some conditions to become more severe (or have an earlier onset) in successive generations. The concept of anticipation might be applicable to AVSDs in that if an intermediate phenotype exists, then with each successive generation, there may be a further shortening of the AVSL. The shortening could continue to the extent of a complete AVSD, where the AVS does not exist. This principle was examined in a large pedigree with total anomalous pulmonary venous return[489]. These individuals were examined for possible trinucleotide repeat expansion due to an increase in the number of cases in more recent generations and the apparent increased penetrance; however, no evidence of a trinucleotide repeat expansion was found.

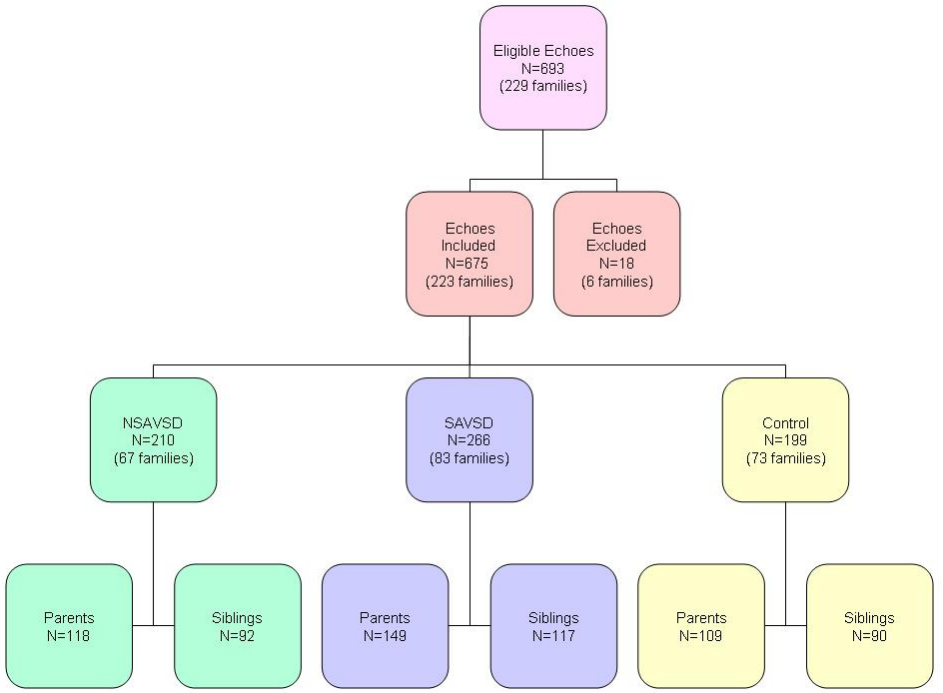
The major goal of this investigation was to measure AVSL in a case-control study in order to determine whether the parents and siblings of a child with a non-syndromic AVSD demonstrate a shorter AVSL, possibly indicating an intermediate phenotype. We hypothesized that a subset of the “unaffected” parents and siblings of case children will have shorter BSA-standardized AVSL than the remainder of the case parents and siblings whose AVSL, in turn, will not be different than the AVSL of parents and siblings in control families.

Methods

Subjects

Case families were those who participated in The Family Study of Endocardial Cushion Defects which was conducted between 1994 and 2004 at the University of Iowa (Figure 3-3).

Figure 3-3: Schematic of Study Population



Note: NSAVSD=non-syndromic atrioventricular septal defect, SAVSD=syndromic (Down syndrome) atrioventricular septal defect.

Numbers in parentheses refer to number of families.

This study evaluated children with an AVSD (both syndromic and non-syndromic) in addition to their parents and siblings. The non-syndromic and syndromic AVSD cases were identified through cardiac catheterization,

echocardiographic, and surgical records at the University of Iowa Hospitals and Clinics and recruited for the study. If the family agreed to participate, a three-generation pedigree was constructed and a health history questionnaire was administered over the phone. The families were then scheduled for echocardiographic examinations and acquisition of a blood sample. Seventy-two families of children with a non-syndromic AVSD, and 83 families of children with an AVSD and Down syndrome, were recruited and examined.

Children free of congenital heart defects and their parents and siblings from Muscatine, Iowa were also recruited to serve as control families. Echocardiograms were obtained in a similar fashion for the families who agreed to participate. Seventy-four control families were recruited and examined. Cases and controls underwent echocardiographic examination by separate sonographers.

Echocardiographic Analysis

The 693 available echocardiograms from the case and control family members were reviewed to measure AVSL in an attempt to define and describe an “intermediate phenotype” of AVSDs. Due to the timeframe of the conduct of the original study, echocardiograms were stored on VHS tapes and were not digitized. A Philips Sonos 5500 echocardiographic machine was utilized for detailed measurements.

The AVSL was measured using the caliper tool as part of the installed software package on the machine. The length of the AVS was defined as the length from the hinge point of the mitral valve to the hinge point of the tricuspid valve along the septum in the apical four-chamber view (Figure 3-1b). Three repeat measurements were made by the primary investigator (SSP) using the same frame. In order to assess reproducibility, two additional four-chamber

views were identified and three measurements of the AVSL were made in each view in a similar fashion for a total of nine measurements of the AVSL.

For the purposes of assessing interrater reliability, repeat measurements (10%) were made by an independent investigator (LTM) with substantial echocardiographic experience. Two repeat measurements of the AVSL were made in each of two views, chosen independently by LTM, for a total of four measurements.

Statistical Analysis

Standardization

Standardization or normalization of the AVSL measurements was performed to account for body size differences by utilizing the estimated BSA of each family member. As no gold standard for BSA estimation exists, both the Mosteller and the DuBois and DuBois formulas were initially utilized and compared:

$$\text{BSA } (m^2) = \sqrt{\text{height (cm)} \times \text{weight (kg)} / 3600} \quad \text{(Mosteller)}^{490}$$

$$\text{BSA } (m^2) = 0.007184 \times \text{height (cm)}^{0.725} \times \text{weight (kg)}^{0.425} \text{ (DuBois and DuBois)}^{491}.$$

The DuBois and DuBois formula for calculating body surface area was originally derived from the measurement of one leg and one arm in nine patients, one of whom was a child. Many investigators have questioned the accuracy of this formula. This formula has been shown to grossly overestimate the surface area of obese people and does not take into account the distortion observed in the thigh and trunk[492, 493]. It has also been shown that the DuBois and DuBois formula underestimates the surface area at lower surface area, with the greatest disparity in newborn infants[493]. Another study examined the accuracy of the DuBois and DuBois formula with a direct measurement using a photodermoplanimeter, an instrument that measures the area of the body

available for absorbing light, which is identical to the area available for radiating heat[494]. This study determined that the two methods were highly correlated, but the area using the formula was systematically lower than the measured area; the underestimate was greatest in small individuals[494].

The Mosteller method was derived based on 81 patients with ages ranging from premature infants to older adults, as a easy-to-remember equation that could be used with a simple calculator[490]. This formula was found to be highly correlated with the DuBois and DuBois formula as well as being applicable to children[495].

Another investigation assessed the DuBois and DuBois and the Mosteller formulas for accuracy using the root mean squared error (RMSE) method of prediction[496]. The RMSE measures concordance between measured and predicted data. The investigators found that the DuBois and DuBois formula systematically underestimates BSA by almost 6% and that the tendency to underestimate BSA is slightly greater in infants than in others, further suggesting that this formula should not be applied to infants and children[496]. It was also determined that the Mosteller formula had a lower RMSE than the DuBois and DuBois formula, suggesting greater accuracy with the Mosteller formula[496].

Reliability

Reliability of the two BSA calculation methods was assessed by construction of a scatterplot and a Bland-Altman plot in addition to performing a paired t-test of the hypothesis of no difference between the sAVSL measurements obtained using each formula.

An intraclass correlation coefficient was estimated using the mean of the three AVSL measurements from each of the three separate views to determine intrarater reliability. Intrarater reliability among the independent investigator's measurements was also assessed using the mean of the two AVSL

measurements from each of the two separate views. As two different sonographers obtained the echocardiograms for the case and control families, two separate intraclass correlation coefficients were calculated to account for sonographer differences. Interrater reliability was assessed by performing paired t-tests using the overall mean measurement (of nine and four measurements, respectively) from each investigator.

Descriptive Analysis

Using the set of measurements obtained by the primary investigator, the mean of the three AVSL measurements from each of the three separate views, i.e., the mean of nine measurements, was determined. Descriptive statistics for gender, age, weight, height, BSA, AVSL, and BSA-standardized AVSL (sAVSL) measurements were estimated for case and control parents and siblings. Means and standard deviations were estimated for continuous variables, while frequencies were determined for categorical variables. Case and control subgroups were compared for differences using analysis of variance (continuous variables) followed by the Student Newman-Keuls post hoc pairwise comparison approach and the chi-square test (categorical variables).

Univariable and Multivariable Regression Analysis

The sAVSL measurements for the group of non-syndromic AVSD case parents were examined using linear regression analysis models to determine if there was an association with age or gender. Those characteristics whose inclusion reached a liberal significance level ($p<0.2$) were retained for additional consideration in multivariable analysis. Multivariable linear regression analysis was used to model the associations with age and gender and the residuals from the model were retained (asAVSL). Each characteristic which had lost significance was removed from the model separately while determining that the reduced model did not fit the data significantly worse than the original model.

Similar analyses were performed for the non-syndromic AVSD case sibling, syndromic AVSD case parent, syndromic AVSD case sibling, control parent, and control sibling groups.

An evaluation of regression diagnostics was performed. Influential points were identified by examination of scatterplots. Following identification, these points were re-evaluated for measurement and/or data entry error.

Admixture Analysis

Admixture analysis was used to test the hypothesis that the overall observed distribution of asAVSL measurements actually reflected the sum of two or more separate component distributions, each of which might represent a different etiology, e.g., a gender effect or a major genetic effect, or a smaller versus a larger number of AVSL shortening alleles.

The distribution of the asAVSL measurements was formally tested for evidence of admixture using the program NOCOM (http://www.genemapping.cn/util.htm)[497]. The initial analysis focused on the measurements for non-syndromic AVSD case parents. The analysis output, when two distributions were assumed, included two estimated means, a common estimated standard deviation, and the proportion of parents estimated to be in each of the component distributions.

Log likelihood statistics were obtained under the null hypothesis, H_0: $\mu_1=\mu_2$, and the alternative hypothesis, H_1: $\mu_1\neq\mu_2$, where μ_i is the mean of the i^{th} component. In order to test whether the fit under H_1 was significantly better than under H_0, the statistic G^2 was calculated where $G^2 = 2[\ln(L_1)-\ln(L_0)]$ and L_i is the maximum likelihood obtained under the i^{th} hypothesis. The usual statistical test based on the chi-square distribution with two degrees of freedom is nonconservative, therefore, the degrees of freedom were determined as $6.08+4.51/\sqrt{n}$, where n was the number of observations[498].

Subsequent admixture analyses focused on non-syndromic AVSD case siblings, syndromic AVSD case parents, syndromic AVSD case siblings, control parents, and control siblings.

Heritability Analysis

For a quantitative trait, familial aggregation (the tendency for a trait to cluster in families) can be examined by estimating correlation coefficients between pairs of relatives, e.g., between parents and children. Significant evidence for familial aggregation suggests that shared genetic and/or shared environmental factors are likely to be involved in the etiology of the trait. The heritability of a trait reflects the magnitude of these correlation coefficients and is the portion of the trait variance that can attributed to additive genetic factors[248]. Heritability has been estimated for a number of quantitative echocardiographic measurements[249-255, 257, 258, 261], but not for AVSL.

The heritability of asAVSL was estimated based on measurements from unaffected siblings and parents of non-syndromic AVSD cases using Analysis Option 19 (Polygenic and QTL Mapping) in MENDEL. The probands were not included in this analysis, as their AVSL reflected their underlying cardiac defect. The heritability of asAVSL was also estimated for the syndromic AVSD and control families.

Results

Participants

Six hundred ninety-three echocardiograms were reviewed using the measurement protocol. Eighteen family members representing six families were excluded due to insufficient information – either the echocardiogram windows were inadequate for reliable measurements of the AVS or height and/or weight data were not available for calculation of BSA. Of the remaining participants, 212

were family members of a non-syndromic case, 266 of a syndromic case, and 199 of a child free from structural heart defects (Figure 3-3).

Standardization

Figure 3-4 shows the association of unadjusted AVSL measurements (for all groups) versus BSA. Univariable analysis was performed to evaluate for an association between AVSL and BSA ($p<0.0001$). Although it is not a tight association, it appears that as the BSA increases during childhood, the AVSL increases; once adult size is reached, the AVSL remains fairly constant. There is a large amount of scatter in these plots, likely due to a tendency toward shorter AVSLs in case families and longer AVSLs in control families. Given the significant association, the AVSL was standardized using the BSA estimate to account for body growth differences.

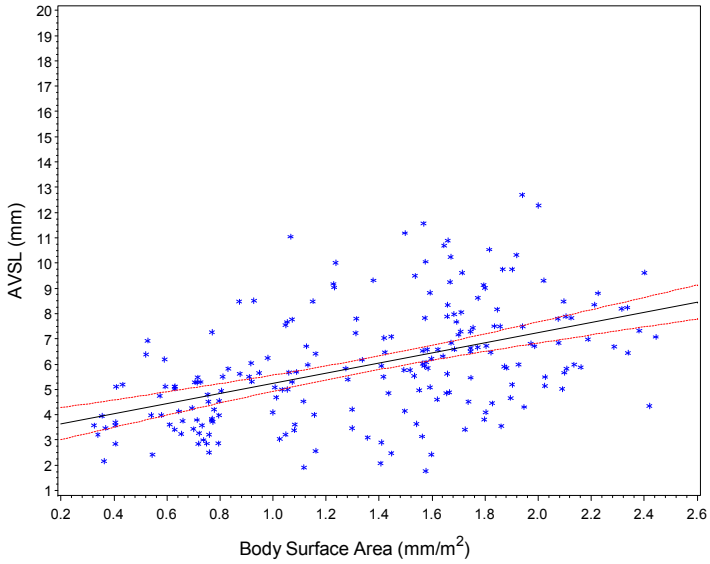
Reliability

The BSA was estimated using both the Mosteller and the DuBois and DuBois formulas as described above. The two BSA estimates were compared using a paired t-test. The mean BSA estimated using the Mosteller method was found to be significantly higher then the BSA estimated using the DuBois and DuBois method ($p<0.0001$). Figure 3-5 shows a scatterplot of the two estimates of BSA with the line of identity, and suggests that the Mosteller method produces slightly higher estimates in both of the tails.

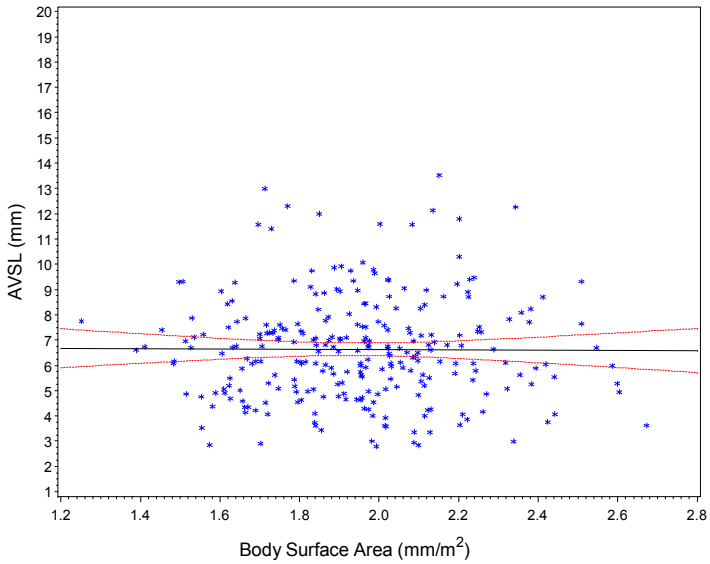
A Bland-Altman plot was also constructed to evaluate for differences between the two methods of estimation which suggests that the magnitude of differences are not constant (Figure 3-6). Therefore, following standardization (the AVSL measurement divided by the BSA estimate), the DuBois and DuBois mean sAVSL was significant longer than the Mosteller mean sAVSL ($p<0.0001$).

For the remainder of the analysis, the BSA estimated using the Mosteller method was utilized as the range of BSA was wide since infants and children were included in this study and this method has been validated for use in these populations[495].

Figure 3-4: Unadjusted Atrioventricular Septal Length versus Body Surface Area by Relationship



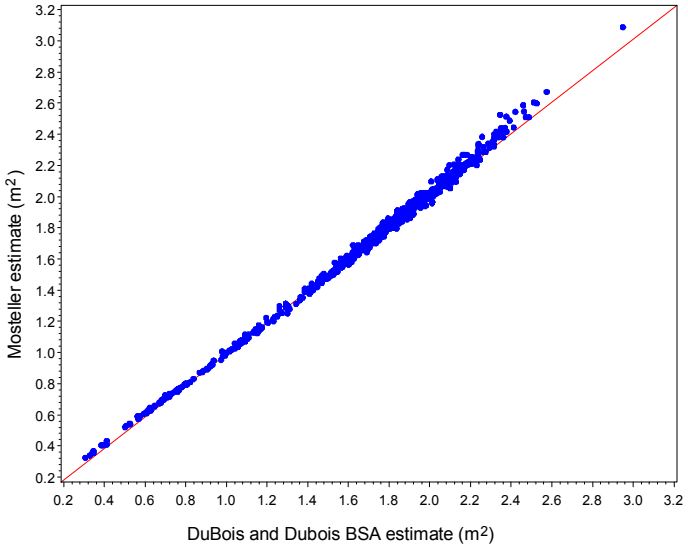
a. Siblings



b. Parents

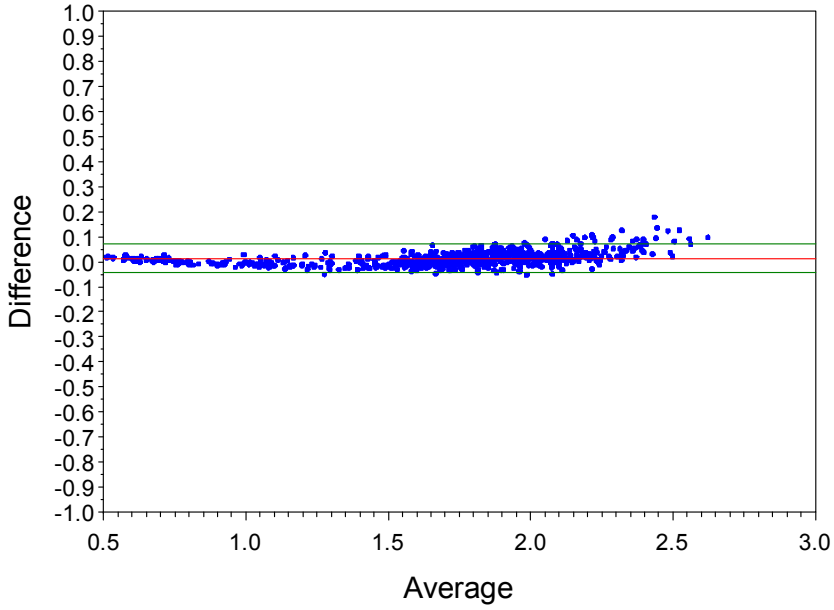
Note: Black line represents the regression line with the 95% confidence interval for the mean in red.

Figure 3-5: Mosteller versus DuBois and DuBois Body Surface Area Estimates



Note: Red line depicts the line of identity, perfect agreement.

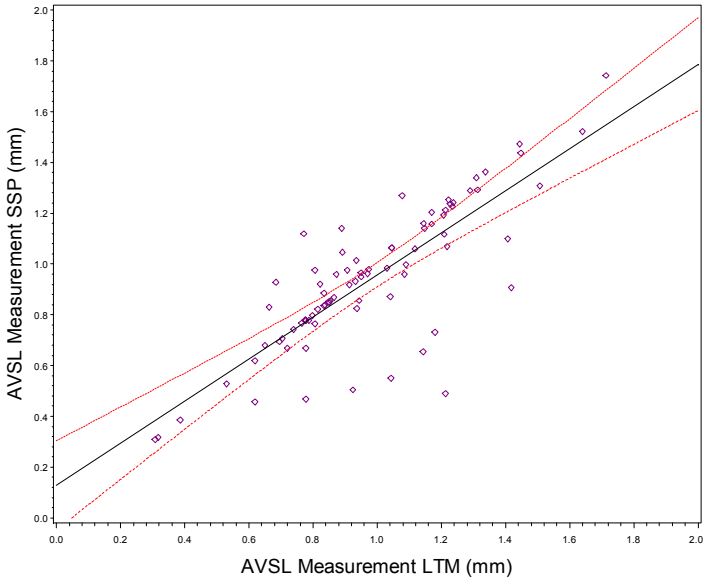
Figure 3-6: Bland-Altman Plot of Body Surface Area Estimating Methods



Note: Red line represents the line of equality. Green lines represent the 95% limits of agreement.

Figure 3-7 shows a scatterplot of the mean AVSL measurements made by the primary and independent investigators. The coefficient of variation between the two investigators was 13.7%, suggesting that the variation of the measurements was small.

Figure 3-7: Mean Atrioventricular Septal Length of Primary versus Independent Investigator



Note: Black line represents the regression line with the 95% confidence interval for the mean in red.

Interrater reliability of these AVSL measurements was assessed using a paired t-test which tested the hypothesis of no difference. The t-statistic was not significant (p=0.57), suggesting that the measurements made by the two investigators were similar. Intrarater reliability of each investigator was also evaluated by estimation of an intraclass correlation coefficient. The overall intraclass correlation coefficient of the three means for the primary investigator (SSP) was 0.979 and the intraclass correlation coefficient of the two means for

the independent investigator (LTM) was 0.983. When the sonographer who obtained the echocardiogram was taken into account, the intraclass correlation coefficient for the case group for the primary investigator was 96.8 and 99.2 for the independent investigator. Similarly, the intraclass correlation coefficient was 95.5 for the control group for the primary investigator and 99.3 for the independent investigator.

Descriptive Analysis

Tables 3-1 and 3-2 describe group characteristics (% male, mean age, height, weight, and AVSL) for each study group. There were no significant differences between the non-syndromic AVSD case, syndromic AVSD case, and control parent groups in terms of gender distribution, age, weight, height, or BSA (Table 3-1). However, the mean sAVSL was significantly different among the three groups of parents ($p<0.0001$). More specifically, the mean sAVSL measurement in the non-syndromic case and syndromic case parent groups was significantly shorter than in the control parent group. Although the mean sAVSL in the non-syndromic AVSD case parent group was shorter than the syndromic AVSD case parent group, the difference was not statistically significant.

No significant differences in gender distribution were noted among the sibling groups (Table 3-2). Significant differences were noted, however, in terms of age, weight, and BSA ($p=0.0014$, $p=0.0002$, and $p=0.0029$, respectively). As case families were ascertained using historical records, many siblings were adults when they were echoed. The non-syndromic AVSD case siblings were significantly older than the syndromic AVSD case and the control siblings and therefore, also had a higher mean weight and BSA. However, the mean sAVSL from the non-syndromic AVSD case sibling group was significantly shorter than the other two sibling groups ($p<0.0001$).

Table 3-1: Group Characteristics of Non-Syndromic AVSD Case, Syndromic AVSD Case, and Control Parents

	NSAVSD parents (N=118)	*SAVSD parents (N=149)*	*Control parents (N=109)*	*p-value*
% Male *	45.76%	48.99%	46.79%	0.86
Mean Age (yrs) ^	38.36 ± 9.32	40.01 ± 10.61	37.83 ± 6.24	0.13
Mean Weight (kg) ^	80.20 ± 18.54	80.04 ± 17.45	77.55 ± 16.92	0.44
Mean Height (cm) ^	171.05 ± 10.17	172.19 ± 9.590	170.82 ± 9.13	0.46
Mean BSA (m^2) ^	1.94 ± 0.26	1.95 ± 0.244	1.91 ± 0.23	0.44
AVSL (mm) ^	6.42 ± 1.98 (2.8-13.0)†	6.81 ± 1.98 (2.9-13.5)⊥	8.90 ± 2.75 (3.1-17.4)	**<0.0001**
Mean sAVSL (mm/m^2) ^	3.36 ± 1.24 †	3.54 ± 1.07⊥	4.68 ± 1.45	**<0.0001**

Note: *=χ^2 test performed to test for differences among groups, ^=ANOVA test performed to test for differences among groups followed by Student Newman-Keuls post hoc comparison approach, AVSD=atrioventricular septal defect, BSA=body surface area, NSAVSD=non-syndromic atrioventricular septal defect, SAVSD=syndromic atrioventricular septal defect, AVSL=atrioventricular septal length, sAVSL=standardized atrioventricular septal length, †=significant difference between NSAVSD and Control groups, ⊥=significant difference between SAVSD and Control groups.

Table 3-2: Group Characteristics of Non-Syndromic AVSD Case, Syndromic AVSD Case, and Control Siblings

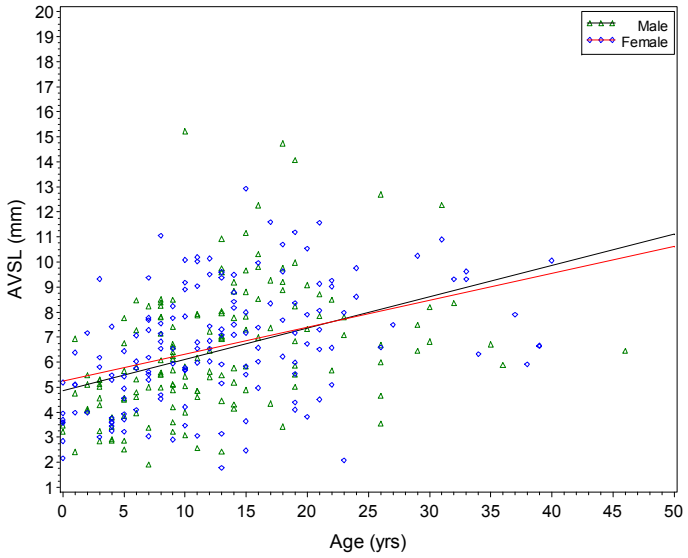
	NSAVSD siblings (N=92)	*SAVSD siblings (N=117)*	*Control siblings (N=90)*	*p-value*
% Male *	52.17%	47.01%	52.22%	0.68
Mean Age (yrs) ^	15.28 ± 10.23† ‡	12.28 ± 8.99	10.81 ± 4.69	**0.0014**
Mean Weight (kg) ^	56.08 ± 32.629† ‡	41.94 ± 22.77	43.02 ± 22.14	**0.0002**
Mean Height (cm) ^	149.60 ± 33.66	140.60 ± 32.91	144.55 ± 25.18	0.12
Mean BSA (m^2) ^	1.49 ± 0.60† ‡	1.26 ± 0.49	1.29 ± 0.43	**0.0029**
AVSL (mm) ^	5.88 ± 2.12 (1.8-10.5)†	6.05 ± 2.35 (1.9-12.7)⊥	7.82 ± 2.36 (2.9-15.2)	**<0.0001**
Mean sAVSL (mm/m^2) ^	4.51± 1.84† ‡	5.38± 2.45⊥	6.43± 2.07	**<0.0001**

Note: *=χ^2 test performed to test for differences among groups, ^=ANOVA test performed to test for differences among groups followed by Student Newman-Keuls post hoc comparison approach, AVSD=atrioventricular septal defect, BSA=body surface area, NSAVSD=non-syndromic atrioventricular septal defect, SAVSD=syndromic atrioventricular septal defect, AVSL=atrioventricular septal length, sAVSL=standardized atrioventricular septal length, †=significant difference between NSAVSD and Control groups, ‡=significant difference between NSAVSD and SAVSD groups, ⊥=significant difference between SAVSD and Control groups.

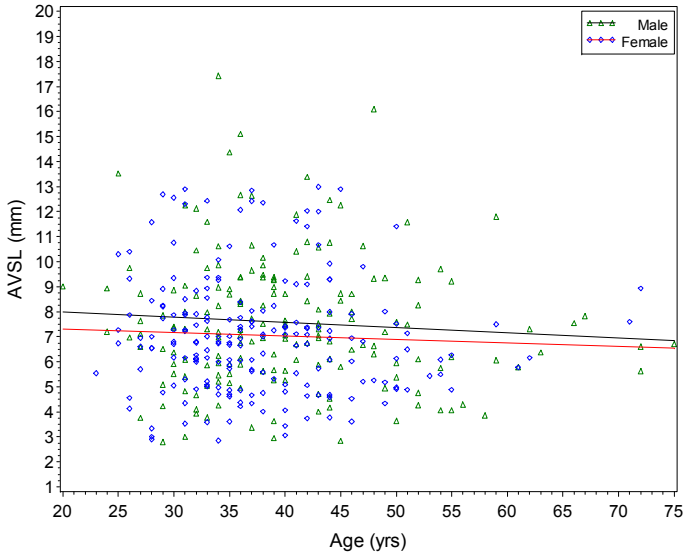
Association of Age and Gender with AVSL

The unadjusted AVSL measurements were examined for association with age by gross inspection for all siblings and parents separately (Figure 3-8).

Figure 3-8: Unadjusted Mean Atrioventricular Septal Length versus Age by Gender and Relationship



a. Siblings



b. Parents

Note: Black line represents the regression line for males. Red line represents the regression line for females.

Similar to the relationship with BSA, these graphs suggest that there is rapid growth of the atrioventricular septum throughout childhood and adolescence, which slows in adulthood.

The relationship between AVSL and age and gender was further evaluated by univariable analysis. Using a liberal significance cut-off of 0.2, younger age and being male were associated with a shorter standardized AVSL by univariable analysis in non-syndromic AVSD parent and sibling groups; younger age and being male were also associated with a shorter standarized AVSL in the syndromic AVSD parent and sibling groups. These characteristics were also significant by univariable analysis in the non-syndromic and syndromic families, which were a combination of the parent and sibling groups. In the control subgroups, age was significantly associated with standardized AVSL in the control siblings and control families by univariable analysis; there was no association with gender.

By multivariable regression analysis, there was a significant association of the standardized AVSL measurements with age and gender among the non-syndromic AVSD siblings and families, along with the syndromic AVSD siblings and families. No associations were seen by multivariable analysis in the non-syndromic or syndromic AVSD case parent groups or in the control parent, sibling, or family groups. Given these results, the sAVSL measurements were adjusted for age and gender, referred to as asAVSL, for the remainder of the analyses.

Tables 3-3 through 3-5 describe the results of univariable and multivariable linear regression analysis for sAVSL. Parameter estimates with standard errors from these models can be found in Appendix Tables A1, A2, and A3.

Table 3-3: Univariable and Multivariable Regression Analysis of Standardized AVSL (p-values) in Non-Syndromic AVSD Case Parents, Siblings, and Families

	Parents		*Siblings*		*Families*	
	Univariable	*Multivariable*	*Univariable*	*Multivariable*	*Univariable*	*Multivariable*
Age	0.0573	NS	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Gender	**0.0336**	NS	0.1877	**0.0468**	0.0555	**0.0217**

Note: AVSL=atrioventricular septal length, AVSD=atrioventricular septal defect, NS=not significant.

Significant p-values are displayed in bold.

Table 3-4: Univariable and Multivariable Regression Analysis of Standardized AVSL (p-values) in Syndromic AVSD Case Parents, Siblings, and Families

	Parents		*Siblings*		*Families*	
	Univariable	*Multivariable*	*Univariable*	*Multivariable*	*Univariable*	*Multivariable*
Age	0.6092	NS	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Gender	0.0584	NS	0.1203	**0.0405**	**0.0323**	**0.0344**

Note: AVSL=atrioventricular septal length, AVSD=atrioventricular septal defect, NS=not significant.

Significant p-values are displayed in bold.

Table 3-5: Univariable and Multivariable Regression Analysis of Standardized AVSL (p-values) in Control Parents, Siblings, and Families

	Parents		*Siblings*		*Families*	
	Univariable	*Multivariable*	*Univariable*	*Multivariable*	*Univariable*	*Multivariable*
Age	0.4759	NS	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Gender	0.6633	NS	0.3144	NS	0.5332	NS

Note: AVSL=atrioventricular septal length, AVSD=atrioventricular septal defect, NS=not significant.

Significant p-values are displayed in bold.

Admixture Analysis

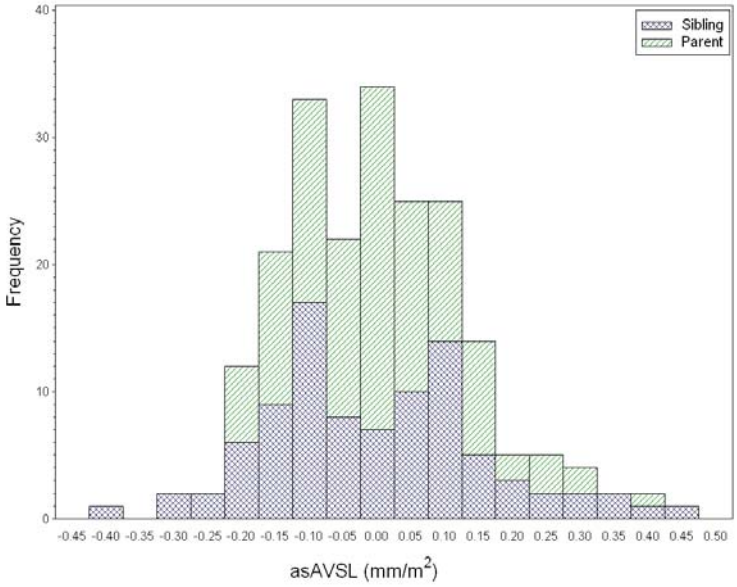
Histograms of the asAVSL measurements were plotted and examined for evidence of admixture (i.e., multiple components) in each group: non-syndromic AVSD case parents and siblings, syndromic AVSD case parents and siblings, control parents and siblings (Figures 3-9). By gross examination, it appears that there are two underlying distributions for asAVSL in the non-syndromic case parents and siblings; the distribution in the syndromic case and control families appear to represent a single distribution.

Likelihood ratio tests were conducted in the each subgroup to formally test for evidence of admixture (Table 3-6). Significant evidence was found for two component distributions in the non-syndromic AVSD case parent and sibling groups ($p=0.0177$ and $p=0.0080$, respectively). The syndromic AVSD case parent and sibling groups, along with the control parent group did not yield evidence for admixture ($p=0.07$, $p=0.11$, and $p=0.15$, respectively). The control sibling group demonstrated evidence for two component distributions, however, the second component only contained two percent of the measurements in the extreme upper tail ($p=0.0109$).

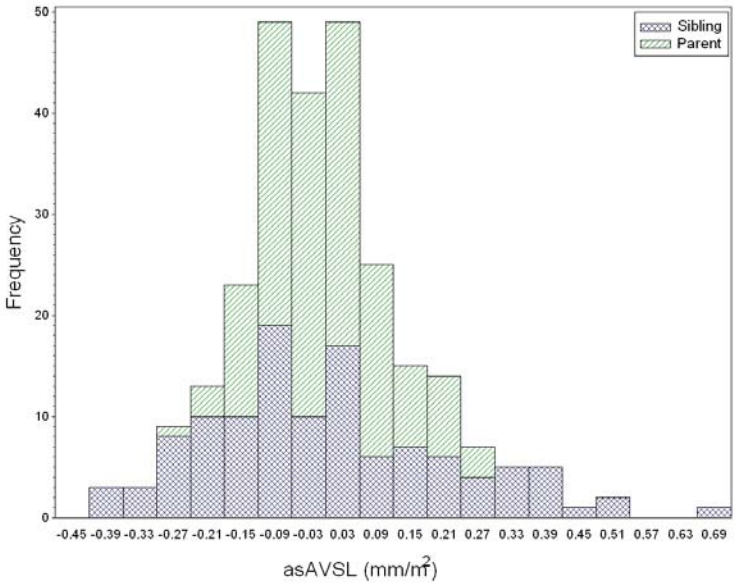
Heritability Analysis

The heritability of the asAVSL was estimated based on the parents and siblings in each group (Table 3-7). It is interesting to note the different heritability estimates in each group. The control families had the highest heritability, 0.68, suggesting that there may be genetic involvement in the AVSL. The non-syndromic and syndromic families had similar heritability estimates, although lower than the control families, 0.44 and 0.43, respectively.

Figure 3-9: Observed Distributions of Age-and Gender-Adjusted, BSA-standardized Atrioventricular Septal Length by Relationship

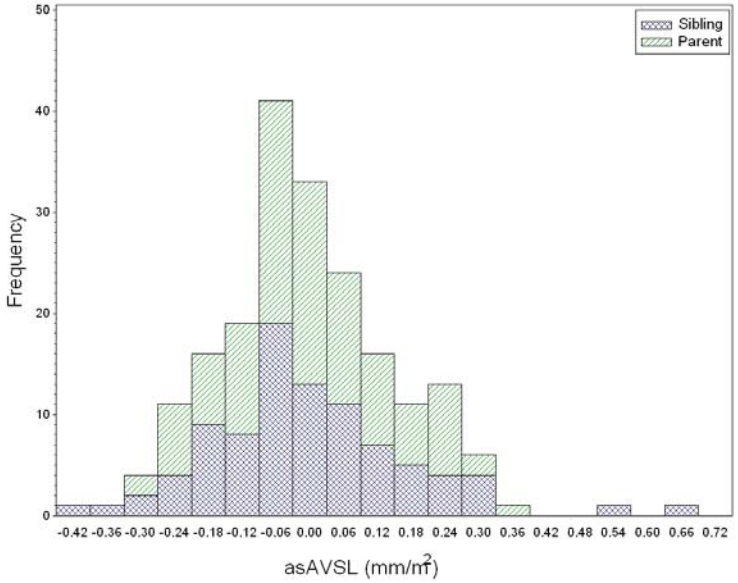


a. Non-syndromic AVSD case families



b. Syndromic AVSD case families

Figure 3-9 Continued



c. Control families

Note: asAVSL=age- and gender-adjusted, BSA-standardized atrioventricular septal length.

Table 3-6: Admixture Analysis of Age-and Gender-Adjusted, BSA-standardized Atrioventricular Septal Length

	NSAVSD		*SAVSD*		*Control*	
	Parents	*Siblings*	*Parents*	*Siblings*	*Parents*	*Siblings*
# of components	2	2	1	1	1	2
Mean 1	-0.022	-0.001	0.000	0.000	0.000	-0.014
Mean 2	0.234	0.096				0.578
Common SD	0.097	0.162	0.105	0.150	0.144	0.153
Proportion 1	0.917	0.907	1	1	1	0.976
Proportion 2	0.083	0.094				0.024
Test Statistic	8.066	9.664	5.296	4.396	3.816	9.044
p-value	**0.0177**	**0.0080**	0.07	0.11	0.15	**0.0109**

Note: NSAVSD=non-syndromic atrioventricular septal defect, SAVSD=syndromic atrioventricular septal defect, SD=standard deviation.

Significant p-values are displayed in bold.

Table 3-7: Heritability Estimates Based on Age-and Gender-Adjusted, BSA-standardized Atrioventricular Septal Length

Group	*N*	*Heritability Estimate*	*SE*
Non-Syndromic AVSD Families	67	0.4430	0.0975
Syndromic AVSD Families	83	0.4239	0.0873
Control Families	73	0.6770	0.0938

Note: N=number of families, SE=standard error.

Discussion

This study evaluated AVSL measurements in parents and siblings of non-syndromic AVSD cases, parents and siblings of syndromic AVSD cases, as well as parents and siblings in families without a history of congenital heart defects. The unadjusted AVSL ranged from 1.8-13.0 mm in the families of non-syndromic AVSD cases, 2.9-17.4 mm in the families of syndromic AVSD cases, and 1.9-13.0 mm in the control families. The unadjusted AVSL measurements of the control families were consistent with previous findings[487]. There was a significant association between the unadjusted AVSL measurements and BSA suggesting the need for adjustment to account for growth. The sAVSL of the control families, however, ranged from 1.8-13.1 mm/m^2, demonstrating a wider range than has been previously reported[27, 28, 487]. In addition, it appears that there is growth of this structure throughout childhood which slows during young adulthood. Although there were sAVSL measurements greater than 8 mm/m^2 (i.e., historical upper limit of normal value), the majority of the measurements were less than 8 mm/m^2 (90.5% in control families).

As there is no gold standard for BSA estimation, two different methods of estimation were utilized. As has been seen in previous investigations, the DuBois and DuBois estimation method for BSA may be less accurate at smaller body surface areas[495, 496]. Although both methods were highly correlated, the Mosteller estimation method was utilized in this investigation as the range of BSA was wide since infants and children were included in this study and the Mosteller method has been shown to provide more accurate estimates in these populations[495].

The major objective of this study was to measure AV septal lengths in a case-control study in order to determine whether the parents and siblings of a child with a non-syndromic AVSD demonstrate a shorter AVSL, possibly

indicating an intermediate phenotype. In comparison to control groups, both parents and siblings of non-syndromic AVSD cases had significantly shorter mean sAVSL. When the mean sAVSL of parents of non-syndromic AVSD cases was compared to the mean for parents of syndromic AVSD cases, the mean of the measurements of the non-syndromic AVSD case parents was shorter although the difference was not significant. There was also a significant mean sAVSL difference between the siblings of non-syndromic AVSD cases and syndromic AVSD cases. These results suggest the possibility of an intermediate phenotype in the relatives of non-syndromic AVSD cases. They also provide additional evidence of distinct etiologies for non-syndromic AVSDs and syndromic AVSDs.

Admixture analysis of asAVSL provided significant evidence for two component distributions in the parents and siblings of non-syndromic AVSD cases. In addition, there was evidence for two component distributions in the control sibling group. After further examination of the distribution of the control sibling AVSL measurements in Figure 3-9c, it appears that the second component follows the natural breakpoint in the observed distribution. The proportion of the measurements in the second group was approximately 2%, suggesting that if a second distribution exists, it represents a very small proportion of individuals with very long AVSLs. There was no evidence for admixture in the parents of syndromic cases or controls.

It is important to note that a large proportion of the measurements in the non-syndromic parents and siblings are estimated to be in the lower distribution (i.e., a large proportion of the data demonstrate a shorter AVSL which is consistent with a downward shift in comparison to the control distribution). Based on the distribution of sAVSL measurements in the control families, possible thresholds for a shortened sAVSL measurement can be suggested.

Possible cut-off points based on the age-adjusted sAVSL measurement distributions in the control male parents and siblings would be 2.93 mm/m^2 for parents and 2.21 mm/m^2 for siblings (2 standard deviations below the mean). Similarly, cut-off points based on the age-adjusted sAVSL measurement distributions in the control female parents and siblings would be 3.15 mm/m^2 for parents and 3.00 mm/m^2 for siblings. In this study, this would include 37.0% of male parents, 8.3% of male siblings, 43.8% of female parents, and 11.4% of female siblings.

The heritability of the echocardiographic measurements of selected cardiac structures has been previously examined[249-255, 257, 258, 261]. For example, the heritability has been demonstrated to be 0.96 for aortic root measurements, 0.72 for sinotubular junction measurements, and 0.57 for aortic valve annulus measurements in first-degree relatives of children with a left ventricular outflow tract obstructive malformation[261]. The closer the estimated heritability is to 1, the more evidence there is indicating genetic involvement in the trait. The estimated heritability of AVSL was high in the control group, suggesting genetic variants or quantitative trait loci shared by relatives likely explain a substantial portion of the familial aggregation. It is interesting to note that the heritability in the non-syndromic and syndromic families was slightly lower, suggesting that possibly the additive model may not be sufficient to explain the inheritance of the length of the AVS in families with an AVSD member. In addition, the syndromic and non-syndromic case family heritability may be different than the control families due to other major genetic effects in addition to polygenic effects. As mentioned previously, two different sonographers obtained the echocardiograms for case and control families. The images of the control families were subjectively sharper, likely due to sonographer experience. These images may have been more accurately measured than the images obtained from the case families

possibly leading to less valid heritability estimates for the case families.

The major strength of this study is innovation. No other study has examined the parents and siblings of AVSD cases with the goal of identifying an intermediate phenotype. The heritability of the AVS has never been examined in families of AVSD cases or controls before.

A major limitation of this study was the echocardiograms. Since the examinations were conducted when echocardiograms were stored on videotape, they precluded more accurate computerized measurements. As the cardiac structure being measured is small, using the caliper tool obscured the hinge points in some cases, likely leading to inaccurate measurements of the AVS. However, the intrarater and interrater reliabilities demonstrated close agreement within each investigator as well as between the two investigators. Also there were two sonographers who performed the studies, and that could influence quality of the imaging in the different study groups. The image quality of the echocardiograms was also affected by the age of the family members; older individuals have an increased distance from the chest wall (i.e., echocardiogram transducer) to the cardiac structure being imaged which leads to decreased sharpness of images compared to younger individuals who have a shorter distance. A final limitation involves the control sample. The families were chosen among those participating in a separate study conducted during the same time period who were recruited from one city in Iowa. This population is a stable, predominantly Caucasian population, as were the case families. Although the control participants were similar to case participants in terms of age, gender, weight, and height, the control participants may not represent the population from which the case families were obtained, as case families were recruited from the entire catchment area of the University of Iowa, not just from one city.

Conclusions

This represents the first study to investigate the possible existence of a shortened atrioventricular septum in parents and siblings of children with a non-syndromic AVSD. The length of the atrioventricular septum was shorter in both the parents and siblings of non-syndromic AVSD cases in comparison to parents and siblings of control children. Siblings of non-syndromic AVSD cases were also found to have a shorter AVSL in comparison to siblings of syndromic AVSD cases. There was no significant difference in the mean sAVSL between the parents of non-syndromic AVSD cases and the parents of syndromic AVSD cases. Evidence for two component distributions from the analysis of case parents and siblings suggests the presence of an intermediate phenotype for non-syndromic AVSD. Measurement criteria for a shortened AVSL were determined based on the distribution of asAVSL measurements in control families. The heritability of AVSL was estimated to be 0.68 in the control families, suggesting the possibility of substantial genetic involvement. Broadening the definition of AVSD to include those with a shortened AVSL may increase the power of genetic association and mapping studies to identify susceptibility genes. In addition, this approach may allow high and low-risk subgroups of unaffected family members to be distinguished, improving the ability to tailor recurrence risk estimates for specific individuals.

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CHAPTER 4
RISK FACTORS ASSOCIATED WITH NON-SYNDROMIC ATRIOVENTRICULAR SEPTAL DEFECTS

Abstract

Background

Atrioventricular septal defects (AVSDs) include a range of anomalies characterized by the involvement of the atrial and/or ventricular septa and the abnormal development of the AV valves. AVSDs most commonly occur in the presence of Down syndrome, but can occur without an identifiable syndrome. Little is known regarding non-genetic risk factors associated with these defects as few risk factors have been observed to have a significant association with non-syndromic AVSDs.

Methods

Using the 1997-2005 National Birth Defects Prevention Study database, a case-control study was performed examining the association between selected parental and environmental risk factors and non-syndromic AVSDs. Exposures of interest were those occurring during the periconceptional period defined as one month before pregnancy through the end of the first trimester. Logistic regression analysis models were used to estimate odds ratios and 95% confidence intervals while controlling for potential confounders. Similar subgroup analyses were performed for complete AVSD, isolated complete AVSD, spectrum AVSD, and isolated spectrum AVSD subgroups.

Results

AVSD case infants were more likely to be premature and born with a birthweight less than 2.5 kilograms than control infants. AVSD case infants were

also more likely to have a family history of birth defects, more specifically, congenital heart defects (CHDs), than control infants. This relationship was seen in all subgroups of case infants.

Women who smoked during the periconceptional period were more likely to have infants with AVSDs than women who did not smoke during this time period, independent of study site, maternal age, maternal race, gestational age, infant birthweight, alcohol consumption during the periconceptional period, and family history of CHDs (adjusted odds ratio (aOR)=1.53, 95% CI 0.98-2.39). Similar findings were noted in the complete and isolated complete subgroups. Women who were exposed to passive smoke during the periconceptional period were also more likely to have infants with AVSDs than women who were unexposed, independent of study site, maternal age, maternal race, gestational age, infant birthweight, active tobacco use, and family history of CHDs (aOR=1.57, 95% CI 1.02-2.44). Similar findings were again noted in the complete and isolated complete subgroups.

No associations were noted between AVSDs and maternal history of a UTI, PID, maternal use of antibacterial, antidepressant, asthma and allergy, or analgesic and antipyretic medications, maternal occupational exposures, and maternal alcohol consumption.

Conclusions

Maternal history of active and passive smoke exposures was associated with AVSDs after controlling for potential confounding factors. Additional investigation into the genetic susceptibilities that could modify these risks on the developing fetal heart could provide more evidence with which clinical and public health primary prevention strategies could be developed.

Introduction

Congenital heart defects (CHDs) constitute a major proportion of clinically significant birth defects and are an important component of pediatric cardiovascular disease, with an estimated prevalence of six to nine per 1000 live births[5-7]. Atrioventricular septal defects (AVSDs), also known as atrioventricular canal defects or endocardial cushion defects, include a range of anomalies characterized by involvement of the atrial septum, the ventricular septum, and one or both of the atrioventricular (AV) valves; they account for approximately 7% of all CHDs[19]. Non-syndromic AVSDs are estimated to occur in approximately one per 10,000 live births[204]. Most non-syndromic AVSDs have been considered to be sporadic or the result of multifactorial inheritance[205].

Little is known regarding non-genetic risk factors for the development of CHDs. There are no published reports of large prospective cohort studies; the majority of information regarding risk factors comes from large population-based case-control studies. While numerous risk factors have been examined for their association with CHDs, very few risk factors have shown a significant association with AVSDs. Those that have include maternal illnesses, maternal medication and non-therapeutic drug use, and maternal and paternal occupational and/or environmental exposures.

In the Baltimore-Washington Infant Study, significant associations were observed between complete AVSDs (N=31) and maternal diabetes (OR=22.8, 95% CI 7.4-70.5), ibuprofen use (OR=2.49, 95% CI 1.42-4.34) and antitussive medication use (OR=6.34, 95% CI 1.86-21.59), heavy maternal cigarette use (>20 cigarettes per day) (OR=2.50, 95% CI 1.21-5.19) and cocaine use (OR=3.45, 95% CI 1.05-11.40), maternal exposure to paint (OR=1.77, 95% CI 1.19-2.63) and varnishes (OR=4.54, 95% CI 1.36-15.18), and paternal exposure to welding (OR=1.82, 95% CI 1.14-2.92) and ionizing radiation (OR=4.54, 95%

CI 1.36-15.18)[274, 288].

Other case-control studies, including those using the National Birth Defects Prevention Study (NBDPS) database, have identified other possible risk factors for non-syndromic AVSDs including maternal history of urinary tract infection during pregnancy (OR=2.29, 95% CI 1.11-4.73), maternal antibiotic use (OR=1.7, 95% CI 1.1-2.6), and moderate (15-24 cigarettes per day) cigarette smoking (OR=2.18, 95% CI 1.04-4.55) during the periconceptional period[328, 428, 486]. A majority of these identified risk factors have not shown consistent associations across different studies, suggesting that additional investigations are warranted. Prior investigations which have utilized the NBDPS database were performed using earlier versions of the dataset with fewer years of data, warranting additional analyses using the most recent database. In addition, the prior analyses were performed using the entire group of CHD cases and did not stratify the larger groups of AVSDs into subgroups based on more specific information regarding type of defect.

We hypothesized that parental and environmental risk factors are associated with non-syndromic AVSDs. While the major objective of this study was to identify risk factors associated with non-syndromic AVSDs, risk factors associated AVSD subgroups were also investigated.

Methods

Subjects

Subjects for this study were identified from The National Birth Defects Prevention Study (NBDPS) database. The NBDPS was designed to identify infants with and without major birth defects and evaluate genetic and environmental factors associated with the occurrence of birth defects[1]. The ongoing case-control study includes case and control infants from birth defect

surveillance registries in ten states (Arkansas, California, Georgia [Centers for Disease Control and Prevention], Iowa, Massachusetts, New Jersey, New York, North Carolina, Texas, and Utah). Cases have one or more of over 30 eligible birth defects. Information for potential cases is reviewed by clinical geneticists at each site to determine study eligibility. Infants with recognized or strongly suspected chromosomal abnormalities or single-gene conditions are excluded from the study. After inclusion in the study, all cases with one specific defect are then classified by clinical geneticists to establish consistency for the defect and to determine whether the defect pattern is isolated or multiple (> 1 major malformation). Cases include all live born (all sites), still born (all sites except New Jersey), or induced abortions (all sites except Massachusetts and New Jersey). Case infants include infants with isolated and multiple defects; infants with multiple eligible defects are included in each defect category.

Infants used as controls (100 per birth year per site) are randomly selected from birth certificate or birth hospital records. Controls are unmatched to cases; they are selected from the same base population as cases, with no major birth defects and an estimated date of delivery within the same year as cases. It has been shown that participating controls are similar to those that did not agree to participate, and are therefore, representative of the base population[1]. To be eligible for the NBDPS, case and control mothers must speak either English or Spanish. Infants who have been adopted or are in foster care are ineligible for the study. As of December 2005, 18,961 cases and 6,807 controls were included in the database. For the purposes of this investigation, case and control infants were eligible NBDPS participants born from October 1997 through December 2005. NBDPS-eligible case infants were diagnosed with an AVSD by echocardiogram, cardiac catheterization, or surgical or autopsy report before 1 year of age.

Each AVSD case was reviewed by one of four NBDPS clinician case classifiers and described as ‘‘simple”, ‘‘associated”, or ‘‘complex’’ depending on the complexity of the cardiac defect[499]. The ‘‘simple’’ CHD category is used to describe either an isolated CHD or a well-defined single entity (for example, tetralogy of Fallot). The ‘‘associated’’ CHD category describes case infants with at least two distinct CHDs (for example, transposition of the great vessels with outflow tract obstruction). CHDs that include three or more cardiac defects are considered ‘‘complex”. CHDs are classified into major categories based on the anatomical lesion: (1) conotruncal, including transposition of the great arteries, tetralogy of Fallot, truncus arteriosus, double-outlet right ventricle, malaligned ventricular septal defects (VSDs), and interrupted aortic arch type B; (2) septal, including VSDs and secundum atrial septal defects (ASDs); (3) right-sided obstructive, including pulmonary valve stenosis, pulmonary atresia, tricuspid atresia, and Ebstein anomaly; (4) left-sided obstructive, including aortic valve stenosis, hypoplastic left heart syndrome and variants, coarctation of the aorta, and interrupted aortic arch types A and C; (5) anomalous pulmonary venous return, including total and partial anomalous pulmonary venous return; and (6) atrioventricular septal defects, including primum ASDs[499]. Sinus venosus ASDs and ASDs not otherwise specified are classified in the septal heart defect category.

As part of the NBDPS, mothers of case and control infants completed a detailed structured interview regarding maternal health during the periconceptional period, pregnancy history, prenatal care, including medical visits, maternal dietary history, including vitamin use, alcohol and tobacco use, parental substance abuse, home environment, maternal occupation and exposures, paternal occupation and exposures, parental demographics, and home water environment, including drinking water. Interviews are targeted for

completion within six months of the infant's estimated date of delivery (EDD) but must be completed no earlier than 6 weeks and no later than 24 months of the EDD.

Variables extracted from the database included study site, maternal age at delivery, maternal height and weight at delivery, maternal education level, maternal race, maternal history of birth defects, infant birth date, infant gender, gestational age, infant birthweight, pregnancy history (number of previous pregnancies, number of live births, number of abortions, number of stillbirths, number of miscarriages), maternal history of fever, maternal alcohol and tobacco use, maternal medication use, maternal folic acid intake, parental illicit drug use, parental job title and exposures, paternal age at delivery, paternal education level, paternal race, parental history of birth defects, family history of birth defects, and family history of CHDs. Interview questions detailing these variables are shown in Appendix Figures A1 through A6b.

Exposures of interest for this analysis occurred during the periconceptional period defined as one month prior to pregnancy through the third month (first trimester) of pregnancy. Urinary tract infection (UTI) and pelvic inflammatory disease (PID) occurrence was based on maternal reporting. The timing of the infection was determined by a dichotomous (yes/no) response. "Exposed" mothers were those who were diagnosed with a UTI or PID during the periconceptional period. "Unexposed" mothers were those who did not report either of these illnesses during the periconceptional period.

Maternal medication use was determined by self-report. Participants were asked to report all medications used during the periconceptional period. As the frequencies of individual medications were very small in the case group, medications were collapsed by drug class. Classes of interest included: (1) anti-infective medications, including antibacterial, antiviral, and antifungal agents, (2)

antidepressant medications, (3) asthma and allergy medications, including bronchodilators, mast-cell stabilizers, leukotriene modifiers, corticosteroids, antitussives, expectorants, and antihistamines, (4) gastrointestinal medications, including antacids, antidiarrheal agents, antiemetics, antiflatulents, and antiulcer agents, and (5) analgesic and antipyretic medications.

Parental illicit drug use was determined by self-report. Participants were asked to report if they had used marijuana, hashish, cocaine, crack, hallucinogens, heroin, or hallucinogenic mushrooms anytime during the pregnancy. If they had used an illicit drug, they were then asked to provide details regarding the timing during pregnancy, amount, and frequency of use. As the frequencies of use of each specific illicit drug were very small in the case group, they were combined into an overall variable indicating whether there was use of any of the above substances. Parents were considered "exposed" if they had used any illicit drug during the pregnancy. "Unexposed" parents were those who did not use any illicit drugs during the pregnancy.

Occupational exposure data were only available for mothers. Participants were asked to report if they had exposure to anesthetic gases, ionizing radiation, heavy metals, solvents, pesticides, herbicides, fungicides, or rat poison. If they answered yes to any of these exposures, they were then asked to provide details regarding the timing during pregnancy, amount, and frequency of exposure. As the frequencies of individual exposures were very small in the case group, they were combined into an overall variable indicating whether there was an exposure to any of the above substances. Mothers were considered exposed if they answered yes to any of the above exposures. Unexposed mothers were those who were not exposed to any of the above substances during pregnancy.

Maternal alcohol consumption was assessed by determining those who reported alcohol use anytime during the periconceptional period. Participants

were asked to report the amount of alcohol they consumed, how frequently they consumed alcohol, and the types of beverages they consumed. The timing of alcohol use was also determined with a dichotomous response. Consistent with other studies using the NBDPS database, the average number of drinks per month was calculated using the variables for the number of days per month multiplied by the number of drinks per day during each of the time points[500]. "Unexposed" mothers were those who did not consume alcohol from one month before pregnancy through the end of the first trimester.

Maternal smoking status (active smoke exposure) was defined as those mothers who reported smoking anytime from one month prior to pregnancy through the end of the first trimester. The timing of maternal tobacco use was also determined. The mothers were asked to report the average amount they smoked each day. "Unexposed" mothers were those who did not report any active smoke exposure during pregnancy. Maternal home and workplace tobacco exposure (passive smoke exposure) was also assessed along with timing of the exposure. A mother was classified as exposed if she had exposure to environmental tobacco smoke at home and/or work. "Unexposed" mothers were those who did not report any passive smoke exposure during pregnancy.

For the purposes of this analysis, cases were those infants with the diagnosis of an AVSD whose mothers completed the NBDPS interview. In addition to the inclusive AVSD case group, there were four major case subgroups including: (1) infants diagnosed with complete AVSDs and (2) infants with a AVSD spectrum diagnosis (diagnoses of partial defects, transitional defects, defects with outflow tract obstruction, unspecified AV septal defects, primum atrial septal defects, and inlet type ventricular septal defects). Each of the major subgroups was also separated based on whether the cardiac defect occurred in conjunction with an extra-cardiac defect (isolated defect), including

(3) infants with isolated complete AVSDs and (4) infants with isolated spectrum defects. Controls were all eligible infants whose mothers completed the NBDPS interview between 1997 and 2005.

Analysis

Descriptive Analysis

Initial analyses were descriptive in nature, and examined and compared the distributions of exposures and covariates in the AVSD case and control groups. Variables of interest also included indicator variables for timing of the exposure to the risk factor. The frequency distributions of these variables were examined. The indicator variables were collapsed into a yes/no variable indicating whether or not the exposure took place during the periconceptional period since the frequencies in individual month indicator categories were very small. Similar analyses were performed for each of the four case subgroups.

Univariable and Multivariable Logistic Regression Analysis

Univariable logistic regression analysis was performed to identify factors associated with AVSDs. Those exposures whose inclusion reached a liberal significance level ($p<0.2$) were retained for additional modeling as well as the variable family history of CHD as it has been previously shown that a family history of CHD places a family at increased risk of recurrence[501]. The current standard of care includes treatment of a UTI with an antibiotic, a number of which are folate antagonists, and therefore antibiotic use and folate intake were included in the final model examining the association between UTI and AVSDs[502].

A multivariable logistic regression model was fit with the retained exposures and confounders. Each exposure that was no longer statistically significant ($p\geq0.05$) was removed from the model separately while determining

that the reduced model did not fit the data significantly worse than the more complete model, by identifying the smallest value of Akaike's Information Criterion (AIC). Changes in parameter estimates were also examined to assess confounding. Interaction terms representing plausible interaction effects were included in a similar manner. Model significance (compared to a model with no explanatory variables) was determined by a likelihood ratio statistic. The Hosmer and Lemenshow goodness-of-fit test was performed using the final model to assess whether the model effectively described the pattern of disease outcome in the sample.

Several potential confounders and effect modifiers were considered for inclusion in the predictive models, including study center, maternal age at delivery, paternal age at delivery, maternal race/ethnicity, paternal race/ethnicity, parity, infant gender, infant gestational age, infant birthweight, family history of birth defects, and family history of CHD. Descriptive analyses of these variables were performed and will be reported by another approved NBDPS project team (unpublished data). Crude and adjusted odds ratios were estimated in order to determine the effect of each potential confounding variable for possible inclusion in the model. Inclusion of potential confounders was determined on the basis of the results of the bivariate analyses and previously published evidence. When necessary, exact methods were utilized.

Subsequent analyses focused on the four separate case subgroups – complete AVSDs, spectrum AVSDs, isolated complete AVSDs, and isolated spectrum AVSDs. Each case subgroup was separately compared to the control group. Each case subgroup analysis was approached in a similar manner as the AVSD case group. Univariable and multivariable logistic regression analysis models were fitted to estimate odds ratios and 95% confidence intervals to describe the strength of the association between each of the above exposures

within each AVSD subgroup. All analyses were performed with SAS 9.2 software (SAS Institute Inc., Cary, NC).

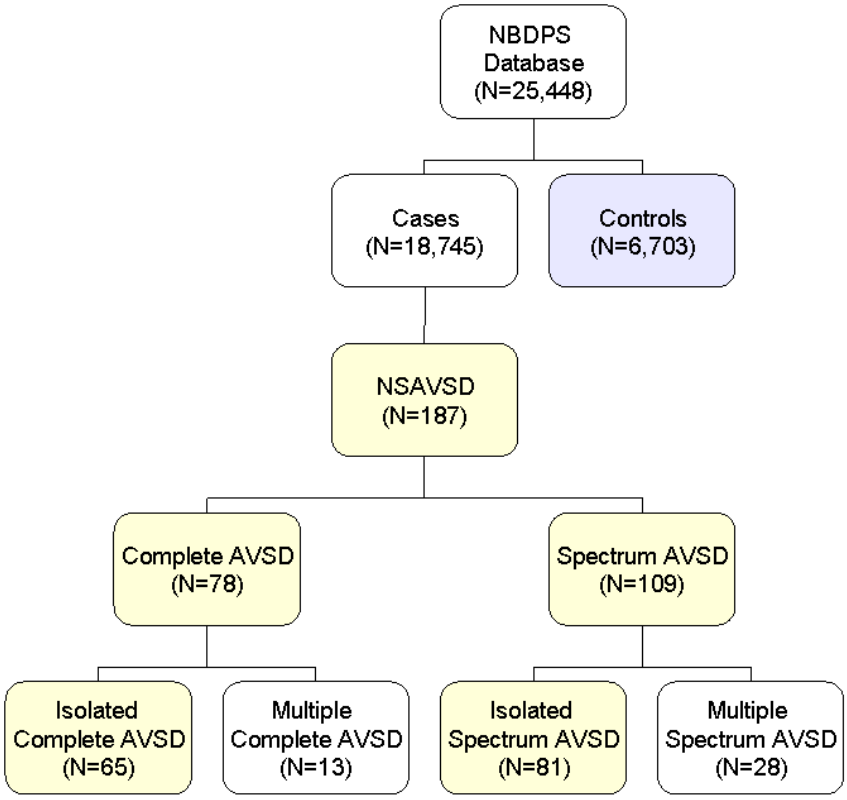
Results

AVSD Group

From October 1997 through December 2005, 189 women who had a live-born infant with an AVSD meeting NBDPS eligibility criteria (case infants) and 6,807 women who had a live-born infant without any birth defect (control infants) were enrolled in the NBDPS. Of these participants, two case and 104 control infants were excluded because their interviews were not complete. The final sample, consisting of 187 case and 6,703 control infants, is illustrated in Figure 4-1.

In this NBDPS sample, the 187 infants diagnosed with an AVSD, were by inclusion into the NBDPS non-syndromic cases. Of these infants, 78 were classified as a complete AVSD. The remaining 109 infants carried the diagnoses of partial AVSDs, transitional AVSDs, AVSDs with outflow tract obstruction, unspecified AVSDs, primum atrial septal defects, or inlet type ventricular septal defects; these infants were placed in the spectrum category. Of the infants who were classified as a complete AVSD, 65 did not have any extra-cardiac defects (isolated), while 13 had other co-existing birth defects (multiple). Of the infants who were placed in the spectrum AVSD category, 81 were isolated defects and 28 were multiple defects.

Figure 4-1: Final Analytical Sample Using the 1997-2005 NBDPS Database (Completed Interviews)



Note: NSAVSD=non-syndromic atrioventricular septal defect, AVSD=atrioventricular septal defect.

Yellow boxes represent separate case groups analyzed in this study. Each group was compared to the control group colored in purple.

Maternal and infant characteristics for the AVSD and control groups are presented in Table 4-1. There were no significant differences between case and control participants with respect to maternal age, maternal body mass index, or infant gender.

Table 4-1: Characteristics of Case and Control Participants

Variable	*Case Participants (N=187) N (%)*	*Control Participants (N=6,703) N (%)*	*Adjusted OR† (95% CI)*
Maternal age			
< 18 years	4 (2.14)	245 (3.65)	0.58 (0.21-1.57)
18-39 years	178 (95.19)	6318 (94.26)	Reference
≥ 40 years	5 (2.67)	140 (2.09)	1.27 (0.51-3.13)
Maternal race			
White	75 (70.09)	2294 (63.21)	Reference
Black	19 (17.76)	442 (21.18)	1.24 (0.74-2.10)
Hispanic	11 (10.28)	783 (21.58)	**0.41 (0.22-0.78)**
Other	2 (1.87)	110 (3.03)	0.56 (0.14-2.33)
Body Mass Index			
Underweight	9 (4.92)	356 (5.53)	0.92 (0.46-1.83)
Normal	99 (54.10)	3593 (55.79)	Reference
Overweight/Obese	75 (40.98)	2491 (38.68)	1.09 (0.81-1.48)
Parity			
Primipara	72 (66.67)	2244 (56.02)	**1.57 (1.04-2.35)**
Multipara	36 (33.33)	1762 (43.98)	Reference
Maternal education			
< High school	15 (8.02)	1128 (16.85)	Reference
High School education	109 (58.29)	3248 (48.52)	**2.52 (1.47-4.35)**
Technical college	13 (6.95)	208 (3.11)	**4.70 (2.20-10.02)**
≥ College education	50 (26.74)	2110 (31.52)	1.79 (1.00-3.20)
Maternal job status			
Employed	155 (82.89)	4825 (72.04)	**1.88 (1.28-2.76)**
Not employed	32 (17.11)	1873 (27.96)	Reference
Gestational age			
< 37 weeks	39 (20.86)	635 (9.47)	**2.52 (1.75-3.61)**
≥ 37 weeks	148 (79.14)	6067 (90.53)	Reference
Birthweight			
< 2.5 kilograms	36 (19.46)	392 (5.87)	**3.88 (2.66-5.66)**
≥ 2.5 kilograms	149 (80.54)	6286 (94.13)	Reference
Gender			
Female	104 (55.61)	3309 (49.04)	1.28 (0.96-1.72)
Male	83 (44.39)	3389 (50.60)	Reference
Family history of birth defects			
Yes	69 (36.90)	1717 (25.86)	**1.68 (1.24-2.27)**
No	118 (63.10)	4923 (74.14)	Reference
Family history of CHD			
Yes	29 (15.51)	212 (3.16%)	**5.62 (3.70-8.54)**
No	158 (84.49)	6491 (96.84)	Reference

Note: CHD=congenital heart defects, †=Odds ratios adjusted for study site.

After adjustment for study site, mothers of infants with AVSDs were more likely to be primiparous (aOR=1.57, 95% CI 1.04-2.35) compared to mothers of infants without a birth defect, and less likely to have listed their race as Hispanic (aOR=0.41, 95% CI 0.22-0.78) compared to control mothers. Additionally, fathers of infants with AVSDs were less likely to have listed their race as Hispanic (aOR=0.53, 95% CI 0.29-0.97) compared to control fathers. Mothers of case infants were also more likely to have completed high school (aOR=2.52, 95% CI 1.47-4.35), a technical program (aOR=4.70, 95% CI 2.20-10.02), or college (aOR=1.90, 95% CI 1.04-3.47); and they were more likely to have been employed during their pregnancy (aOR=1.88, 95% CI 1.28-2.76) in comparison to control mothers. Infants with AVSDs were more likely to be premature (aOR=2.52, 95% CI 1.75-3.61) and have a birthweight less than 2.5 kilograms (aOR=3.88, 95% CI 2.66-5.66) than control infants. Infants with AVSDs were also more likely to have a family history of birth defects (aOR=1.68, 95% CI 1.24-2.27) and have a family history of CHD in a first-degree relative (aOR=5.62, 95% CI 3.70-8.54) than control infants.

Complete AVSD Subgroup

Maternal and infant characteristics for the complete AVSD subgroup and the control group are presented in Appendix Table A4. There were no significant differences between case and control participants with respect to maternal age, maternal race, body mass index, parity, or infant gender.

Mothers of infants with complete AVSDs were more likely to have completed high school (aOR=2.95, 95% CI 1.26-6.89) or a technical program (aOR=3.61, 95% CI 1.01-12.91) than mothers of control infants; they were also more likely to have been employed during their pregnancy (aOR=2.16, 95% CI 1.16-4.00). Infants with complete AVSDs were more likely to be premature

(aOR=2.88, 95% CI 1.69-4.91) and have a birthweight less than 2.5 kilograms (aOR=2.92, 95% CI 1.57-5.45) than control infants. Infants with complete AVSDs were also more likely to have a family history of birth defects (aOR=1.90, 95% CI 1.20-3.00) and have a family history of CHD in a first-degree relative (aOR=6.73, 95% CI 3.71-12.20) than control infants.

Isolated Complete AVSD Subgroup

Maternal and infant characteristics for the isolated complete AVSD subgroup and the control group are presented in Appendix Table A5. There were no significant differences between case and control participants with respect to maternal age, maternal race, body mass index, parity, gestational age, or infant gender.

Mothers of infants with isolated complete AVSDs were more likely to have completed high school (aOR=3.62, 95% CI 1.01-12.93); they were also more likely to have been employed during their pregnancy (aOR=1.94, 95% CI 1.01-3.72). Infants with isolated complete AVSDs were more likely to be born with a birthweight less than 2.5 kilograms (aOR=2.58, 95% CI 1.27-5.26) than control infants. Infants with isolated complete AVSDs were also more likely to have a family history of birth defects (aOR=1.92, 95% CI 1.16-3.16) and have a family history of CHD in a first-degree relative (aOR=6.99, 95% CI 3.68-13.29) than control infants.

Spectrum AVSD Subgroup

Maternal and infant characteristics for the spectrum AVSD subgroup and the control group are presented in Appendix Table A6. There were no significant differences between case and control participants with respect to maternal age, maternal race, maternal body mass index, or infant gender.

Mother of infants with spectrum AVSDs were more likely to be primiparous (aOR=2.29, 95%CI 1.32-3.99). Mothers of infants with spectrum AVSDs were also more likely to have completed high school (aOR=2.24, 95% CI 1.11-4.54) or a technical program (aOR=5.42, 95% CI 2.13-13.83) than mothers of control infants; they were also more likely to have been employed during their pregnancy (aOR=1.72, 95% CI 1.05-2.80). Infants with spectrum AVSDs were more likely to be premature (aOR=2.27, 95% CI 1.40-3.68) and be born with a birthweight less than 2.5 kilograms (aOR=4.64, 95% CI 2.91-7.39) than control infants. Infants with spectrum AVSDs were also more likely to have a family history of birth defects (aOR=1.53, 95% CI 1.03-2.28) and have a family history of CHD in a first-degree relative (aOR=4.88, 95% CI 2.78-8.56) than control infants.

Isolated Spectrum AVSD Subgroup

Maternal and infant characteristics for the isolated spectrum AVSD subgroup and the control group are presented in Appendix Table A7. There were no significant differences between case and control participants with respect to maternal age, body mass index, gestational age, or infant gender.

Mother of infants with isolated spectrum AVSDs were more likely to be primiparous (aOR=2.87, 95%CI 1.42-5.78) than mothers of control infants. Mothers of infants with isolated spectrum AVSDs were less likely to be of Hispanic origin (aOR=0.34, 95% CI 0.12-0.95). Mothers of infants with isolated spectrum AVSDs were also more likely to have completed a technical program (aOR=5.43, 95% CI 1.88-15.63) than mothers of control infants; they were also more likely to have been employed during their pregnancy (aOR=1.84, 95% CI 1.03-3.28). Infants with isolated spectrum AVSDs were more likely to be born with a birthweight less than 2.5 kilograms (aOR=3.11, 95% CI 1.70-5.69) than control infants. Infants with isolated spectrum AVSDs were also more likely to

have a family history of birth defects (aOR=1.68, 95% CI 1.07-2.65) and have a family history of CHD in a first-degree relative (aOR=5.31, 95% CI 2.83-9.95) than control infants.

Risk Factor Identification

Urinary Tract Infection (UTI)

No association was noted between a reported history of a UTI during the periconceptional period and AVSDs (aOR=1.36, 95% CI 0.74-2.50). Similar findings were noted with the complete (aOR=1.16, 95% CI 0.45-3.01), isolated complete (aOR=1.01, 95% CI 0.34-3.04), spectrum (aOR=1.52, 95% CI 0.70-3.32), and isolated spectrum (aOR=1.22, 95% CI 0.52-2.88) subgroups. Table 4-2 displays the odds ratios adjusted for study site and the odds ratios adjusted for study site, maternal age, maternal race, gestational age, infant birthweight, antibiotic use, maternal folate intake, and family history of CHDs.

Pelvic Inflammatory Disease (PID)

Due to a small number of case and control mothers (1 case mother, 15 control mothers) reporting a history of PID during the periconceptional period, further analysis was not performed.

Medication Use

No association was observed between use of an antibacterial medication during the periconceptional period and AVSDs (aOR=1.48, 95% CI 0.96-2.29). Similar findings were noted with the complete (aOR=1.51, 95% CI 0.77-2.94), isolated complete (aOR=1.05, 95% CI 0.45-2.45), spectrum (aOR=1.46, 95% CI 0.83-2.58), and isolated spectrum (aOR=1.71, 95% CI 0.92-3.18) subgroups. Table 4-3 displays the odds ratios adjusted for study site and the odds ratios adjusted for study site, maternal age, maternal race, gestational age, infant birthweight, maternal folate intake, and family history of CHDs.

Mothers who reported use of an antiviral medication during the periconceptional period were more likely to have an infant with an AVSD (aOR=3.30, 95% CI 1.00-10.85). Similar findings were noted with the complete (aOR=8.05, 95% CI 2.42-26.83) and isolated complete (aOR=6.32, 95% CI 1.48-26.93) subgroups. Due to limited sample sizes, the spectrum and isolated spectrum subgroups could not be analyzed. Table 4-4 displays the odds ratios following adjustment for study site and following adjustment for study site, maternal age, maternal race, gestational age, infant birthweight, and family history of CHDs.

No association was noted between maternal use of an antifungal medication during the periconceptional period and AVSDs (aOR=3.20, 95% CI 0.97-10.51), although there was a suggestion of an association. However, significant associations were observed in the spectrum (aOR =5.58, 95% CI 1.69-18.45) and isolated spectrum (aOR=4.97, 95% CI 1.17-21.07) subgroups. Table 4-5 displays the odds ratios adjusted for study site and the odds ratios adjusted for study site, maternal age, maternal race, gestational age, infant birthweight, and family history of CHDs.

No association was observed maternal use of an antidepressant medication during the periconceptional period and AVSDs (aOR=1.19, 95% CI 0.58-2.44). Similar findings were noted with the complete (OR=1.43, 95% CI 0.52-3.94), isolated complete (aOR=1.27, 95% CI 0.40-4.07), spectrum (aOR=1.01, 95% CI 0.37-2.78), and isolated spectrum (aOR=1.02, 95% CI 0.32-3.27) subgroups. Table 4-6 displays the odds ratios adjusted for study site and the odds ratios adjusted for study site, maternal age, maternal race, gestational age, infant birthweight, and family history of CHDs.

No association was noted between use of an asthma or allergy medication during the periconceptional period and AVSDs (aOR=0.88, 95% CI 0.60-1.29).

Similar findings were noted with the complete (aOR=1.15, 95% CI 0.67-1.98), isolated complete (aOR=1.35, 95% CI 0.77-2.39), spectrum subgroup (aOR=0.70, 95% CI 0.41-1.19), and isolated spectrum (aOR=0.77, 95% CI 0.43-1.41) subgroups. Table 4-7 displays the odds ratios adjusted for study site and the odds ratios adjusted for study site, maternal age, maternal race, gestational age, infant birthweight, and family history of CHDs.

Mothers who reported use of a gastrointestinal medication including antacids, antidiarrheal agents, antiemetics, antiflatulents, and antiulcer agents during the periconceptional period were more likely to have an infant with an AVSD (aOR=1.47, 95% CI 1.07-2.03). While the magnitude of the odds ratio was similar in the complete (aOR=1.45, 95% CI 0.90-2.37), isolated complete (aOR=1.42, 95% CI 0.83-2.43), spectrum (aOR=1.49, 95% CI 0.98-2.26), and the isolated spectrum (aOR=1.35, 95% CI 0.82-2.21) subgroups, the findings were not significant. Table 4-8 displays the odds ratios adjusted for study site and the odds ratios adjusted for study site, maternal age, maternal race, gestational age, infant birthweight, and family history of CHDs.

No association was observed between use of an analgesic or antipyretic medication during the periconceptional period and AVSDs (aOR=1.13, 95% CI 0.83-1.54). Similar findings were noted with the complete (aOR=1.35, 95% CI 0.83-2.20), isolated complete (aOR=1.29, 95% CI 0.76-2.19), spectrum (aOR=1.00, 95% CI 0.67-1.49), and isolated spectrum (aOR=1.19, 95% CI 0.74-1.91) subgroups. Table 4-9 displays the odds ratios adjusted for study site and the odds ratios adjusted for study site, maternal age, maternal race, gestational age, infant birthweight, and family history of CHDs.

Table 4-2: History of a Urinary Tract Infection during the Periconceptional Period

*Case Group **	*Cases N (%)*	*Crude OR† (95% CI)*	*Adjusted OR‡ (95% CI)*
AVSD Group (N=44)	19 (43.18)	1.36 (0.74-2.50)	0.98 (0.40-2.41)
Complete AVSD (N=18)	7 (38.89)	1.16 (0.45-3.01)	1.23 (0.30-5.04)
Isolated Complete AVSD (N=14)	5 (35.71)	1.01 (0.34-3.04)	1.47 (0.32-6.67)
Spectrum AVSD (N=26)	12 (46.15)	1.52 (0.70-3.32)	0.84 (0.27-2.67)
Isolated Spectrum AVSD (N=22)	9 (40.91)	1.22 (0.52-2.88)	0.83 (0.24-2.81)

Note: AVSD=atrioventricular septal defect, *=each case group was compared to the control group (positive response in 451/1263=35.71%, remaining 5452 responses were “don’t know” and treated as missing data), †=ORs were adjusted for study site, ‡=ORs were adjusted for study site, maternal age, maternal race, gestational age, infant birthweight, maternal antibiotic use, maternal folate intake, and family history of congenital heart defects.

Table 4-3: Antibacterial Medication Use during the Periconceptional Period

*Case Group **	*Cases N (%)*	*Crude OR† (95% CI)*	*Adjusted OR‡ (95% CI)*
AVSD Group (N=187)	24 (12.83)	1.48 (0.96-2.29)	1.46 (0.85-2.53)
Complete AVSD (N=78)	10 (12.82)	1.51 (0.77-2.94)	1.10 (0.45-2.70)
Isolated Complete AVSD (N=65)	6 (9.23)	1.05 (0.45-2.45)	0.70 (0.21-2.34)
Spectrum AVSD (N=109)	14 (12.84)	1.46 (0.83-2.58)	1.73 (0.88-3.40)
Isolated Spectrum AVSD (N=81)	12 (14.81)	1.71 (0.92-3.18)	1.77 (0.84-3.72)

Note: AVSD=atrioventricular septal defect, *=each case group was compared to the control group (positive response in 606/6703=9.04%), †=ORs were adjusted for study site, ‡=ORs were adjusted for study site, maternal age, maternal race, gestational age, infant birthweight, maternal folate intake, and family history of congenital heart defects.

Table 4-4: Antiviral Medication Use during the Periconceptional Period

*Case Group **	*Cases N (%)*	*Crude OR† (95% CI)*	*Adjusted OR‡ (95% CI)*
AVSD Group (N=187)	3 (1.6)	3.30 (1.00-10.85)	4.23 (0.92-19.42)
Complete AVSD (N=78)	3 (3.85)	**8.05 (2.42-26.83)**	**10.49 (2.20-50.07)**
Isolated Complete AVSD (N=65)	2 (3.08)	**6.32 (1.48-26.93)**	6.14 (0.77-49.18)
Spectrum AVSD (N=109)	0	Not calculable	Not calculable
Isolated Spectrum AVSD (N=81)	0	Not calculable	Not calculable

Note: AVSD=atrioventricular septal defect, *=each case group was compared to the control group (positive response in 33/6703=0.49%), †=ORs were adjusted for study site, ‡=ORs were adjusted for study site, maternal age, maternal race, gestational age, infant birthweight, and family history of congenital heart defects.

Table 4-5: Antifungal Medication Use during the Periconceptional Period

*Case Group **	*Cases N (%)*	*Crude OR† (95% CI)*	*Adjusted OR‡ (95% CI)*
AVSD Group (N=187)	3 (1.6)	3.20 (0.97-10.51)	1.51 (0.19-11.80)
Complete AVSD (N=78)	0	Not calculable	Not calculable
Isolated Complete AVSD (N=65)	0	Not calculable	Not calculable
Spectrum AVSD (N=109)	3 (2.75)	**5.58 (1.69-18.45)**	2.75 (0.35-21.53)
Isolated Spectrum AVSD (N=81)	2 (2.47)	**4.97 (1.17-21.07)**	Not calculable

Note: AVSD=atrioventricular septal defect, *=each case group was compared to the control group (positive response in 34/6703=0.51%), †=ORs were adjusted for study site, ‡=ORs were adjusted for study site, maternal age, maternal race, gestational age, infant birthweight, and family history of congenital heart defects.

Table 4-6: Antidepressant Medication Use during the Periconceptional Period

*Case Group **	*Cases N (%)*	*Crude OR† (95% CI)*	*Adjusted OR‡ (95% CI)*
AVSD Group (N=187)	8 (4.28)	1.19 (0.58-2.44)	1.65 (0.69-3.94)
Complete AVSD (N=78)	4 (5.13)	1.43 (0.52-3.94)	1.93 (0.57-6.48)
Isolated Complete AVSD (N=65)	3 (4.62)	1.27 (0.40-4.07)	1.66 (0.38-7.18)
Spectrum AVSD (N=109)	4 (3.67)	1.01 (0.37-2.78)	1.48 (0.45-4.90)
Isolated Spectrum AVSD (N=81)	3 (3.70)	1.02 (0.32-3.27)	1.24 (0.29-5.24)

Note: AVSD=atrioventricular septal defect, *=each case group was compared to the control group (positive response in 243/6703=3.63%), †=ORs were adjusted for study site, ‡=ORs were adjusted for study site, maternal age, maternal race, gestational age, infant birthweight, and family history of congenital heart defects.

Table 4-7: Asthma and Allergy Medication Use during the Periconceptional Period

*Case Group **	*Cases N (%)*	*Crude OR† (95% CI)*	*Adjusted OR‡ (95% CI)*
AVSD Group (N=187)	33 (17.65)	0.88 (0.60-1.29)	0.91 (0.55-1.51)
Complete AVSD (N=78)	17 (21.79)	1.15 (0.67-1.98)	1.06 (0.51-2.22)
Isolated Complete AVSD (N=65)	16 (24.62)	1.35 (0.77-2.39)	1.22 (0.55-2.69)
Spectrum AVSD (N=109)	16 (14.68)	0.70 (0.41-1.19)	0.82 (0.41-1.64)
Isolated Spectrum AVSD (N=81)	13 (16.05)	0.77 (0.43-1.41)	0.82 (0.38-1.76)

Note: AVSD=atrioventricular septal defect, *=each case group was compared to the control group (positive response in 1312/6703=19.57%), †=ORs were adjusted for study site, ‡=ORs were adjusted for study site, maternal age, maternal race, gestational age, infant birthweight, and family history of congenital heart defects.

Table 4-8: Gastrointestinal Medication Use during the Periconceptional Period

*Case Group **	*Cases N (%)*	*Crude OR† (95% CI)*	*Adjusted OR‡ (95% CI)*
AVSD Group (N=187)	55 (29.41)	**1.47 (1.07-2.03)**	1.39 (0.89-2.16)
Complete AVSD (N=78)	23 (29.49)	1.45 (0.90-2.37)	1.47 (0.76-2.83)
Isolated Complete AVSD (N=65)	19 (29.23)	1.42 (0.83-2.43)	1.41 (0.68-2.94)
Spectrum AVSD (N=109)	32 (29.36)	1.49 (0.98-2.26)	1.33 (0.74-2.37)
Isolated Spectrum AVSD (N=81)	22 (27.16)	1.35 (0.82-2.21)	1.27 (0.65-2.46)

Note: AVSD=atrioventricular septal defect, *=each case group was compared to the control group (positive response in 1482/6703=22.11%), †=ORs were adjusted for study site, ‡=ORs were adjusted for study site, maternal age, maternal race, gestational age, infant birthweight, and family history of congenital heart defects.

Table 4-9: Analgesic and Antipyretic Medication Use during the Periconceptional Period

*Case Group **	*Cases N (%)*	*Crude OR† (95% CI)*	*Adjusted OR‡ (95% CI)*
AVSD Group (N=187)	126 (67.38)	1.13 (0.83-1.54)	1.07 (0.70-1.62)
Complete AVSD (N=78)	55 (70.51)	1.35 (0.83-2.20)	1.11 (0.58-2.11)
Isolated Complete AVSD (N=65)	45 (69.23)	1.29 (0.76-2.19)	0.93 (0.47-1.85)
Spectrum AVSD (N=109)	71 (65.14)	1.00 (0.67-1.49)	1.03 (0.60-1.78)
Isolated Spectrum AVSD (N=81)	56 (69.14)	1.19 (0.74-1.91)	1.07 (0.57-2.00)

Note: AVSD=atrioventricular septal defect, *=each case group was compared to the control group (positive response in 4326/6703=64.54%), †=ORs were adjusted for study site, ‡=ORs were adjusted for study site, maternal age, maternal race, gestational age, infant birthweight, and family history of congenital heart defects.

Illicit Drug Use

No association was observed between reported use of an illicit drug by mothers during the periconceptional period and AVSDs (aOR=1.07, 95% CI 0.56-2.04). Similar findings were noted with the complete (aOR=1.32, 95% CI 0.53-3.29), isolated complete (aOR=1.62, 95% CI 0.65-4.07), spectrum (aOR=0.90, 95% CI 0.36-2.21), and isolated spectrum (aOR=1.22, 95% CI 0.49-3.03) subgroups. Table 4-10 displays the odds ratios adjusted for study site and the odds ratios adjusted for study site, maternal age, maternal race, gestational age, infant birthweight, maternal alcohol consumption, active smoke exposure, and family history of CHDs.

No association was observed between reported use of an illicit drug by fathers during the periconceptional period and AVSDs (aOR=1.19, 95% CI 0.75-1.87). Similar findings were noted with the complete (aOR=1.48, 95% CI 0.78-2.82), isolated complete (aOR=1.67, 95% CI 0.85-3.29), spectrum (aOR=0.99, 95% CI 0.53-1.85), and isolated spectrum (aOR=1.24, 95% CI 0.64-2.42) subgroups. Table 4-11 displays the odds ratios adjusted for study site and the odds ratios adjusted for study site, maternal age, maternal race, gestational age, infant birthweight, and family history of CHDs.

Maternal Occupational Exposures

No association was observed between exposure to anesthetic gases, ionizing radiation, heavy metals, solvents, pesticides, herbicides, fungicides, or rat poison and AVSDs (aOR=0.95, 95% CI 0.46-1.95). Similar findings were noted with the complete (aOR=1.13, 95% CI 0.41-3.13), isolated complete (aOR=1.41, 95% CI 0.51-3.94), spectrum (aOR=0.82, 95% CI 0.30-2.24), and isolated spectrum (aOR=0.81, 95% CI 0.25-2.60) subgroups. Table 4-12 displays the odds ratios adjusted for study site and the odds ratios adjusted for study site, maternal age, maternal race, gestational age, infant birthweight,

maternal education, and family history of CHDs.

Alcohol Consumption

No association was observed between alcohol use during the periconceptional period and AVSDs (aOR=0.98, 95% CI 0.72-1.32). Similar findings were noted with the complete subgroup (aOR=1.15, 95% CI 0.73-1.82), the isolated complete subgroup (aOR=1.03, 95% CI 0.62-1.72), the spectrum subgroup (aOR=0.87, 95% CI 0.58-1.29), and the isolated spectrum subgroup (aOR=0.99, 95% CI 0.63-1.56). Table 4-13 displays the odds ratios adjusted for study site and the odds ratios adjusted for study site, maternal age, maternal race, gestational age, infant birthweight, active smoke exposure, and family history of CHDs.

Tobacco Smoke Exposure

Mothers who had infants with AVSDs were more likely than mothers who had infants without birth defects to have reported active smoke exposure during the month prior to conception through the first trimester of the pregnancy (aOR=1.53, 95% CI 1.09-2.13). Similar findings were noted in the complete subgroup (aOR=2.26, 95% CI 1.47-3.79) and the isolated complete subgroup (aOR=2.50, 95% CI 1.49-4.19). No association was observed in the spectrum subgroup (aOR=1.06, 95% CI 0.66-1.70) or the isolated spectrum subgroup (aOR=1.18, 95% CI 0.70-2.01). The association between active tobacco smoke exposure and AVSDs was independent of potential confounding factors, such as study site, maternal age, maternal race, alcohol consumption during the periconceptional period, and family history of CHDs. Table 4-14 displays the odds ratios adjusted for study site and the odds ratios adjusted for study site, maternal age, maternal race, gestational age, infant birthweight, maternal alcohol consumption, and family history of CHDs.

Mothers who had infants with AVSDs were also more likely than mothers

who had infants without birth defects to have reported passive smoke exposure during the periconceptional period (aOR=1.52, 95% CI 1.12-2.07). Similar findings were noted in the complete subgroup (aOR=2.32, 95% CI 1.47-3.65) and the isolated complete subgroup (aOR=2.17, 95% CI 1.32-3.58).

The association between active and passive tobacco smoke exposure was also examined. Mothers who reported both active and passive tobacco smoke exposures were more likely to have an infant with an AVSD (OR=1.92, 95% CI 1.31-2.83) compared to mothers who did not report either exposure. Mothers who reported active smoke exposure were also more likely to report passive smoke exposure (OR=7.33, 95% CI 6.43-8.35).

The association between passive tobacco smoke exposure and AVSDs was independent of potential confounding factors, such as study site, maternal age, maternal race, gestational age, infant birthweight, active smoke exposure, and family history of CHDs. Table 4-15 displays the odds ratios adjusted for study site and the odds ratios adjusted for study site, maternal age, maternal race, gestational age, infant birthweight, active smoke exposure, and family history of CHDs.

Table 4-10: Maternal Illicit Drug Use during the Periconceptional Period

*Case Group **	*Cases N (%)*	*Crude OR† (95% CI)*	*Adjusted OR‡ (95% CI)*
AVSD Group (N=187)	10 (5.35)	1.07 (0.56-2.04)	0.53 (0.18-1.51)
Complete AVSD (N=78)	5 (6.41)	1.32 (0.53-3.29)	0.47 (0.11-2.08)
Isolated Complete AVSD (N=65)	5 (7.69)	1.62 (0.65-4.07)	0.66 (0.14-2.99)
Spectrum AVSD (N=109)	5 (4.59)	0.90 (0.36-2.21)	0.57 (0.13-2.49)
Isolated Spectrum AVSD (N=81)	5 (6.17)	1.22 (0.49-3.03)	0.18 (0.18-3.45)

Note: AVSD=atrioventricular septal defect, *=each case group was compared to the control group (positive response in 336/6699=5.02%, remaining 4 responses were “don’t know” and treated as missing data), †=ORs adjusted for study site, ‡=ORs were adjusted for study site, maternal age, maternal race, gestational age, infant birthweight, maternal alcohol consumption, active smoke exposure, and family history of congenital heart defects.

Table 4-11: Paternal Illicit Drug Use during the Periconceptional Period

*Case Group **	*Cases N (%)*	*Crude OR† (95% CI)*	*Adjusted OR‡ (95% CI)*
AVSD Group (N=184)	22 (11.96)	1.19 (0.75-1.87)	1.36 (0.76-2.43)
Complete AVSD (N=77)	11 (14.29)	1.48 (0.78-2.82)	1.89 (0.86-4.14)
Isolated Complete AVSD (N=64)	10 (15.63)	1.67 (0.85-3.29)	**2.43 (1.08-5.44)**
Spectrum AVSD (N=107)	11 (10.28)	0.99 (0.53-1.85)	0.98 (0.42-2.32)
Isolated Spectrum AVSD (N=79)	10 (12.66)	1.24 (0.64-2.42)	1.31 (0.55-3.13)

Note: AVSD=atrioventricular septal defect, *=each case group was compared to the control group (positive response in 680/6624=10.27%, remaining 79 responses were “don’t know” and treated as missing data), †=ORs adjusted for study site, ‡=ORs were adjusted for study site, maternal age, maternal race, gestational age, infant birthweight, and family history of congenital heart defects.

Table 4-12: Maternal Occupational Exposure during the Periconceptional Period

*Case Group **	*Cases N (%)*	*Crude OR† (95% CI)*	*Adjusted OR‡ (95% CI)*
AVSD Group (N=155)	8 (5.16)	0.95 (0.46-1.95)	0.89 (0.35-2.25)
Complete AVSD (N=66)	4 (6.06)	1.13 (0.41-3.13)	1.29 (0.39-4.29)
Isolated Complete AVSD (N=54)	4 (7.41)	1.41 (0.51-3.94)	1.77 (0.52-5.97)
Spectrum AVSD (N=89)	4 (4.49)	0.82 (0.30-2.24)	0.63 (0.15-2.66)
Isolated Spectrum AVSD (N=67)	3 (4.48)	0.81 (0.25-2.60)	0.41 (0.06-3.00)

Note: AVSD=atrioventricular septal defect, *=each case group was compared to the control group (positive response in 263/4822=5.46%, remaining 1881 responses were “don’t know” and treated as missing data), †=ORs adjusted for study site, †=ORs were adjusted for study site, ‡=ORs were adjusted for study site, maternal age, maternal race, gestational age, infant birthweight, maternal education, and family history of congenital heart defects.

Table 4-13: Maternal Alcohol Consumption during the Periconceptional Period

*Case Group **	*Cases N (%)*	*Crude OR† (95% CI)*	*Adjusted OR‡ (95% CI)*
AVSD Group (N=187)	68 (36.36)	0.98 (0.72-1.32)	0.87 (0.58-1.30)
Complete AVSD (N=78)	31 (39.74)	1.15 (0.73-1.82)	1.04 (0.57-1.91)
Isolated Complete AVSD (N=65)	24 (36.92)	1.03 (0.62-1.72)	0.79 (0.40-1.58)
Spectrum AVSD (N=109)	37 (33.94)	0.87 (0.58-1.29)	0.74 (0.43-1.28)
Isolated Spectrum AVSD (N=81)	30 (37.04)	0.99 (0.63-1.56)	0.87 (0.48-1.59)

Note: AVSD=atrioventricular septal defect, *=each case group was compared to the control group (positive response in 2466/6680=36.93%, remaining 23 responses were “don’t know” and treated as missing data), †=ORs adjusted for study site, ‡=ORs were adjusted for study site, maternal age, maternal race, gestational age, infant birthweight, maternal active smoke exposure, and family history of congenital heart defects.

Table 4-14: Active Tobacco Smoke Exposure during the Periconceptional Period

*Case Group **	*Cases N (%)*	*Crude OR† (95% CI)*	*Adjusted OR‡ (95% CI)*
AVSD Group (N=186)	49 (26.34)	**1.52 (1.09-2.13)**	1.53 (0.98-2.39)
Complete AVSD (N=78)	27 (34.62)	**2.36 (1.47-3.79)**	**2.21 (1.18-4.17)**
Isolated Complete AVSD (N=65)	23 (35.38)	**2.50 (1.49-4.19)**	**2.18 (1.07-4.43)**
Spectrum AVSD (N=108)	22 (20.37)	1.06 (0.66-1.70)	1.13 (0.61-2.11)
Isolated Spectrum AVSD (N=80)	18 (22.50)	1.18 (0.70-2.01)	0.95 (0.46-1.95)

Note: AVSD=atrioventricular septal defect, *=each case group was compared to the control group (positive response in 1272/6701=18.99%, remaining 2 responses were “don’t know” and treated as missing data), †=ORs adjusted for study site, ‡=ORs were adjusted for study site, maternal age, maternal race, gestational age, infant birthweight, alcohol consumption during the periconceptional period, and family history of congenital heart defects.

Table 4-15: Passive Tobacco Smoke Exposure during the Periconceptional Period

*Case Group **	*Cases N (%)*	*Crude OR† (95% CI)*	*Adjusted OR‡ (95% CI)*
AVSD Group (N=186)	64 (34.41)	**1.52 (1.12-2.07)**	**1.57 (1.02-2.44)**
Complete AVSD (N=78)	34 (43.59)	**2.32 (1.47-3.65)**	**2.28 (1.19-4.36)**
Isolated Complete AVSD (N=65)	27 (41.54)	**2.17 (1.32-3.58)**	1.75 (0.85-3.61)
Spectrum AVSD (N=108)	30 (27.78)	1.09 (0.71-1.67)	1.17 (0.65-2.13)
Isolated Spectrum AVSD (N=80)	26 (32.50)	1.35 (0.84-2.16)	1.43 (0.74-2.76)

Note: AVSD=atrioventricular septal defect, *=each case group was compared to the control group (positive response in 1715/6686=25.66%, remaining 17 responses were “don’t know” and treated as missing data), †=ORs adjusted for study site, ‡=ORs were adjusted for study site, maternal age, maternal race, gestational age, infant birthweight, active smoke exposure, and family history of congenital heart defects.

Discussion

The main objective of this study was to identify risk factors associated with AVSDs. The findings from this population-based case-control study suggest that mothers who had infants with AVSDs were more likely to have smoked within the periconceptional period when compared to mothers who had babies without a birth defect. More specifically, the findings also suggest that mothers who had infants with either complete AVSDs or isolated complete AVSDs were more likely to have smoked during the periconceptional period than control mothers. This finding was independent of study site, maternal age, maternal race, gestational age, infant birthweight, maternal alcohol consumption, and family history of CHDs. These findings are consistent with a previous study conducted using the NBDPS database which showed an increased risk of AVSDs in infants of mothers who smoked 15-24 cigarettes a day compared with mothers who did not smoke[428]. No association between active smoke exposure and spectrum AVSDs and isolated spectrum AVSDs was identified.

This study represents the first study to evaluate the association between passive smoke exposure and AVSDs. Mothers of infants with AVSDs were more likely to have had passive smoke exposure during the periconceptional period relative to mothers of infants without birth defects. Similar to active smoke exposure, mothers who had infants with either complete AVSDs or isolated complete AVSDs were more likely to have smoked during the periconceptional period than control mothers. This finding was independent of study site, maternal age, maternal race, gestational age, infant birthweight, maternal active smoke exposure, and family history of CHDs.

Further studies examining maternal and fetal genetic susceptibilities that could modify the harmful effects of maternal tobacco use and maternal passive smoke exposure are needed. Genetic polymorphisms within the nitric oxide

synthase (*NOS*) gene are known to be associated with birth defects including cleft lip and/or palate, gastroschisis, and limb deficiency defects[503-505]. It has also been demonstrated that *NOS* isoforms are present early in embryonic cardiac development[506]. A study of infants from the California Birth Defects Monitoring Program database examined single nucleotide polymorphisms in the *NOS3* gene and observed that infants with conotruncal defects born to mothers who smoked cigarettes during the periconceptional period were more likely to carry the *NOS3* polymorphisms (922A>G) and/or (298G>T) compared to infants whose mothers did not smoke[507]. Another population-based study performed in The Netherlands observed that mothers of infants with conotruncal defects were more likely to carry the *eNOS* 894G>T variant and have smoked during pregnancy compared to mothers who had infants with other structural heart defects, but did not smoke during pregnancy[508]. While these investigations were conducted in infants with conotruncal defects, the findings of gene-environment interaction effects demonstrate the importance of additional investigations of the associations between structural heart defects, maternal smoking, and genetic variants that may modify the effect of smoking on the developing fetal heart.

It is interesting to note the difference in magnitude of odds ratios and confidence intervals between the complete AVSD and spectrum subgroups throughout this study, which suggests that complete AVSDs are different from the diagnoses included in the spectrum group which may have a different etiology. As the spectrum group is not a clinical diagnostic group, but rather a categorization scheme developed for this investigation, it is possible that there is a difference in the etiology for the spectrum group. However, in order to be considered as a case in this study, the defect was categorized within the large grouping of AVSDs, implying some similarity of the cases. This difference warrants further investigation of complete AVSDs versus the remainder of the

AVSD diagnoses.

Although a statistically significant association was not observed between AVSDs and antibacterial medication use, there was a suggestion of an association. Moderate associations were observed between AVSDs and maternal report of antiviral medication use and maternal report of antifungal medication use. A study using the 1997-2003 NBDPS database examined the relationship between maternal antifungal medication use and birth defects. Although AVSDs were not specifically examined, an increased risk of CHDs was noted in mothers who used antifungal medications compared to control mothers[509]. To date, no studies have examined the association between antiviral medication use and AVSDs. Additional investigations are warranted to examine the association between specific antiinfective agents and AVSDs for improved prevention strategies.

Maternal use of gastrointestinal medications, including antacids, antidiarrheal agents, antiemetics, antiflatulents, and antiulcer agents, during the periconceptional period was also associated with AVSDs. No associations were seen in the subgroups likely due to limited sample sizes. To date, no studies have identified an association between gastrointestinal medications and AVSDs.

No associations were noted between AVSDs and UTI, PID, antidepressant medications, asthma and allergy medications, analgesic and antipyretic medications, maternal occupational exposures, maternal alcohol consumption, and parental illicit drug use using the 1997-2005 NBDPS database.

The strengths of this study include the use of the NBDPS database, which represents the largest population-based, case-control study of major cardiovascular malformations conducted in the United States. The NBDPS has a geographically and ethnically diverse population which reduces the risk of selection bias. As has been previously reported, the NBDPS controls are similar

to all live births in the United States[1]. The cases are reviewed and verified by clinical geneticists improving the accuracy of correct case classification. Additionally, there is rigorous review of the abstracted medical chart data by an expert panel of clinicians in order to maximize the homogeneity of case classification.

Limitations of the NBDPS must be considered. A major limitation of this study was small sample sizes when stratified by subgroups. Recall bias was also of concern due to the retrospective data collection. Passive smoke exposure was determined by maternal self-reports without independent biochemical validation. The classification of the malformation relies on correct coding by each center. Due to a variety of codes used for atrial and ventricular septal defects, some of which are not atrioventricular septal defects, there is a chance that there were some infants who had a partial defect which was miscoded and would not have been evaluated by a heart classifier. This would result in a reduction in the actual numbers. The surveillance method of each participating center in the NBDPS also varies. Some centers perform surveillance across certain portions of the state, while others include the entire state. This may result in under-representation of certain race/ethnic groups, or socioeconomic classes.

Conclusions

This study identified an association between active tobacco use and AVSDs. Passive smoke exposure during the periconceptional period was also associated with infant AVSDs. Additional investigations are warranted to identify possible gene-environment interaction effects that may modify these risks in order to develop improved primary prevention strategies.

CHAPTER 5
PROGNOSTIC FACTORS FOR LEFT ATRIOVENTRICULAR VALVE REOPERATION FOLLOWING PRIMARY ATRIOVENTRICULAR SEPTAL DEFECT REPAIR

Abstract

Background

While the results of atrioventricular septal defect (AVSD) repair have improved dramatically since the first repair, development of significant left atrioventricular (AV) valve regurgitation continues to occur in some patients following surgery, necessitating additional surgical interventions, including valve replacement. Descriptions of the medical course of these patients are sparse and consistent risk factors for AV valve replacement have not yet been identified. The aim of this study was to identify prognostic factors for left AV valve replacement in patients following primary AVSD repair.

Methods

Using the Pediatric Cardiac Care Consortium database, descriptive analyses of reoperation characteristics were performed in patients with previously-repaired AVSDs. A prosthetic valve size to body weight ratio was calculated for each patient who underwent valve replacement. Univariable and multivariable linear regression analyses were performed to identify factors associated with time to reoperation and time to replacement. Survival analysis was performed to evaluate for differences in survival between repair reoperation and replacement reoperation subgroups. Cox proportional hazards models were also developed to aid in the identification of significant covariables associated with in-hospital death.

Results

A total of 370 patients were included in the study – 243 underwent left AV valve repair reoperation, 127 replacement reoperation. Median time to first reoperation following primary repair was 0.67 years in the repair reoperation subgroup and 0.18 years in the replacement reoperation subgroup; median time to valve replacement following primary repair was 0.37 years. Multivariable age-, weight-, and AVSD repair era-adjusted predictors of earlier time to valve replacement included presence of Down syndrome and postoperative mitral valve stenosis. Thirty-day post-reoperation survival of patients undergoing left AV valve replacement reoperation (78%) was significantly poorer compared to those undergoing repair reoperation (88%) (p=0.0002). Multivariable age-, weight-, and AVSD repair era-adjusted predictors of in-hospital death following valve replacement included the presence of Down syndrome (hazard ratio 2.16, 95% confidence interval 1.11-4.20) and larger prosthetic valve size to weight ratio (hazard ratio 1.63 per mm/kg, 95% confidence interval 1.24-2.15). Receiver operating characteristic analysis was performed which identified an optimal cut-off of prosthetic valve size to body weight ratio of 3 mm/kg.

Conclusions

A prosthetic valve size to body weight ratio greater than 3 mm/kg is a predictor of in-hospital death following left AV valve replacement. Additionally, patients who have previously undergone primary AVSD repair and have Down syndrome are at greater risk of death following left AV valve replacement.

Introduction

Atrioventricular septal defects (AVSDs), also known as atrioventricular canal defects or endocardial cushion defects, include a range of anomalies

characterized by involvement of the atrial septum, the ventricular septum, and one or both of the atrioventricular (AV) valves; they account for approximately 7% of all congenital heart defects[19].

AVSDs are typically surgically repaired between four and six months of age. The timing of the repair is optimally carried out prior to the development of pulmonary vascular disease. The results of surgical repair for complete AVSDs have improved markedly since the first repair was performed in 1955[31-34]. To date, three main surgical techniques have been employed in the repair of AVSDs – a one-patch method, a two-patch method, and a modified one-patch method. Following its description in 1997, the modified one-patch method has been demonstrated to be superior to the other methods in terms of patient morbidity and mortality[35].

Although the overall results of repair have improved in terms of survival, there continues to be development of hemodynamically significant left AV valve regurgitation (LAVVR) following AVSD repair at a rate of 6 to 14%[36-39]. Previously reported factors associated with the development of LAVVR following primary AVSD repair include preoperative LAVVR, dysplastic AV valves, and the absence of Down syndrome[33, 35, 43].

LAVVR is initially medically managed, but when persistent, surgical management must be explored. Hemodynamically significant LAVVR with the need for reoperation occurs in 4 to 15% of patients following primary repair of the AVSD[39, 45-47]. Surgical options to address persistent LAVVR include repair via valvuloplasty, annuloplasty, or a combination of valvuloplasty and annuloplasty versus left AV valve replacement.

The management of LAVVR is especially problematic as the effect of mechanical valve placement and its complications must be weighed judiciously. In patients with AVSDs, there is an unpredictable location of the AV node which

renders the node vulnerable to injury during reoperations on the left AV valve[34]. A serious complication related to the uncertain location of the AV node is the development of complete heart block requiring pacemaker implantation. The reported incidence of complete heart block after left AV valve replacement varies between 20 and 30%[49, 510]. Other complications of valve replacement include thromboembolism, prosthetic valve infection, bleeding, paravalvular leak, need for multiple mechanical valve replacements due to growth with increasing age, and subsequent reoperation[35, 38]. Early mortality rates following left AV valve replacement, defined as less than 30 days following surgery, have been shown to range from 22 to 36%[510, 511]. One-year survival rates following left AV valve replacement have also been reported to range from 52 to 90%[45, 511]. A multi-institutional study examined multiple risk factors predictive of in-hospital death following left AV valve replacement in children with all types of congenital heart defects. The investigators identified the presence of complete AVSD morphology, the presence of Shone's syndrome, and an increased ratio of prosthetic valve size to body weight as significant prognostic factors associated with in-hospital death[50].

To date, no other studies have examined early outcomes of left AV valve replacement, or predictors of in-hospital death following left AV valve replacement, in patients who have undergone primary repair of an AVSD. We hypothesized that prognostic factors, such as patient age at first repair, weight at first repair, AVSD morphology, length of time between surgeries, and/or presence of Down syndrome, are associated with the outcome of left AV valve replacement in patients with previously-repaired AVSDs.

Methods

Patients

The Pediatric Cardiac Care Consortium (PCCC) database was utilized for this analysis. The PCCC is a consortium of approximately 47 university-based hospitals in 20 states in the United States and two international centers. The PCCC Data Center, established in 1982, is a collaborative, voluntary effort of pediatric cardiologists and cardiothoracic surgeons from a variety of medical centers to gather and analyze data regarding operative results from procedures performed to diagnose or repair congenital heart defects. The major advantage of the PCCC is a collective pooling of data across cardiac centers that allows for statistical analysis and comparison not routinely possible at a single center because of inadequate sample size. The PCCC collects information on each child who undergoes a cardiac catheterization, electrophysiologic study, and/or cardiac operation, or dies with a cardiac malformation. Follow-up data are available if a patient had a subsequent procedure performed at a consortium institution. Follow-up data can also be ascertained by contacting the member institutions for any given study. For the purposes of this investigation, member institutions were not contacted to obtain additional information and therefore follow-up data regarding long-term survival were not available.

All patients evaluated and/or followed for an AVSD at PCCC member institutions between 1982 and 2007 were eligible for inclusion in the study. Those patients who underwent a biventricular AVSD repair were included. Patients who underwent single-ventricle palliation, repair of tetralogy of Fallot with AVSD, or were diagnosed with isomerism of atrial appendages were excluded. Expired patients followed by PCCC member institutions prior to their demise who met inclusion criteria were included in the database. Information was obtained from existing data and records, diagnostic test results, and surgical

and/or catheter intervention procedure reports. An intake form used by the PCCC is shown in Appendix Figure A7.

Variables extracted from the PCCC database included gender, presence of Down syndrome, morphology of AVSD (partial or complete), Rastelli classification[512], presence of left AV valve (mitral) cleft, patient age and weight at primary repair, date of primary repair, length of cross-clamp time during primary repair, type of primary repair, closure of left AV valve cleft, LAVVR pre- and post-primary repair, presence of left AV valve stenosis, type of reoperation (repair versus replacement), patient ages and weights at subsequent surgeries, dates of subsequent surgeries, length of cross-clamp time during subsequent surgeries, total number of surgical procedures, prosthetic valve size, morbidities, patient outcome, and surgical volume of the institution. Patients were placed into subgroups based on type of reoperation. Patients were included in the repair reoperation subgroup if they had only undergone repair reoperations on the left AV valve. Patients were included in the replacement reoperation subgroup if they ever underwent a replacement reoperation regardless of the number of previous repair reoperation attempts on the left AV valve.

Analysis

Descriptive Analysis

Descriptive statistics for demographic, anatomic, operative, and outcome variables were calculated, including means, standard deviations, 95% confidence intervals, and minimum and maximum values for all continuous variables. Due to the non-normality of some variables, the median and 25^{th} and 75^{th} percentiles were reported. If necessary, these variables were transformed for analysis. Frequency counts and percentages were used to describe categorical variables.

Demographic, anatomic, operative, and outcome characteristic differences

between the reoperation subgroup of patients who underwent valve replacement and those who underwent repair were examined using Student's t-test (continuous variables) and chi-square tests (categorical variables). Similar analyses were performed to compare patients who survived to discharge and patients who died prior to discharge for each reoperation subgroup.

Univariable and Multivariable Linear Regression Analysis

Univariable linear regression analysis was performed to identify factors associated with time to reoperation. Those factors whose inclusion reached a liberal significance level ($p<0.2$) were retained for additional consideration in multivariable analysis. Each factor that lost significance was removed from the model separately while determining that the reduced model did not fit the data significantly worse than the more complete model, by identifying the smallest value of Akaike's Information Criterion (AIC). Changes in parameter estimates were also examined to assess confounding. Interaction effects, such as between Down syndrome and morphology, were investigated for inclusion in the final model. A similar analysis was conducted to identify factors associated with time to replacement.

Survival Analysis

Survival analysis was performed to evaluate differences in early outcomes, survival to discharge or in-hospital death, between the repair and replacement reoperation subgroups. Censored observations were patients who were discharged or transferred to another institution without a subsequent surgery in the study time period; events or failures were in-hospital deaths. Survival curves were constructed based on therapy, repair versus replacement, and were compared using a log-rank test.

Cox proportional hazards models were constructed to aid in the identification of significant covariates associated with in-hospital mortality in the

reoperation group. Those factors whose inclusion reached a liberal significance level ($p<0.2$) by univariable analysis were retained for additional consideration in multivariable analysis. A multivariable Cox proportional hazards regression model was fit with the retained factors. Each factor that lost significance was removed from the model separately while determining that the reduced model did not fit the data significantly worse than the more complete model, by identifying the smallest AIC and monitoring changes in parameter estimates. Indicator variables were constructed to allow for inclusion of variables with more than two categories in the regression models. Interaction effects were also examined for inclusion in the final model. A similar analysis was performed to identify factors associated with in-hospital death in the replacement subgroup. All analyses were performed using SAS 9.2 software, Cary, NC.

Results

Reoperation Cohort

A total of 370 children who met criteria for inclusion into the study comprise the Reoperation Cohort. Table 5-1 displays descriptive statistics for characteristics examined in the entire group. Of the 370 children, 42% were male. Down syndrome was an associated diagnosis in 64% of the patients. Nine percent of patients received care at an institution with a small surgical volume (where the institution performed less than 100 heart surgeries per year), 36% at an institution with a medium volume (where the institution performed between 100 and 199 heart surgeries per year), 39% at an institution with a large volume (where the institution performed between 200 and 299 heart surgeries per year), and 16% at an institution with a very large surgical volume (where the institution performed more than 300 heart surgeries per year).

Table 5-1: Demographic, Anatomic, Operative, and Outcome Characteristics of the Reoperation Cohort (N=370)

Characteristic	*N (%)*	*Median*	*25th %-ile*	*75th %-ile*
Male	154 (42)			
Down syndrome present	227 (61)			
Institutional surgical volume				
Small (<100 surgeries)	34 (9)			
Medium (100-199 surgeries)	131 (36)			
Large (200-299 surgeries)	145 (39)			
Very large (≥300 surgeries)	60 (16)			
Complete AVSD morphology	337 (93)			
Rastelli classification (N=195)				
Type A	115 (59)			
Type B	27 (14)			
Type C	53 (27)			
Left AV valve cleft present	266 (78)			
Preop LAVVR (n=273)				
None-mild	193 (71)			
Mild-moderate	52 (19)			
Moderate-severe	28 (10)			
AVSD repair era				
1982-1989	52 (14)			
1990-1998	184 (50)			
1990-2007	134 (36)			
Type of AVSD repair				
One-patch	100 (28)			
Two-patch	213 (60)			
Modified one-patch	23 (6)			
Other repair	23 (6)			
Age at AVSD repair (yrs)		0.44	0.29	0.67
Weight at AVSD repair (kg)		5.00	4.24	6.10
CC time AVSD repair (min)		74	58	94
Cleft closure at AVSD repair	239 (68)			
Postop LAVVR				
None-mild	12 (4)			
Mild-moderate	75 (22)			
Moderate-severe	254 (74)			
Postop mitral stenosis	44 (12)			
Postop complete heart block	41 (11)			
Time to first reoperation (yrs)		0.35	0.06	2.40
Age at first reoperation (yrs)		1.16	0.46	3.45
Weight at first reoperation (kg)		7.13	5.07	12.50
Survived to discharge	317 (85)			

Note: %-ile=percentile, AVSD=atrioventricular septal defect, AV=atrioventricular, preop=preoperative, LAVVR=left atrioventricular valve regurgitation, CC time=cross-clamp time, postop=postoperative.

Anatomic characteristics of the heart defect were also described. A majority of the patients, 93%, had a complete AVSD. Rastelli classification[512] was available in 53% of the Cohort. Type A classification in which the superior bridging leaflet is attached to the ventricular septum by chordal insertions was noted in 59% of those patients; Type B classification in which the superior bridging leaflet is attached over the ventricular septum by an anomalous papillary muscle of the right ventricle was seen in 14%; Type C classification where the superior bridging leaflet is free-floating was noted in 27%. In addition, a mitral valve cleft was reported in 78%. LAVVR was reported preoperatively in 83% of the Cohort with moderate to severe LAVVR accounting for 10% of the reports.

Operative characteristics examined included characteristics related to the primary AVSD repair as well as the subsequent reoperations. For 14% of patients, the primary repair of their AVSD was performed during 1982-1989, 50% of patients underwent repair during 1990-1998, and 36% of patients underwent repair during 1999-2007. Median age at the time of primary repair was 0.44 years; median weight was 5.00 kg. The median cross-clamp or ischemic time during the primary repair was 74 minutes. The type of surgical repair employed was also described in 93% of the Cohort; of these patients, 28% underwent a one-patch repair, 60% a two-patch repair, and 6% a modified one-patch repair. The remaining 6% underwent another type of repair e.g., atrial septal defect patch placement with suture closure of the ventricular septal defect. The mitral valve cleft, if present, was closed in 68% of patients at the time of the primary repair of the AVSD. Postoperative LAVVR was noted in 99% of patients with moderate to severe LAVVR accounting for 74% of the reports. Postoperative mitral stenosis was noted in 12% of the Cohort. There were 50 operative morbidities, equaling a complication rate of 14%, reported in the Cohort. Of

these reports, there were 41 instances of complete heart block, 5 instances of paravalvular leak, 3 instances of prosthetic valve thrombosis, and 1 instance of a left ventricular pseudoaneurysm. The median time to first reoperation (repair) of the left AV valve was 0.35 years. Median age at first repair reoperation was 1.16 years and median weight was 7.13 kg.

Overall, 85% of the Cohort survived to discharge following any reoperation of the left AV valve; the remaining 15% died prior to their discharge. The median time to death following reoperation was 0.02 years (7.3 days) in the group of patients who died prior to discharge.

Left AV Valve Repair Subgroup

Two hundred forty-three Cohort members underwent a repair reoperation of the left AV valve following primary AVSD repair. Table 5-2 displays demographic, anatomic, operative, and outcome characteristics for this subgroup. Of these patients, 39% were male; 70% had Down syndrome as an associated diagnosis. Ten percent of patients received care at an institution with a small surgical volume, 40% at an institution with a medium surgical volume, 34% at an institution with a large surgical volume, and 16% at an institution with a very large surgical volume.

Complete AVSD morphology was noted in 92% of the repair subgroup. Of those patients were Rastelli classification was available, a slight majority of patients (53%) who underwent a repair reoperation were classified as Rastelli type A. A mitral cleft was reported in 80% and moderate to severe preoperative LAVVR was seen in 8% of this subgroup.

Table 5-2: Demographic, Anatomic, Operative, and Outcome Characteristics of the Repair Reoperation Subgroup (N=243)

Characteristic	*N (%)*	*Median*	*25th %-ile*	*75th %-ile*
Male	97 (39)			
Down syndrome present	169 (70)			
Institutional surgical volume				
Small (<100 surgeries)	25 (10)			
Medium (101-199 surgeries)	97 (40)			
Large (200-299 surgeries)	83 (34)			
Very large (≥300 surgeries)	38 (16)			
Complete AVSD morphology	221 (92)			
Rastelli classification (N=129)				
Type A	64 (53)			
Type B	17 (14)			
Type C	39 (33)			
Left AV valve cleft present	182 (80)			
Preop LAVVR (N=177)				
None-mild	126 (71)			
Mild-moderate	37 (21)			
Moderate-severe	14 (8)			
AVSD repair era				
1982-1989	32 (13)			
1990-1998	116 (48)			
1990-2007	95 (39)			
Type of AVSD repair				
One-patch	51 (21)			
Two-patch	150 (63)			
Modified one-patch	20 (8)			
Age at AVSD repair (yrs)		0.43	0.27	0.67
Weight at AVSD repair (kg)		5.00	4.02	6.10
CC time at AVSD repair (min)		76.5	62	95
Cleft closure at AVSD repair	159 (69)			
Postop LAVVR				
None-mild	11 (5)			
Mild-moderate	61 (27)			
Moderate-severe	156 (68)			
Postop mitral stenosis	9 (4)			
Postop complete heart block	13 (5)			
Time to first reoperation (yrs)		0.67	0.08	3.27
Age at first reoperation (yrs)		1.23	0.49	4.12
Weight at first reoperation (kg)		7.67	5.20	14.50
CC time at first reoperation (min)		45.5	32	64
Survived to discharge	228 (94)			

Note: %-ile=percentile, AVSD=atrioventricular septal defect, AV=atrioventricular, preop=preoperative, LAVVR=left atrioventricular valve regurgitation, CC time=cross-clamp time, postop=postoperative.

A two-patch repair was performed in 63% of these patients. Median age at the time of primary AVSD repair was 0.43 years and median weight was 5.00 kg. The median cross-clamp time during the primary repair was 76.5 minutes. The mitral valve cleft, if present, was closed in 69% of the repair reoperation subgroup. Postoperative moderate to severe LAVVR was reported in 68%; four percent of patients were also noted to have postoperative mitral stenosis. Postoperative complete heart block was seen in 5% of this subgroup. The median time to first reoperation (repair) of the left AV valve was 0.67 years. Median age at first repair reoperation was 1.23 years and median weight was 7.67 kg. Median cross-clamp time was 45.5 minutes. Eighty-nine percent of patients in the repair reoperation subgroup survived to discharge.

Left AV Valve Replacement Subgroup

One hundred twenty-seven of the 370 Reoperation Cohort members (34%) underwent replacement of their left AV valve. Table 5-3 displays the demographic, anatomic, operative, and outcome characteristics for this subgroup. Of the patients who underwent left AV valve replacement: 49% were male; Down syndrome was an associated diagnosis in 54% of the patients. Seven percent of patients in the replacement reoperation subgroup received care at an institution with a small surgical volume; 27% at an institution with a medium surgical volume, 49% at an institution with a large surgical volume, and 17% at an institution with a very large surgical volume.

A majority of the patients (94%) had a complete AVSD. The majority of patients (68%) who underwent a replacement reoperation were classified as Rastelli type A. In addition, a mitral valve cleft was reported in 76%. Moderate to severe LAVVR was reported prior to primary repair in 15% of patients.

Table 5-3: Demographic, Anatomic, Operative, and Outcome Characteristics of the Replacement Subgroup (N=127)

Characteristic	*N (%)*	*Median*	*25th %-ile*	*75th %-ile*
Male	60 (49)			
Down syndrome present	58 (46)			
Institutional surgical volume				
Small (<100 surgeries)	9 (7)			
Medium (101-199 surgeries)	34 (27)			
Large (200-299 surgeries)	62 (49)			
Very large (≥300 surgeries)	22 (17)			
Complete AVSD morphology	116 (94)			
Rastelli classification (N=75)				
Type A	51 (68)			
Type B	10 (13)			
Type C	14 (19)			
Left AV valve cleft present	84 (76)			
Preop LAVVR (N=96)				
None-mild	67 (70)			
Mild-moderate	15 (16)			
Moderate-severe	14 (15)			
AVSD repair era				
1982-1989	20 (16)			
1990-1998	68 (54)			
1990-2007	39 (31)			
Type of AVSD repair				
One-patch	49 (41)			
Two-patch	63 (52)			
Modified one-patch	3 (2)			
Age at AVSD repair (yrs)		0.44	0.32	0.63
Weight at AVSD repair (kg)		4.96	4.42	5.97
CC time at AVSD repair (min)		67.5	54.5	93.5
Cleft closure at AVSD repair	80 (68)			
Postop LAVVR				
None-mild	1 (1)			
Mild-moderate	14 (12)			
Moderate-severe	98 (87)			
Postop mitral stenosis	35 (28)			
Postop complete heart block	28 (22)			
Time to first reoperation (yrs)		0.17	0.04	1.19
Age at first reoperation (yrs)		0.92	0.41	2.52
Weight at first reoperation (kg)		6.45	4.90	10.20
CC time at first reoperation (min)		69	48	95

Table 5-3 Continued

Characteristic	*N (%)*	*Median*	*25th %-ile*	*75th %-ile*
Time to replacement (yrs)		0.37	0.05	2.39
Valve size to body weight (mm/kg)		2.82	1.65	3.60
Survived to discharge	89 (70)			

Note: %-ile=percentile, AVSD=atrioventricular septal defect, AV=atrioventricular, preop=preoperative, LAVVR=left atrioventricular valve regurgitation, CC time=cross-clamp time, postop=postoperative.

Median age of these patients at the time of primary repair was 0.44 years and median weight was 4.96 kg. The median cross-clamp or ischemic time during the primary repair was 67.5 minutes; 41% of patients underwent a one-patch repair, 52% a two-patch repair, and 2% a modified one-patch repair. The mitral valve cleft, if present, was closed in 68% of patients at the time of primary AVSD repair. Moderate to severe postoperative LAVVR was seen in 87% of patients in this subgroup; postoperative mitral stenosis was reported in 28%; and postoperative complete heart block was seen in 33% of this subgroup. The median time to first reoperation (either repair or replacement) was 0.17 years. Median age at first reoperation was 0.92 years; median weight at first reoperation was 6.45 kg. The majority of patients who eventually had their left AV valve replaced underwent replacement during the first reoperation (57%). The median time to replacement was 0.37 years. St. Jude (76%) and Carbomedics (16%) prosthetic valves comprised the majority of prosthetic valve replacements. Valve sizes ranged from 14 mm to 31 mm. Seventy percent of the left AV valve replacement subgroup survived to discharge.

The descriptive characteristics of the repair reoperation and replacement subgroups were examined for differences. Table 5-4 lists the characteristics examined and the comparative p-values. Comparison of the replacement and repair subgroups revealed significant differences in terms of the proportion of patients with Down syndrome, surgical volume of institution, type of primary

AVSD repair, presence of postoperative LAVVR, frequency of postoperative mitral stenosis, frequency of complete heart block, time to first reoperation, age at first reoperation, weight at first reoperation, cross-clamp time during first reoperation, and survival to discharge.

Table 5-4: Characteristics of Patients Undergoing Left AV Valve Repair versus Replacement Following Primary AVSD Repair

Characteristic	*p-value*
Male (%)	0.07
Down syndrome present (%)	**<0.0001**
Institutional surgical volume	**0.0211**
Complete AVSD morphology (%)	0.44
Rastelli classification (%)	0.08
Left AV valve cleft present (%)	0.38
Preop LAVVR (%)	0.16
AVSD repair era (%)	0.28
Type of AVSD repair (%)	**0.0007**
Age at AVSD repair (yrs)	0.32
Weight at AVSD repair (kg)	0.33
CC time at AVSD repair (min)	0.13
Cleft closure at AVSD repair (%)	0.89
Postop LAVVR (%)	**0.0010**
Postop mitral stenosis (%)	**<0.0001**
Postop complete heart block (%)	**<0.0001**
Time to first reoperation (yrs)	**0.0002**
Age at first reoperation (yrs)	**0.0239**
Weight at first reoperation (kg)	**0.0190**
CC time at first reoperation (min)	**<0.0001**
Survived to discharge (%)	**<0.0001**

Note: AVSD=atrioventricular septal defect, AV=atrioventricular, preop=preoperative, LAVVR=left atrioventricular valve regurgitation, CC time=cross-clamp time, postop=postoperative.

Significant p-values are displayed in bold.

Patients with co-existing Down syndrome were more frequent in the repair reoperation subgroup (70%) than in the replacement reoperation subgroup

(54%). The majority of care (40%) was received at an institution classified as medium (performing 101-199 cardiac surgeries per year) in the repair reoperation subgroup, while the majority of the replacement reoperation subgroup (49%) received care at a large institution (performing 200-299 cardiac surgeries per year). The two-patch method was performed for the majority of patients who were in the repair reoperation subgroup (63%), while the one-patch (41%) and two-patch (52%) methods were utilized equally in the replacement reoperation subgroup. Moderate to severe postoperative LAVVR occurred in 68% of patients in the repair reoperation subgroup and in 87% of patients in the replacement reoperation subgroup. Postoperative mitral stenosis occurred more frequently in the replacement reoperation subgroup (28%) in comparison to the repair reoperation subgroup (4%); postoperative complete heart block was also more frequent in the replacement reoperation subgroup (22%) compared to the repair subgroup (5%). Time to first reoperation, age at first reoperation, and weight at first reoperation were significantly longer (higher) in the repair reoperation subgroup in comparison to the replacement reoperation subgroup. The cross-clamp time during the first reoperation was significantly shorter in the repair reoperation subgroup. Survival to discharge was significantly greater in the repair reoperation subgroup in comparison to the replacement reoperation subgroup (89 versus 70%).

Factors Associated with Time to Reoperation/Replacement

Univariable and multivariable results from the analysis of time to reoperation, which was log transformed due to non-normality, are presented in Table 5-5. Parameter estimates with standard errors for the fitted linear regression models can be found in Appendix Table A8. Significant associations ($p<0.05$) were identified between earlier time to reoperation and later eras of

AVSD repair, earlier age at AVSD repair, smaller weight at AVSD repair, closure of the mitral valve cleft during the primary AVSD repair, presence of moderate to severe postoperative LAVVR, and presence of complete heart block following AVSD repair. In the multivariable model, closure of the mitral valve cleft, moderate to severe postoperative LAVVR, and presence of postoperative complete heart block were associated with earlier time to reoperation after adjusting for age and weight at AVSD repair as well as era of AVSD repair (R^2=13.4%).

Univariable and multivariable results from the analysis of time to replacement, which was log transformed due to non-normality, are also presented in Table 5-5. Parameter estimates with standard errors for these fitted linear regression models can be found in Appendix Table A9. Significant univariable associations were identified between earlier time to replacement and presence of Down syndrome, more than mild preoperative LAVVR, recent eras of AVSD repair, longer cross-clamp time during primary AVSD repair, and postoperative mitral stenosis. After adjusting for age and weight at the time of the primary AVSD repair as well as era of repair, presence of Down syndrome and presence of postoperative mitral stenosis were associated with earlier time to replacement in the multivariable model (R^2=27.3%).

Survival to Discharge

Demographic, anatomic, and operative characteristics were examined by outcome (survival to discharge versus in-hospital death) for each subgroup. Differences among the characteristics were also examined by outcome for each subgroup. Appendix Tables A10 and A11 display characteristics examined and comparative p-values.

Table 5-5: Univariable and Multivariable Regression Analyses (p-values) of Time to Reoperation and Time to Replacement

Characteristic	*Time to Reoperation (N=370)*		*Time to Replacement (N=127)*	
	Univariable	*Multivariable*	*Univariable*	*Multivariable*
Male	0.27		0.93	
Down syndrome present	0.56		**0.0067**	**0.0089**
Institutional surgical volume				
Small (<100 surgeries)	reference		reference	
Medium (101-199 surgeries)	0.17	NS	0.79	
Large (200-299 surgeries)	0.48		0.17	NS
Very large (>300 surgeries)	0.48		0.92	
Complete AVSD morphology	0.19	NS	0.33	
Rastelli classification				
Type A	reference		reference	
Type B	0.32		0.99	
Type C	0.81		0.54	
Left AV valve cleft present	0.74		0.84	
Preop LAVVR				
None-Mild	reference		reference	
Mild-Moderate	0.82		0.11	NS
Moderate-Severe	0.27		0.11	NS
AVSD repair era				
1982-1989	reference		reference	
1990-1998	**0.0466**	0.24	**0.0162**	**0.0160**
1999-2007	**<0.0001**	**0.0003**	**0.0002**	**0.0001**
Type of AVSD repair				
One-patch	0.16		0.21	
Two-patch	0.93		0.30	
Modified one-patch	reference		reference	

Table 5-5 Continued

Characteristic	*Time to Reoperation (N=370)*		*Time to Replacement (N=127)*	
	Univariable	*Multivariable*	*Univariable*	*Multivariable*
Age at AVSD repair (yrs)	**0.0103**	0.29	0.59	0.42
Weight at AVSD repair (kg)	**0.0028**	0.24	0.94	0.88
CC time at AVSD repair (min)	0.07	Not included	**0.0071**	Not included
Cleft closure at AVSD repair	**0.0246**	**0.0159**	0.64	
Postop LAVVR				
None-Mild	reference		reference	
Mild-Moderate	0.64		**0.0120**	NS
Moderate-Severe	**0.0498**	**0.0004**	**0.0066**	NS
Postop mitral stenosis	0.09	NS	**0.0027**	**0.0296**
Postop complete heart block	**0.0046**	**0.0020**	0.49	

Note: AVSD=atrioventricular septal defect, AV=atrioventricular, preop=preoperative, LAVVR=left atrioventricular valve regurgitation, CC time=cross-clamp time, postop=postoperative.

Significant p-values are displayed in bold. P-values not in bold type represent factors that were adjusted for in final model. NS=not significant. Cross-clamp time was not included in model fitting due to large number of missing values.

Of the 127 patients who underwent left AV valve replacement, 89 (70%) survived to hospital discharge. Of the survivors, 40% had previously undergone a one-patch repair, 50% a two-patch repair, and 2% a modified one-patch repair. Nineteen percent underwent primary AVSD repair during 1982-1989, 53% during 1990-1998, and 28% during 1999-2007. Postoperative complete heart block was reported in 27% of survivors. Among the survivors, 2% underwent replacement during their primary AVSD repair, 55% during their first reoperation, 36% during a second reoperation, and 7% during a third reoperation.

Of the 38 patients who died prior to hospital discharge (30%), 40% had previously undergone a one-patch repair, 57% a two-patch repair, and 3% a modified one-patch repair. Eight percent underwent primary AVSD repair during 1982-1989, 55% during 1990-1998, and 37% during 1999-2007. Postoperative complete heart block was reported in 11% of non-survivors. Among the patient mortalities, 16% underwent replacement during their primary AVSD repair, 60% during their first reoperation, 21% during a second reoperation, and 3% during their third reoperation.

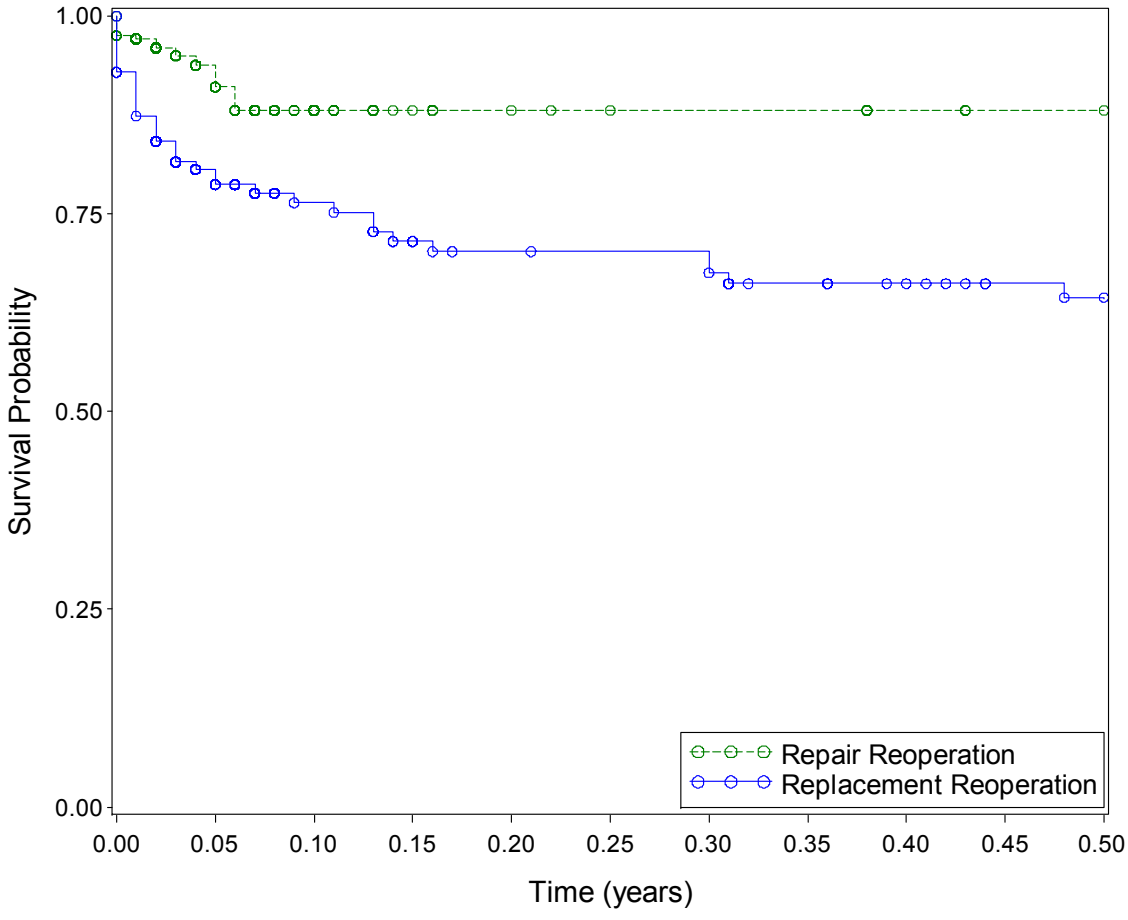
Replacement surgery occurred during the first reoperation for the majority of patients who ultimately underwent a replacement reoperation. The highest in-hospital mortality rate was seen in patients who underwent left AV valve replacement during the primary AVSD repair (Appendix Figure A8). The rate of in-hospital deaths decreased as the valve replacement was performed further out in time from primary AVSD repair (Appendix Figure A9).

Patients who underwent a repair reoperation and survived to discharge had a longer time to first reoperation and were older and weighed more at the time of first reoperation. Patients who underwent a replacement reoperation and survived to discharge were older at the time of primary repair, had a longer time to first reoperation, were older and weighed more at the time of the first

reoperation, had a longer time to valve replacement, and a smaller prosthetic valve size to body weight ratio.

Survival analysis was performed to evaluate differences in survival between the repair and replacement subgroups. Survival for the repair reoperation subgroup was 97% at 7 days, 94% at 2 weeks, 88% at 1 month, 88% at 6 months, and 88% at 1 year (Figure 5-1). Survival for the replacement reoperation subgroup was 87% at 7 days, 82% at 2 weeks, 78% at 1 month, 64% at 6 months, and 64% at 1 year.

Figure 5-1: Survival Curves of Repair and Replacement Subgroups



Note: Open circles represent censored observations.

Figure 5-1 demonstrates that survival for the replacement subgroup decreases dramatically during the first two months following surgery. The difference between survival of the two subgroups remains fairly constant following that time with the replacement subgroup survival significantly lower than the repair reoperation subgroup based on the log-rank test (p=0.0002).

Prognostic Factors for In-Hospital Death

Univariable and multivariable predictors of in-hospital death for the Reoperation Cohort and replacement subgroup are presented in Table 5-6. Parameter estimates with standard errors for the fitted Cox proportional hazards models can be found in Appendix Tables A12 and A13.

Significant univariable associations ($p<0.2$) were identified between in-hospital death and complete AVSD morphology, Rastelli type C classification relative to type A, most recent era of AVSD repair, earlier age and smaller weight at both primary AVSD and reoperation surgeries, longer cross-clamp time during AVSD repair, presence of postoperative complete heart block, shorter time interval to reoperation, and undergoing a replacement reoperation by univariable analysis. In the multivariable model, after adjusting for age and weight at the time of the reoperation and era of primary AVSD repair, factors predictive of in-hospital death following reoperation included earlier age at primary AVSD repair (hazard ratio 0.36 per year, 95% CI 0.12-0.92), undergoing a replacement surgery (hazard ratio 3.29, 95% CI 1.75-6.21), and earlier age at reoperation (hazard ratio 0.75 per year, 95% CI 0.58-0.96). Interaction effects between Down syndrome and type of repair and surgical era and type of repair were considered for inclusion in the multivariable model, but were not significant.

When analysis was restricted to patients undergoing replacement reoperation, significant univariable associations ($p<0.2$) were identified between

in-hospital death and Down syndrome, care received at an institution with a large surgical volume relative to an institution with a small volume, Rastelli type C classification relative to type A, later eras of AVSD repair, longer cross-clamp time during AVSD repair, presence of postoperative complete heart block, shorter interval times to reoperation and replacement, earlier age and smaller weight at both reoperation and replacement surgeries, smaller prosthetic valve size, and larger prosthetic valve size to body weight ratio. In the multivariable model after adjusting for age and weight at the time of the replacement surgery in addition to era of AVSD repair, factors predictive of in-hospital death following replacement reoperation included Down syndrome (hazard ratio 2.16, 95% CI 1.11-4.20) and larger prosthetic valve size to weight ratio (hazard ratio 1.63 per mm/kg, 95% CI 1.24-2.15). Interaction effects between Down syndrome and type of repair and surgical era and type of repair considered for inclusion in the multivariable model, but were not significant.

Receiver-operating characteristic (ROC) curve analysis was used to determine an optimal cut-off of the prosthetic valve size to body weight ratio by maximizing the sum of sensitivity and specificity, which is equivalent to maximizing the difference between the sensitivity with the prognostic factor and the sensitivity that the prognostic factor would have if it did no better than random chance[513]. Using this criterion, the optimal cut-off was determined to be a prosthetic valve size to body weight ratio of 3 mm/kg; this was associated with a sensitivity of 0.84 and a specificity of 0.58 (Figure 5-2).

Table 5-6: Univariable and Multivariable Regression Analyses (p-values) of Outcome in Reoperation Cohort and Replacement Subgroup

Characteristic	*Reoperation Cohort (N=370)*		*Replacement Subgroup (N=127)*	
	Univariable	*Multivariable*	*Univariable*	*Multivariable*
Male	0.24		0.48	
Down syndrome present	0.27		**0.0422**	**0.0233**
Institutional surgical volume				
Small (<100 surgeries)	reference		reference	
Medium (101-199 surgeries)	0.54		0.68	
Large (200-299 surgeries)	0.37		0.11	NS
Very large (>300 surgeries)	0.52		0.53	
Complete AVSD morphology	0.18	NS	0.43	
Rastelli classification				
Type A	reference		reference	
Type B	0.36		0.46	
Type C	0.05	NS	0.17	
Left AV valve cleft present	0.53		0.76	
Preop LAVVR				
None-Mild	reference		reference	
Mild-Moderate	0.98		0.68	
Moderate-Severe	0.79		0.99	
AVSD repair era				
1982-1989	reference		reference	
1990-1998	0.30	0.54	0.15	0.34
1999-2007	0.18	0.56	0.08	0.53
Type of AVSD repair				
One-patch	0.06	NS	0.26	
Two-patch	**0.0489**	NS	0.20	
Modified one-patch	reference		reference	

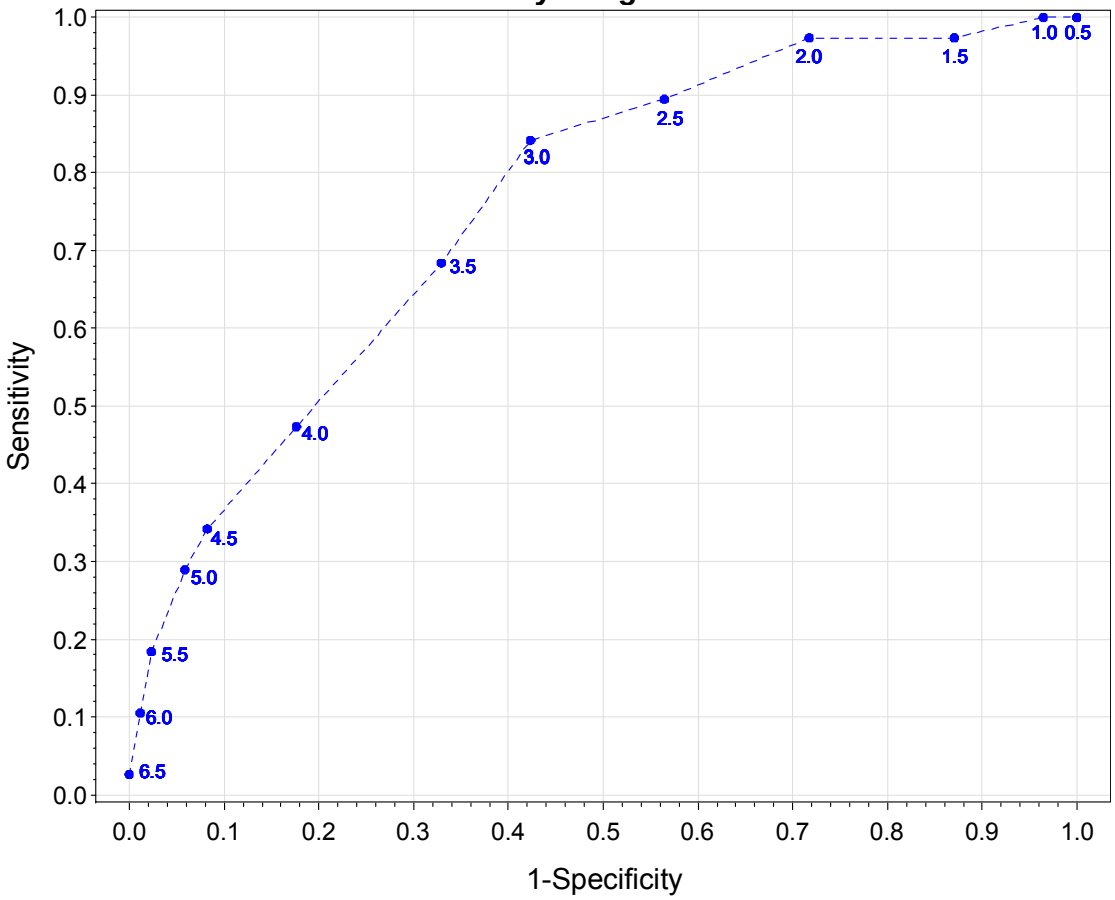
Table 5-6 Continued

Characteristic	*Reoperation Cohort (N=370)*		*Replacement Subgroup (N=127)*	
	Univariable	*Multivariable*	*Univariable*	*Multivariable*
Age at AVSD repair (yrs)	**0.0196**	**0.0492**	0.29	
Weight at AVSD repair (kg)	**0.0136**	NS	0.21	
CC time at AVSD repair (min)	0.07	Not included	**0.0071**	Not included
Cleft closure at AVSD repair	0.71		0.64	
Postop LAVVR				
None-Mild	reference		reference	
Mild-Moderate	0.48			
Moderate-Severe	0.98		0.38	
Postop mitral stenosis	0.64		0.10	
Postop complete heart block	0.13	NS	**0.0441**	
Time to first reoperation (yrs_	**0.0129**	NS	**0.0311**	
Age at first reoperation (yrs)	**0.0071**	**0.0241**	**0.0125**	
Weight at first reoperation (kg)	**0.0147**	0.67	**0.0056**	
Replacement reoperation performed	**0.0003**	**0.0002**	NA	
Time to replacement (yrs)	NA		**0.0079**	
Age at replacement (yrs)	NA		**0.0025**	0.69
Weight at replacement (kg)	NA		**0.0006**	0.26
Prosthetic valve size (mm)	NA		**0.0091**	
Valve size to body weight ratio (mm/kg)	NA		**<0.0001**	**0.0005**

Note: AVSD=atrioventricular septal defect, AV=atrioventricular, preop=preoperative, LAVVR=left atrioventricular valve regurgitation, CC time=cross-clamp time, postop=postoperative, wt=weight, NA=not applicable.

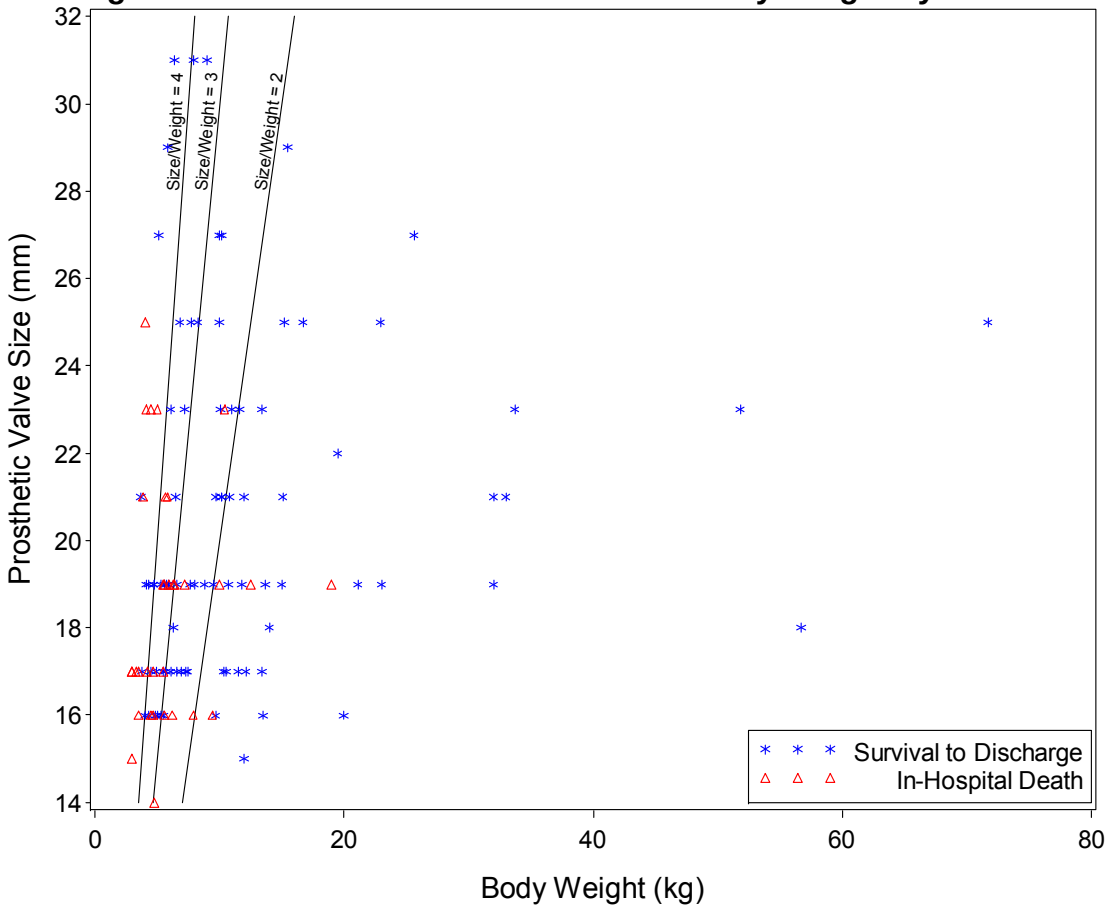
Significant p-values are displayed in bold. P-values not in bold type represent factors that were adjusted for in final model. NS=not significant. Cross-clamp time was not included in model fitting due to large number of missing values.

Figure 5-2: Receiver Operating Characteristic Curve for Prosthetic Valve Size to Body Weight Ratio



Note: Point labels refer to prosthetic valve size to body weight ratio.

Figure 5-3: Prosthetic Valve Size versus Body Weight by Outcome



Note: Lines representing constant size to weight ratios are plotted for reference.

The threshold value estimated by ROC curve analysis was further examined by constructing a dichotomous variable using a prosthetic valve size to body weight ratio of 3 mm/kg to include in multivariable models. The dichotomous variable remained significant (p=0.0002) in the multivariable model which included the presence of Down syndrome after adjustment for age and weight at replacement as well as era of primary AVSD repair (hazard ratio 2.9, 95% CI 1.46-5.75).

Figure 5-3 shows the prosthetic valve size versus body weight by outcome (in-hospital death and survival to discharge). Lines representing constant

prosthetic valve size to body weight ratios are also displayed. This scatterplot demonstrates that a majority of in-hospital deaths following valve replacement occurred in patients with a valve size to body weight ratio greater than 3 mm/kg.

Discussion

The first objective of this study was to describe children who underwent repair reoperation or replacement reoperation of the left AV valve following primary AVSD repair in terms of patient characteristics, as well as anatomic, operative, and outcome data. With stratification of the patients by type of reoperation, significant differences were noted between the repair reoperation and replacement reoperation subgroups. The replacement reoperation subgroup had a greater frequency of postoperative mitral stenosis. The mean age and weight of the replacement reoperation subgroup at the first reoperation were significantly less than the mean age and weight of the repair reoperation subgroup, possibly indicating that those who underwent a replacement surgery were sicker children. The mean time to first reoperation was also significantly lower in the replacement reoperation subgroup than in the repair reoperation subgroup. The frequency of Down syndrome was lower in the replacement reoperation subgroup, possibly due to prior practices of non-aggressiveness in treating patients with Down syndrome[514].

Multivariable factors associated with earlier time to reoperation included cleft closure during primary repair, moderate to severe postoperative LAVVR, and postoperative complete heart block. The association between earlier time to reoperation and cleft closure during primary repair likely reflects the routine practice of cleft closure when the LAVVR is severe. Multivariable factors associated with earlier time to replacement included Down syndrome and postoperative mitral stenosis.

The second objective of this study was to describe surgical outcomes and identify poor prognostic factors following left AV valve replacement in these children. Previous studies have described mortality rates as high as 52% following left AV valve replacement[49]. Complete heart block has also been reported to occur in approximately 30% of patients following left AV valve replacement. The present study identified an in-hospital mortality rate of 30%, with complete heart block occurring in 22% of patients following left AV valve replacement, consistent with previous reports.

Identification of poor prognostic factors should be used to improve decision-making when there is a choice among therapeutic strategies, thereby improving long-term results. Although the current practice is to treat all children with non-complex AVSDs aggressively, whether or not they also have Down syndrome, the association between Down syndrome and survival following surgical interventions for AVSD remains unclear. One study identified Down syndrome as a prognostic factor for better surgical outcomes following primary AVSD repair[515]. Another epidemiologic study reported that infants with AVSD and Down syndrome had a higher overall mortality rate following repair (20%) compared to non-Down syndrome infants with AVSD (5%)[516]. More recent studies have found no significant association between Down syndrome and mortality following repair[517-519]. To date, no studies have examined the association between Down syndrome and mortality following left AV valve replacement in patients who have previously undergone AVSD repair. The data from this study identified Down syndrome as an important predictor of in-hospital mortality.

An additional poor prognostic factor was an increased prosthetic valve size to body weight ratio. This would suggest that a larger prosthesis in a small infant is problematic and raises the concern that the disparity between the

prosthetic valve size and the left heart structure size may lead to anatomic obstruction of the left ventricular outflow tract, restriction of the prosthetic valve leaflets, and/or injury to the conduction system. As there is a general perception that left AV valve replacement in children is associated with a high mortality, it is possible that surgeons may attempt to oversize the prosthetic valve in order to allow for patient growth and delay additional valve replacements. Oversizing the left AV valve may increase the disparity between the prosthetic valve and the left heart which, given the results from this study, may be associated with increased mortality following replacement. The results of the present study confirmed the previous finding of increased prosthetic valve size to body weight ratio as a predictor of death in a study which evaluated left AV valve replacement in patients with all types of congenital heart defects[50].

ROC curve analysis was performed to determine a threshold value for the prosthetic valve size to body weight ratio. The cut-off was found to be 3 mm/kg with a sensitivity of 0.80 and a specificity of 0.57. The sensitivity, or true positive rate, represents the probability that there is a death following valve replacement when the ratio is greater than 3 mm/kg. The specificity, or true negative rate, represents the probability that the there is a survival to discharge when the ratio is less than 3 mm/kg.

The strengths of this study include the use of the PCCC database. This is a large, multi-institutional database which reduces the risk of selection bias as patients are representative of all regions of the United States. An additional strength of this study is that this is the largest investigation to date of left AV valve replacement following AVSD repair. A major limitation of this study is the availability of data. The data are collected by a voluntary registry which limits the complexity of the available data. For example, height information was unavailable for all patients in the replacement reoperation subgroup. Therefore,

body mass index could not calculated and used for standardization of the prosthetic valve size. As this registry relies on the submission of data from participating institutions, complete reports are not available for all patients. There are copies of operative and some echocardiogram reports, but no computerized format for these data has been implemented, thus hindering variable selection and completeness of data. Many of the variables included in this study are not available for all patients (e.g., AVSD primary repair and subsequent surgery cross-clamp times). An additional limitation of this study is the limited follow-up data available for each patient. Routine follow-up data submission is not performed; survival was determined by status of the last reoperation contained in the database. Long-term survival, therefore, was unable to be determined accurately.

Conclusions

This represents the first study evaluating left AV valve replacements in patients who have undergone primary AVSD repair. Significant findings include a lower frequency of patients with Down syndrome undergoing left AV valve replacement versus repair and a higher frequency of postoperative mitral valve stenosis in patients who underwent left AV valve replacement. Multivariable age-, weight, and surgical era-adjusted predictors of earlier time to valve replacement included presence of Down syndrome and postoperative mitral stenosis. Survival of patients following left AV valve replacement was found to be significantly worse compared to those who underwent repair. Multivariable predictors of in-hospital death following valve replacement included the presence of Down syndrome and a prosthetic valve size to body weight ratio greater than 3 mm/kg. The ability to predict outcomes following left AV valve replacement in patients who have previously undergone primary AVSD repair may be useful in choosing between

valve repair and replacement strategies.

CHAPTER 6
STUDY CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

While congenital heart defects (CHDs) constitute a major proportion of clinically significant birth defects, knowledge regarding etiologies, both genetic and environmental, and prognostic factors remains relatively unknown. This dissertation research focused on a specific congenital heart defect, atrioventricular septal defects (AVSDs), using three different databases to conduct the research.

The first paper, “Is a Shorter Atrioventricular Septal Length an Intermediate Phenotype in the Spectrum of Non-Syndromic Atrioventricular Septal Defects?”, focused on determining if a shorter atrioventricular septal length might be an intermediate phenotype in the spectrum of non-syndromic atrioventricular septal defects. Using the Family Study of Endocardial Cushion Defects, the atrioventricular septal length (AVSL) was measured in echocardiograms of parents and siblings of non-syndromic and syndromic AVSD case children, as well as in parents and siblings of control children. Following standardization of the AVSL by body surface area, the standardized AVSL (sAVSL) was found to be significantly shorter in the parents and siblings of AVSD case children in comparison to the parents and siblings of control children. There was no significant difference between the means of the sAVSL in parents of non-syndromic AVSD case children and parents of syndromic AVSD case children, although there was a trend of a shorter mean in the parents of non-syndromic AVSD cases. Siblings of non-syndromic AVSD cases were noted to have a shorter mean sAVSL when compared to the mean of siblings of syndromic AVSD cases. The finding of different AVSL measurements in family members of non-syndromic and syndromic AVSD cases provides additional evidence of distinct etiologies for each defect.

The distributions of the age- and gender-adjusted BSA-standardized AVSL (asAVSL) for each study group were also tested for evidence of multiple component distributions. There was significant evidence for two components in the parents and siblings of non-syndromic AVSD cases. Evidence for two component distributions from this analysis suggests the presence of an intermediate phenotype for non-syndromic AVSD, with a majority of the population in the distribution with the lower (shorter) asAVSL measurements. Based on the distribution of sAVSL measurements in the control families, possible thresholds for a shortened sAVSL measurement can be suggested. Possible cut-off points based on the age-adjusted sAVSL measurement distributions in control families would be 2.93 mm/m^2 for male parents and 2.21 mm/m^2 for male siblings, 3.15 mm/m^2 for female parents and 3.00 mm/m^2 for female siblings (2 standard deviations below the mean). .

Heritability of asAVSL was estimated for each study group. The high estimated heritability in the control group (0.68), suggests that shared genetic variants or quantitative trait loci likely explain a substantial portion of the familial aggregation. It is interesting to note that the heritability in the non-syndromic and syndromic families was slightly lower (0.44 and 0.43, respectively), suggesting that the additive (polygenic) model may not be sufficient to explain the inheritance of AVS length. In addition, the syndromic and non-syndromic case family heritability may be different than the control family heritability due to major genetic effects.

The second paper, “Risk Factors Associated with Non-Syndromic Atrioventricular Septal Defects”, identified parental and environmental risk factors associated with non-syndromic AVSDs using the National Birth Defects Prevention Study database. Subgroup analyses investigating risk factor associations within the complete AVSD, isolated complete AVSD, spectrum

AVSD, and isolated spectrum AVSD subgroups, were also performed.

There was suggestive evidence of associations between AVSDs and antibacterial, antiviral, antifungal, and gastrointestinal medication use. No associations were observed between AVSDs and urinary tract infection, pelvic inflammatory disease, antidepressant medication use, allergy and asthma medication use, analgesic and antipyretic medication use, maternal occupational exposures, maternal alcohol consumption, or parental illicit drug use during the periconceptional period.

Women who reported smoking during the periconceptional period were more likely to have an infant with an AVSD than women who did not smoke during the same time period. This relationship was independent of potential confounding factors, including study site, maternal age, maternal race, alcohol consumption during the periconceptional period, and family history of CHDs. This association was also noted in the complete AVSD and isolated complete AVSD subgroups. In addition, women who reported passive smoke exposure, either at home or work, during the periconceptional period, were more likely to have an infant with an AVSD. This association was seen in the complete AVSD and isolated complete AVSD subgroups, and was independent of potential confounding factors, including study site, maternal age, maternal race, gestational age, infant birthweight, active smoke exposure, and family history of CHDs.

Future investigations into the genetic susceptibilities that could modify these risks on the developing fetal heart will provide further evidence with which primary prevention strategies can be developed. Additional investigations are needed to evaluate the association between specific medications and AVSDs for improved prevention strategies.

The third paper, “Left Atrioventricular Valve Reoperation Following

Primary Atrioventricular Septal Defect Repair", focused on reoperations of the left atrioventricular (AV) valve following primary AVSD repair. Using the Pediatric Cardiac Care Consortium database, descriptive analyses of reoperation characteristics were performed. Significant findings included a lower frequency of patients with Down syndrome who underwent left AV valve replacement versus repair and a higher frequency of postoperative mitral valve stenosis in patients who underwent left AV valve replacement.

Multivariable age- and weight-adjusted predictors of earlier time to reoperation included closure of the mitral valve cleft during the primary AVSD repair, presence of moderate to severe postoperative left AV valve regurgitation following primary AVSD repair, and presence of postoperative complete heart block following primary AVSD repair. Multivariable age- and weight-adjusted predictors of earlier time to replacement included Down syndrome and postoperative mitral valve stenosis following primary AVSD repair.

Factors predictive of in-hospital death following reoperation included earlier age at primary AVSD repair, undergoing a replacement reoperation, and earlier age at reoperation. Factors predictive of in-hospital death following replacement included Down syndrome and larger prosthetic valve size to body weight ratio. A prosthetic valve size to body weight ratio lower than 3 mm/kg was defined as the optimal cut-off using receiver-operating characteristic analysis. The ability to predict outcomes following left AV valve replacement in patients who have previously undergone primary AVSD repair may be useful in choosing between valve replacement and repair strategies.

These three studies represent investigations into the etiologies of AVSDs and prognostic factors of AVSD treatment outcomes. As has been detailed in the preceding chapters, detailed knowledge regarding this defect remains largely unknown due to the relative rarity of AVSDs.

Future studies of an intermediate phenotype described in Chapter 3 should include a replication study involving parents and siblings of non-syndromic AVSD cases using digitized echocardiograms. This approach will provide more precise measurements as the echocardiographic technology has continued to improve since the original study was conducted. Additional directions include further investigations regarding the transmission of shortening alleles under the threshold model. It may be that both parents in the non-syndromic families have a slightly shorter septum and have each transmitted a number of AVSL shortening alleles that in combination are sufficient to result in an AVSD phenotype in their offspring, or that one of the parents with a much shorter septum transmitted a sufficient number of AVSL shortening alleles in addition to those transmitted by the parent with a "normal" septum. This may aid in the identification of genetic variants associated with the shortened AVSL phenotype. Additionally, identification of an intermediate phenotype will aid in the identification of susceptibility genes for AVSDs by increasing the power of genetic association and mapping studies as relatives of case children who do not have a defect that is part of the recognized spectrum of AVSDs could now be classified as affected using an expanded definition.

Based on the findings from the study described in Chapter 4 which examined risk factors associated with AVSDs, future studies should include detailing the timing of the exposures, extent of exposures, as well as specific exposures (e.g., specific medications within the larger class). These studies are difficult to perform due to small numbers of cases with positive responses. Genetic susceptibilities should also be investigated to aid in developing primary prevention strategies.

Future investigations of the prognostic risk factors include performing a replication study with a larger sample size; however, this would be a

considerable challenge since there are limited databases which contain information regarding congenital heart disease surgeries. Additional risk factors such as race, by-pass time, and mitral valve annulus size that were unavailable in the database utilized for the study in Chapter 5 might be included in the analyses.

These studies have added information regarding a possible intermediate phenotype. This investigation was innovative as an intermediate phenotype in parents of AVSD children had not been previously considered. These studies have also demonstrated evidence of additional environmental risk factors associated with AVSDs. To date, no other studies had identified the relationship between passive smoke exposure and AVSDs. Finally, these studies identified prognostic risk factors for left AV valve replacement following primary AVSD repair, which had not been previously examined.

In summary, by adding to our knowledge of the AVSD familial and environmental risk factors from these studies and future investigations, we will be able to (1) improve genetic counseling, (2) identify other family members for genetic testing, (3) begin to devise primary prevention strategies, and (4) improve treatment modalities. By recognizing prognostic factors which influence survival, optimal patient care can be devised which will not only improve treatment modalities, but also long-term survival.

APPENDIX

Table A1: Univariable and Multivariable Regression Analysis of Standardized AVSL (ß Parameter Estimates and Standard Errors) in Non-Syndromic AVSD Case Parents, Siblings, and Families

	Parents		*Siblings*		*Families*	
	Univariable	*Multivariable*	*Univariable*	*Multivariable*	*Univariable*	*Multivariable*
Age	-0.0023 (0.0012)	NS	-0.0081 (0.0017)	-0.0085 (0.0017)	-0.0048 (0.0006)	-0.0048 (0.0006)
Gender	0.0486 (0.0226)	NS	0.0508 (0.0383)	0.0687 (0.0341)	0.0427 (0.0222)	0.0459 (0.0198)

Note: AVSL=atrioventricular septal length, AVSD=atrioventricular septal defect, NS=not significant.

Data are expressed as ß Parameter Estimate (Standard Error).

Table A2: Univariable and Multivariable Regression Analysis of Standardized AVSL (ß Parameter Estimates and Standard Errors) in Syndromic AVSD Case Parents, Siblings, and Families

	Parents		*Siblings*		*Families*	
	Univariable	*Multivariable*	*Univariable*	*Multivariable*	*Univariable*	*Multivariable*
Age	-0.0004 (0.0008)	NS	-0.0120 (0.0023)	-0.0122 (0.0023)	-0.0060 (0.0006)	-0.0059 (0.0006)
Gender	0.0330 (0.0173)	NS	0.0707 (0.0452)	0.0818 (0.0405)	0.0531 (0.0247)	0.0457 (0.0215)

Note: AVSL=atrioventricular septal length, AVSD=atrioventricular septal defect, NS=not significant.

Data are expressed as ß Parameter Estimate (Standard Error).

Table A3: Univariable and Multivariable Regression Analysis of Standardized AVSL (ß Parameter Estimates and Standard Errors) in Control Parents, Siblings, and Families

	Parents		*Siblings*		*Families*	
	Univariable	*Multivariable*	*Univariable*	*Multivariable*	*Univariable*	*Multivariable*
Age	-0.0016 (0.0022)	NS	-0.0222 (0.0041)	NS	-0.0067 (0.0008)	NS
Gender	0.0122 (0.0279)	NS	0.0443 (0.0438)	NS	0.0173 (0.0278)	NS

Note: AVSL=atrioventricular septal length, AVSD=atrioventricular septal defect, NS=not significant.

Data are expressed as ß Parameter Estimate (Standard Error).

Figure A1: Selected Sections from the NBDPS Interview Questionnaire – UTI and PID

MATERNAL HEALTH-INFECTIONS

B47. Between (-3) and ([DOIB] / [DOPT]), did you have any of the following illnesses…? READ LIST

A. a kidney, bladder, or urinary tract infection?
YES 1
NO 2
DK 8

B. or did you have pelvic inflammatory disease or PID?
YES 1
NO 2
DK 8

IF NO TO BOTH A AND B, SKIP TO B59.
FOR EACH YES, ASK B48-B58.

	B48. Was the (infection/ PID) diagnosed by a doctor?	B49. During which months did you have the illness? MO	YES	NO	DK	B50. When you were sick with (infection/PID), did you have a fever?
A. kidney, bladder, or urinary tract infection (UTI)	YES ..... 1 NO ..... 2 DK 8	B3	1	2	8	YES ..... 1 NO....(SKIP TO B53) ... 2 DK....(SKIP TO B53) ... 8
		B2	1	2	8	
		B1	1	2	8	
		P1	1	2	8	
		P2	1	2	8	
		P3	1	2	8	
		T2	1	2	8	
		T3	1	2	8	
B. PID	YES ..... 1 NO ..... 2 DK 8	B3	1	2	8	YES ..... 1 NO....(SKIP TO B53) ... 2 DK....(SKIP TO B53) ... 8
		B2	1	2	8	
		B1	1	2	8	
		P1	1	2	8	
		P2	1	2	8	
		P3	1	2	8	
		T2	1	2	8	
		T3	1	2	8	

Figure A2: Selected Sections from the NBDPS Interview Questionnaire – Active Tobacco Smoke Exposure

SECTION E: TOBACCO-MOTHER

E1. The next questions are about tobacco use. Did you ever smoke cigarettes?

YES 1
NO ..... (SKIP TO E5) 2
DK ..... (SKIP TO E5) 8

E2. At any time from (-3) to (DOIB), did you smoke cigarettes?

YES 1
NO ..... (SKIP TO E5) 2
DK ..... (SKIP TO E5) 8

E3. During which months did you smoke?
CIRCLE FOR EACH MONTH. DO NOT CODE SHADED AREA.

MO	YES (ASK E4)	NO	DK	E4. During (SPECIFY MONTH) about how many cigarettes did you smoke a day?/Did you continue to smoke that many cigarettes through (LAST MONTH STATED)?
B3	1	2	8	<1/DAY ..... 01 1/DAY ..... 02 2-4/DAY ..... 03 ½ PACK (5-14) ..... 04 1 PACK(15-24) ..... 05 1 ½ PACK (25-34) ..... 06 2 PACK (35-44) ..... 07 >2 PACK ..... 08 DK 98
B2	1	2	8	<1/DAY ..... 01 1/DAY ..... 02 2-4/DAY ..... 03 ½ PACK (5-14) ..... 04 1 PACK(15-24) ..... 05 1 ½ PACK (25-34) ..... 06 2 PACK (35-44) ..... 07 >2 PACK ..... 08 DK 98
B1	1	2	8	<1/DAY ..... 01 1/DAY ..... 02 2-4/DAY ..... 03 ½ PACK (5-14) ..... 04 1 PACK(15-24) ..... 05 1 ½ PACK (25-34) ..... 06 2 PACK (35-44) ..... 07 >2 PACK ..... 08 DK 98
P1	1	2	8	<1/DAY ..... 01 1/DAY ..... 02 2-4/DAY ..... 03 ½ PACK (5-14) ..... 04 1 PACK(15-24) ..... 05 1 ½ PACK (25-34) ..... 06 2 PACK (35-44) ..... 07 >2 PACK ..... 08 DK 98
P2	1	2	8	<1/DAY ..... 01 1/DAY ..... 02 2-4/DAY ..... 03 ½ PACK (5-14) ..... 04 1 PACK(15-24) ..... 05 1 ½ PACK (25-34) ..... 06 2 PACK (35-44) ..... 07 >2 PACK ..... 08 DK 98

Figure A2 Continued

MO	YES (ASK E4)	NO	DK	E4. During (SPECIFY MONTH) about how many cigarettes did you smoke a day?/Did you continue to smoke that many cigarettes through (LAST MONTH STATED)?
P3	1	2	8	<1/DAY ..... 01 1/DAY ..... 02 2-4/DAY ..... 03 ½ PACK (5-14) ..... 04 1 PACK(15-24) ..... 05 1 ½ PACK (25-34) ..... 06 2 PACK (35-44) ..... 07 >2 PACK ..... 08 DK 98
T2	1	2	8	<1/DAY ..... 01 1/DAY ..... 02 2-4/DAY ..... 03 ½ PACK (5-14) ..... 04 1 PACK(15-24) ..... 05 1 ½ PACK (25-34) ..... 06 2 PACK (35-44) ..... 07 >2 PACK ..... 08 DK 98
T3	1	2	8	<1/DAY ..... 01 1/DAY ..... 02 2-4/DAY ..... 03 ½ PACK (5-14) ..... 04 1 PACK(15-24) ..... 05 1 ½ PACK (25-34) ..... 06 2 PACK (35-44) ..... 07 >2 PACK ..... 08 DK 98

Figure A3: Selected Sections from the NBDPS Interview Questionnaire – Passive Tobacco Smoke Exposure

TOBACCO-HOUSEHOLD

E5. Did anyone in your household smoke cigarettes in your home between (-3) and (DOIB)?

YES 1
NO ..... (SKIP TO E7) 2
DK ..... (SKIP TO E7) 8

E6. During which months did someone smoke in your home? CIRCLE FOR EACH MONTH. DO NOT CODE SHADED AREA.

MO	YES	NO	DK
B3	1	2	8
B2	1	2	8
B1	1	2	8
P1	1	2	8
P2	1	2	8
P3	1	2	8
T2	1	2	8
T3	1	2	8

TOBACCO-WORKPLACE

E7. Did anyone smoke cigarettes near you at a workplace or school you may have attended during that year?

YES 1
NO ..... (SKIP TO F1) 2
DK ..... (SKIP TO F1) 8

E8. During which months from (-3) to (DOIB) did someone smoke near you at work/school? CIRCLE FOR EACH MONTH. DO NOT CODE SHADED AREA.

MO	YES	NO	DK
B3	1	2	8
B2	1	2	8
B1	1	2	8
P1	1	2	8
P2	1	2	8
P3	1	2	8
T2	1	2	8
T3	1	2	8

Figure A4: Selected Sections from the NBDPS Interview Questionnaire – Alcohol Use

SECTION F: ALCOHOL

F1. Now I'm going to ask you some questions about drinking alcoholic beverages. We define an alcoholic drink as one beer, one glass of wine, one mixed drink, or one shot of liquor. Between (-3) and (DOIB), did you drink any wine, beer, mixed drinks or shots of liquor?

YES.....1
NO.....(SKIP TO G1).....2
DK.....(SKIP TO G1).....8
RF.....(SKIP TO G1).....7

MO	F2. During which months did you drink any alcoholic beverages? **CIRCLE FOR EACH MONTH. DO NOT CODE SHADED AREA.** YES (ASK F3-F5)	NO (NXT)	DK (NXT)	F3. In the (3rd/2nd/1st month before pregnancy, 1st/2nd/3rd…9th month of pregnancy), on average, how many **days** did you drink alcoholic beverages? (DK = 98) (RF = 97) # DAYS	F4. On those days that you drank alcoholic beverages, on average, how many drinks did you have per day? (DK = 98) (RF = 97) # DRINKS	F5. What was the greatest number of drinks you had on one occasion in (MONTH)? (DK = 98) (RF = 97) # DRINKS
B3	1	2	8	☐☐	☐☐	☐☐
B2	1	2	8	☐☐	☐☐	☐☐
B1	1	2	8	☐☐	☐☐	☐☐
P1	1	2	8	☐☐	☐☐	☐☐
P2	1	2	8	☐☐	☐☐	☐☐
P3	1	2	8	☐☐	☐☐	☐☐
T2	1	2	8	☐☐	☐☐	☐☐
T3	1	2	8	☐☐	☐☐	☐☐

F6. On the days that you drank alcohol, what type(s) of alcohol did you usually drink? READ CHOICES.

	YES	NO	RF	DK
a. Beer	1	2	7	8
b. Wine	1	2	7	8
c. Mixed drink	1	2	7	8
d. Shot liquor	1	2	7	8
e. Other alcohol	1	2	7	8

SPECIFY: _____

Figure A5: Selected Sections from the NBDPS Interview Questionnaire – Substance Abuse

SECTION G: SUBSTANCE ABUSE-FATHER

IF FATHER UNKNOWN, SKIP TO G7.
Now I am going to ask you about recreational drug use.

G1. Between (-3) and (DOIB), did (NOIB)'s father use any of the following recreational or street drugs?

	YES	NO	RF	DK
a. Marijuana	1	2	7	8
b. Hash	1	2	7	8
c. Cocaine	1	2	7	8
d. Crack	1	2	7	8
e. Hallucinogens like LSD or 'acid'	1	2	7	8
f. Heroin	1	2	7	8
g. Hallucinogenic Mushrooms	1	2	7	8

G2. Between (-3) and (DOIB), did (NOIB)'s father use anything else to get high?

YES 1
NO 2
DK 8
RF 7

G3. What did he use? / Anything else? SPECIFY.

SPECIFY: _____

FOR EACH "YES" ITEM FROM G1 AND G3, ASK G4 TO G6. IF ALL NO OR DK, SKIP TO G7.

Figure A5 Continued

SUBSTANCE ABUSE-MOTHER

Now I would like to ask you about any recreational drugs you may have used.

G7. Between (-3) and (DOIB), did you use any of the following recreational or street drugs?

	YES	NO	RF	DK
a. Marijuana	1	2	7	8
b. Hash	1	2	7	8
c. Cocaine	1	2	7	8
d. Crack	1	2	7	8
e. Hallucinogens like LSD or 'acid'	1	2	7	8
f. Heroin	1	2	7	8
g. Hallucinogenic Mushrooms	1	2	7	8

G8. Between (-3) and (DOIB), did you use anything else to get high?

YES 1
NO 2
DK 8
RF 7

G9. What did you use? / Anything else? SPECIFY.

SPECIFY: _____

FOR EACH "YES" ITEM FROM G7 AND G9, ASK G10 TO G12. IF ALL NO OR DK, SKIP TO H1.

Figure A6: Selected Sections from the NBDPS Interview Questionnaire – Maternal Occupational Exposures

MOTHER'S OCCUPATION-2

I12. In your job(s) between (-3) and (DOIB) did you work with or make (READ CHOICES)? CONDITION	YES	NO (NXT)	DK (NXT)	I13. What was the name of the product?	I14. During which months did you use (PRODUCT)? MO	YES	NO	DK	I15. How many hours or minutes per week were you around the product? <ONCE/WK = 995 ONE TIME EXPOSURE = 996 MIN	HRS	DK
a. anesthetic gases.....	1	2	8	_____ DK..... ❑	B3	1	2	8	❑❑	1	2 ❑
					B2	1	2	8	❑❑	1	2 ❑
					B1	1	2	8	❑❑	1	2 ❑
					P1	1	2	8	❑❑	1	2 ❑
					P2	1	2	8	❑❑	1	2 ❑
					P3	1	2	8	❑❑	1	2 ❑
					T2	1	2	8	❑❑	1	2 ❑
					T3	1	2	8	❑❑	1	2 ❑
b. ionizing radiation, such as x-rays.....	1	2	8	_____ DK..... ❑	B3	1	2	8	❑❑	1	2 ❑
					B2	1	2	8	❑❑	1	2 ❑
					B1	1	2	8	❑❑	1	2 ❑
					P1	1	2	8	❑❑	1	2 ❑
					P2	1	2	8	❑❑	1	2 ❑
					P3	1	2	8	❑❑	1	2 ❑
					T2	1	2	8	❑❑	1	2 ❑
					T3	1	2	8	❑❑	1	2 ❑
c. heavy metals (such as lead, mercury, nickel)	1	2	8	_____ DK..... ❑	B3	1	2	8	❑❑	1	2 ❑
					B2	1	2	8	❑❑	1	2 ❑
					B1	1	2	8	❑❑	1	2 ❑
					P1	1	2	8	❑❑	1	2 ❑
					P2	1	2	8	❑❑	1	2 ❑
					P3	1	2	8	❑❑	1	2 ❑
					T2	1	2	8	❑❑	1	2 ❑
					T3	1	2	8	❑❑	1	2 ❑

Figure A6 Continued

I12. In your job(s) between (-3) and (DOIB) did you work with or make (READ CHOICES)? CONDITION	YES	NO (NXT)	DK (NXT)	I13. What was the name of the product?	I14. During which months did you use (PRODUCT)? MO	YES	NO	DK	I15. How many hours or minutes per week were you around the product? <ONCE/WK = 995 ONE TIME EXPOSURE = 996	MIN	HRS	DK
d. pesticides, herbicides, fungicides, insecticides or rat poison	1	2	8	_____ DK ❑	B3	1	2	8	❑❑	1	2	❑
					B2	1	2	8	❑❑	1	2	❑
					B1	1	2	8	❑❑	1	2	❑
					P1	1	2	8	❑❑	1	2	❑
					P2	1	2	8	❑❑	1	2	❑
					P3	1	2	8	❑❑	1	2	❑
					T2	1	2	8	❑❑	1	2	❑
					T3	1	2	8	❑❑	1	2	❑
e. solvents like paint thinners, auto fluids, toluene, carbon disulfide or carbon tetrachloride	1	2	8	_____ DK ❑	B3	1	2	8	❑❑	1	2	❑
					B2	1	2	8	❑❑	1	2	❑
					B1	1	2	8	❑❑	1	2	❑
					P1	1	2	8	❑❑	1	2	❑
					P2	1	2	8	❑❑	1	2	❑
					P3	1	2	8	❑❑	1	2	❑
					T2	1	2	8	❑❑	1	2	❑
					T3	1	2	8	❑❑	1	2	❑

Table A4: Characteristics of Complete AVSD Case and Control Participants

Variable	*Case Participants (N=78) N (%)*	*Control Participants (N=6703) N (%)*	*Adjusted OR† (95% CI)*
Maternal age			
< 18 years	2 (2.56)	245 (3.65)	0.72 (0.18-2.94)
18-39 years	73 (93.59)	6318 (94.26)	Reference
≥ 40 years	3 (3.85)	140 (2.09)	1.85 (0.58-5.95)
Maternal race			
White	32(68.09)	2294 (63.21)	Reference
Black	10 (21.28)	442 (21.18)	1.55 (0.75-3.23)
Hispanic	4 (8.51)	783 (21.58)	0.36 (0.12-1.01)
Other	1 (2.13)	110 (3.03)	0.66 (0.09-4.86)
Body Mass Index			
Underweight	4 (5.19)	356 (5.53)	1.17 (0.41-3.30)
Normal	35 (45.45)	3593 (55.79)	Reference
Overweight/Obese	38 (49.35)	2491 (38.68)	1.57 (0.92-2.68)
Parity			
Primipara	22 (53.66)	2244 (56.02)	0.92 (0.49-1.70)
Multipara	19 (46.34)	1762 (43.98)	Reference
Maternal education			
< High school	6 (7.69)	1128 (16.85)	Reference
High School education	51 (65.38)	3248 (48.52)	**2.95 (1.26-6.89)**
Technical college	4 (5.13)	208 (3.11)	**3.61 (1.01-12.91)**
≥ College education	17 (21.79)	2110 (31.52)	1.48 (0.58-3.77)
Maternal job status			
Yes	66 (84.62)	4825 (72.04)	**2.16 (1.16-4.00)**
No	12 (15.38)	1873 (27.96)	Reference
Gestational age			
< 37 weeks	18 (23.08)	635 (9.47)	**2.88 (1.69-4.91)**
≥ 37 weeks	60 (76.92)	6067 (90.53)	Reference
Birthweight			
< 2.5 grams	12 (15.38)	392 (5.87)	**2.92 (1.57-5.45)**
≥ 2.5 grams	66 (84.62)	6286 (94.13)	Reference
Gender			
Female	45 (57.69)	3309 (49.04)	1.40 (0.89-2.19)
Male	33 (42.31)	3389 (50.60)	Reference
Family history of birth defects			
Yes	31 (39.74)	1717 (25.86)	**1.90 (1.20-3.00)**
No	47 (60.26)	4923 (74.14)	Reference
Family history of CHD			
Yes	14 (17.95)	212 (3.16)	**6.73 (3.71-12.20)**
No	64 (82.05)	6491 (96.84)	Reference

Note: CHD=congenital heart defects. †=Odds ratios adjusted for study site.

Table A5: Characteristics of Isolated Complete AVSD Case and Control Participants

Variable	*Case Participants (N=65) N (%)*	*Control Participants (N=6703) N (%)*	*Adjusted OR† (95% CI)*
Maternal age			
< 18 years	2 (3.08)	245 (3.65)	0.88 (0.22-3.64)
18-39 years	60 (92.31)	6318 (94.26)	Reference
≥ 40 years	3 (4.62)	140 (2.09)	2.26 (0.70-7.30)
Maternal race			
White	25 (65.79)	2294 (63.21)	Reference
Black	9 (23.68)	442 (21.18)	1.64 (0.75-3.63)
Hispanic	3 (7.89)	783 (21.58)	0.32 (0.10-1.07)
Other	1 (2.63)	110 (3.03)	0.86 (0.96-6.41)
Body Mass Index			
Underweight	3 (4.69)	356 (5.53)	0.99 (0.30-3.27)
Normal	31 (48.44)	3593 (55.79)	Reference
Overweight/Obese	30 (46.88)	2491 (38.68)	1.29 (0.70-2.36)
Parity			
Primipara	20 (64.52)	2244 (56.02)	1.45 (0.69-3.03)
Multipara	11 (35.48)	1762 (43.98)	Reference
Maternal education			
< High School	6 (9.23)	1128 (16.85)	Reference
High School education	40 (61.54)	3248 (48.52)	2.31 (0.98-5.47)
Technical college	4 (6.15)	208 (3.11)	**3.62 (1.01-12.93)**
≥ College education	15(23.08)	2110 (31.52)	1.29 (0.50-3.35)
Maternal job status			
Yes	54 (83.08)	4825 (72.04)	**1.94 (1.01-3.72)**
No	11 (16.92)	1873 (27.96)	Reference
Gestational age			
< 37 weeks	10 (13.85)	635 (9.47)	1.75 (0.89-3.45)
≥ 37 weeks	55 (84.62)	6067 (90.53)	Reference
Birthweight			
< 2.5 grams	9 (13.85)	392 (5.87)	**2.58 (1.27-5.26)**
≥ 2.5 grams	56 (86.15)	6286 (94.13)	Reference
Gender			
Female	37 (56.92)	3309 (49.04)	1.35 (0.83-2.21)
Male	28 (43.08)	3389 (50.60)	Reference
Family history of birth defects			
Yes	2 (40.00)	1717 (25.86)	**1.92 (1.16-3.16)**
No	39 (60.00)	4923 (74.14)	Reference
Family history of CHD			
Yes	12 (18.46)	212 (3.16)	**6.99 (3.68-13.29)**
No	53 (81.54)	6491 (96.84)	Reference

Note: CHD=congenital heart defects. †=Odds ratios adjusted for study site.

Table A6: Characteristics of Spectrum AVSD Case and Control Participants

Variable	*Case Participants (N=109) N (%)*	*Control Participants (N=6703) N (%)*	*Adjusted OR† (95% CI)*
Maternal age			
< 18 years	2 (1.83)	245 (3.65)	0.48 (0.12-1.97)
18-39 years	105 (96.33)	6318 (94.26)	Reference
≥ 40 years	2 (1.83)	140 (2.09)	0.86 (0.21-3.54)
Maternal race			
White	43 (71.67)	2294 (63.21)	Reference
Black	9 (15.00)	442 (21.18)	1.01 (0.48-2.12)
Hispanic	7 (11.67)	783 (21.58)	0.45 (0.20-1.02)
Other	1 (1.67)	110 (3.03)	0.49 (0.07-3.62)
Body Mass Index			
Underweight	5 (4.72)	356 (5.53)	0.78 (0.31-1.95)
Normal	64 (60.38)	3593 (55.79)	Reference
Overweight/Obese	37 (34.90)	2491 (38.68)	0.73 (0.44-1.23)
Parity			
Primipara	50 (74.63)	2244 (56.02)	**2.29 (1.32-3.99)**
Multipara	17 (25.37)	1762 (43.98)	Reference
Maternal education			
< High School	9 (8.26)	1128 (16.85)	Reference
High School education	58 (53.21)	3248 (48.52)	**2.24 (1.11-4.54)**
Technical college	9 (8.26)	208 (3.11)	**5.42 (2.13-13.83)**
≥ College education	33 (30.28)	2110 (31.52)	2.01 (0.96-4.21)
Maternal job status			
Yes	89 (81.65)	4825 (72.04)	**1.72 (1.05-2.80)**
No	20 (18.35)	1873 (27.96)	Reference
Gestational age			
< 37 weeks	21 (19.27)	635 (9.47)	**2.27 (1.40-3.68)**
≥ 37 weeks	88 (80.73)	6067 (90.53)	Reference
Birthweight			
< 2.5 grams	24 (22.43)	392 (5.87)	**4.64 (2.91-7.39)**
≥ 2.5 grams	83 (77.57)	6286 (94.13)	Reference
Gender			
Female	59 (54.13)	3309 (49.04)	1.21 (0.83-1.78)
Male	50 (45.87)	3389 (50.60)	Reference
Family history of birth defects			
Yes	38 (34.86)	1717 (25.86)	**1.53 (1.03-2.28)**
No	71 (65.14)	4923 (74.14)	Reference
Family history of CHD			
Yes	15 (13.76)	212 (3.16)	**4.88 (2.78-8.56)**
No	94 (86.24)	6491 (96.84)	Reference

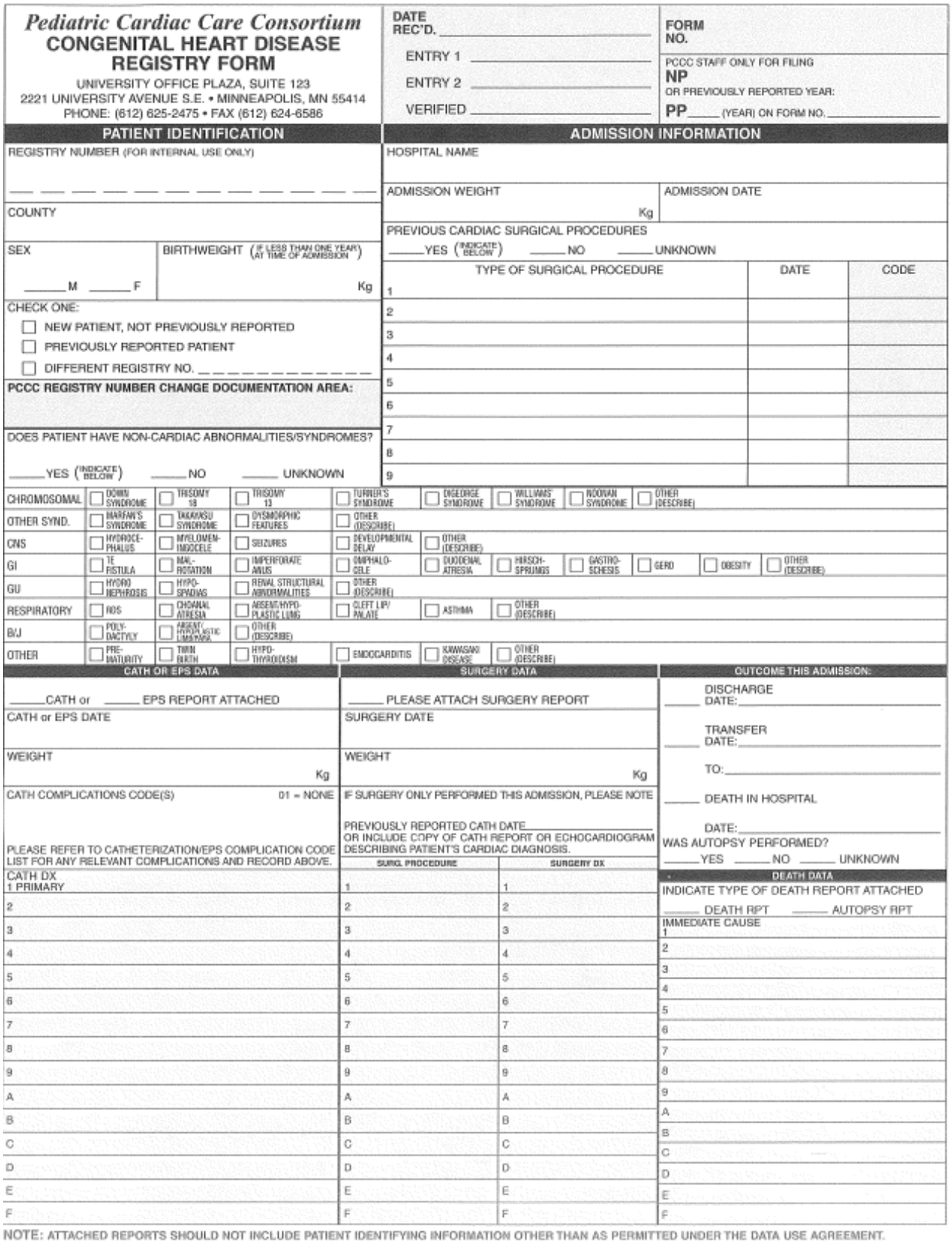
Note: CHD=congenital heart defects. †=Odds ratios adjusted for study site.

Table A7: Characteristics of Isolated Spectrum AVSD Case and Control Participants

Variable	*Case Participants (N=81) N (%)*	*Control Participants (N=6703) N (%)*	*Adjusted OR† (95% CI)*
Maternal age			
< 18 years	2 (2.47)	245 (3.65)	0.64 (0.16-2.61)
18-39 years	79 (97.53)	6318 (94.26)	Reference
≥ 40 years	0	140 (2.09)	Not calculable
Maternal race			
White	35 (76.09)	2294 (63.21)	Reference
Black	7 (15.22)	442 (21.18)	1.04 (0.46-2.39)
Hispanic	4 (8.70)	783 (21.58)	0.34 (0.12-0.95)
Other	0	110 (3.03)	Not calculable
Body Mass Index			
Underweight	4 (5.06)	356 (5.53)	0.84 (0.30-2.36)
Normal	47 (59.49)	3593 (55.79)	Reference
Overweight/Obese	28 (35.45)	2491 (38.68)	0.79 (0.44-1.41)
Parity			
Primipara	37 (78.72)	2244 (56.02)	**2.87 (1.42-5.78)**
Multipara	10 (21.28)	1762 (43.98)	Reference
Maternal education			
< High School	7 (8.64)	1128 (16.85)	Reference
High School education	44 (54.32)	3248 (48.52)	2.19 (0.98-4.87)
Technical college	7 (8.64)	208 (3.11)	**5.43 (1.88-15.63)**
≥ College education	23 (28.40)	2110 (31.52)	1.82 (0.78-4.26)
Maternal job status			
Yes	67 (82.72)	4825 (72.04)	**1.84 (1.03-3.28)**
No	14 (17.28)	1873 (27.96)	Reference
Gestational age			
< 37 weeks	10 (12.35)	635 (9.47)	1.34 (0.69-2.60)
≥ 37 weeks	71 (87.65)	6067 (90.53)	Reference
Birthweight			
< 2.5 grams	13 (16.25)	392 (5.87)	**3.11 (1.70-5.69)**
≥ 2.5 grams	67 (83.75)	6286 (94.13)	Reference
Gender			
Female	46 (56.79)	3309 (49.04)	1.35 (0.87-2.10)
Male	35 (43.21)	3389 (50.60)	Reference
Family history of birth defects			
Yes	30 (37.04)	1717 (25.86)	**1.68 (1.07-2.65)**
No	51 (62.96)	4923 (74.14)	Reference
Family history of CHD			
Yes	12 (14.81)	212 (3.16)	**5.31 (2.83-9.95)**
No	69 (85.19)	6491 (96.84)	Reference

Note: CHD=congenital heart defects. †=Odds ratios adjusted for study site.

Figure A7: Intake Form Used by the Pediatric Cardiac Care Consortium



Pediatric Cardiac Care Consortium
CONGENITAL HEART DISEASE REGISTRY FORM
UNIVERSITY OFFICE PLAZA, SUITE 123
2221 UNIVERSITY AVENUE S.E. • MINNEAPOLIS, MN 55414
PHONE: (612) 625-2475 • FAX (612) 624-6586

DATE REC'D. _____ FORM NO.
ENTRY 1 _____
ENTRY 2 _____
VERIFIED _____
PCCC STAFF ONLY FOR FILING
NP
OR PREVIOUSLY REPORTED YEAR:
PP____ (YEAR) ON FORM NO. _____

PATIENT IDENTIFICATION

REGISTRY NUMBER (FOR INTERNAL USE ONLY)
___ ___ ___ ___ ___ ___ ___ ___ ___ ___ ___ ___

COUNTY

SEX
_____M _____F

BIRTHWEIGHT (IF LESS THAN ONE YEAR) AT TIME OF ADMISSION
Kg

CHECK ONE:
☐ NEW PATIENT, NOT PREVIOUSLY REPORTED
☐ PREVIOUSLY REPORTED PATIENT
☐ DIFFERENT REGISTRY NO. _ _ _ _ _ _ _ _ _ _ _ _

PCCC REGISTRY NUMBER CHANGE DOCUMENTATION AREA:

DOES PATIENT HAVE NON-CARDIAC ABNORMALITIES/SYNDROMES?
_____YES (INDICATE BELOW) _____NO _____UNKNOWN

CHROMOSOMAL	☐ DOWN SYNDROME	☐ TRISOMY 18	☐ TRISOMY 13	☐ TURNER'S SYNDROME	☐ DIGEORGE SYNDROME	☐ WILLIAMS' SYNDROME	☐ NOONAN SYNDROME	☐ OTHER (DESCRIBE)			
OTHER SYND.	☐ MARFAN'S SYNDROME	☐ TAKAYASU SYNDROME	☐ DYSMORPHIC FEATURES	☐ OTHER (DESCRIBE)							
CNS	☐ HYDROCEPHALUS	☐ MYELOMENINGOCELE	☐ SEIZURES	☐ DEVELOPMENTAL DELAY	☐ OTHER (DESCRIBE)						
GI	☐ TE FISTULA	☐ MAL-ROTATION	☐ IMPERFORATE ANUS	☐ OMPHALOCELE	☐ DUODENAL ATRESIA	☐ HIRSCHSPRUNGS	☐ GASTROSCHESIS	☐ GERD	☐ OBESITY	☐ OTHER (DESCRIBE)	
GU	☐ HYDRONEPHROSIS	☐ HYPOSPADIAS	☐ RENAL STRUCTURAL ABNORMALITIES	☐ OTHER (DESCRIBE)							
RESPIRATORY	☐ RDS	☐ CHOANAL ATRESIA	☐ ABSENT/HYPOPLASTIC LUNG	☐ CLEFT LIP/PALATE	☐ ASTHMA	☐ OTHER (DESCRIBE)					
B/J	☐ POLYDACTYLY	☐ ARSENT/HYPOPLASTIC LIMB/HAND	☐ OTHER (DESCRIBE)								
OTHER	☐ PREMATURITY	☐ TWIN BIRTH	☐ HYPOTHYROIDISM	☐ ENDOCARDITIS	☐ KAWASAKI DISEASE	☐ OTHER (DESCRIBE)					

ADMISSION INFORMATION

HOSPITAL NAME

ADMISSION WEIGHT
Kg

ADMISSION DATE

PREVIOUS CARDIAC SURGICAL PROCEDURES
_____YES (INDICATE BELOW) _____NO _____UNKNOWN

	TYPE OF SURGICAL PROCEDURE	DATE	CODE
1			
2			
3			
4			
5			
6			
7			
8			
9			

CATH OR EPS DATA

_____CATH or _____ EPS REPORT ATTACHED

CATH or EPS DATE

WEIGHT
Kg

CATH COMPLICATIONS CODE(S) 01 = NONE

PLEASE REFER TO CATHETERIZATION/EPS COMPLICATION CODE LIST FOR ANY RELEVANT COMPLICATIONS AND RECORD ABOVE.

	CATH DX 1 PRIMARY
1	
2	
3	
4	
5	
6	
7	
8	
9	
A	
B	
C	
D	
E	
F	

SURGERY DATA

_____ PLEASE ATTACH SURGERY REPORT

SURGERY DATE

WEIGHT
Kg

IF SURGERY ONLY PERFORMED THIS ADMISSION, PLEASE NOTE

PREVIOUSLY REPORTED CATH DATE_____
OR INCLUDE COPY OF CATH REPORT OR ECHOCARDIOGRAM DESCRIBING PATIENT'S CARDIAC DIAGNOSIS.

	SURG. PROCEDURE	SURGERY DX
1		
2		
3		
4		
5		
6		
7		
8		
9		
A		
B		
C		
D		
E		
F		

OUTCOME THIS ADMISSION:

_____ DISCHARGE DATE:_____

_____ TRANSFER DATE:_____

TO:_____

_____ DEATH IN HOSPITAL

DATE:_____

WAS AUTOPSY PERFORMED?
_____YES _____NO _____UNKNOWN

DEATH DATA

INDICATE TYPE OF DEATH REPORT ATTACHED
_____ DEATH RPT _____ AUTOPSY RPT

	IMMEDIATE CAUSE
1	
2	
3	
4	
5	
6	
7	
8	
9	
A	
B	
C	
D	
E	
F	

NOTE: ATTACHED REPORTS SHOULD NOT INCLUDE PATIENT IDENTIFYING INFORMATION OTHER THAN AS PERMITTED UNDER THE DATA USE AGREEMENT.

Table A8: Univariable and Multivariable Regression Analysis of Time to Reoperation (ß Parameter Estimates and Standard Errors) (N=370)

	Univariable		*Multivariable*	
Characteristic	*ß estimate*	*SE (ß)*	*ß estimate*	*SE (ß)*
Male	0.3366	0.3068		
Down syndrome present	0.1814	0.3127		
Institutional surgical volume				
Small (<100 surgeries)	Reference			
Medium (101-199 surgeries)	0.7649	0.5554		
Large (200-299 surgeries)	0.3869	0.5499		
Very large (>300 surgeries)	0.4471	0.6195		
Complete AVSD morphology	-0.7723	0.5900		
Rastelli classification				
Type A	Reference			
Type B	-0.6028	0.6026		
Type C	-0.1140	0.4679		
Left AV valve cleft present	-0.1309	0.3884		
Preop LAVVR				
None-mild	Reference			
Mild-moderate	-0.1093	0.4742		
Moderate-severe	-0.6761	0.6137		
AVSD repair era				
1982-1989	Reference			
1990-1998	-0.8752	0.4383	-0.5687	0.4853
1999-2007	-2.1293	0.4559	-1.874	0.5100
Type of AVSD repair				
One-patch	0.7302	0.5132		
Two-patch	0.0419	0.4684		
Modified one-patch	Reference			

Table A8 Continued

	Univariable		*Multivariable*	
Characteristic	*ß estimate*	*SE (ß)*	*ß estimate*	*SE (ß)*
Age at AVSD repair (yrs)	-0.3975	0.1541	-0.4179	0.3928
Weight at AVSD repair (kg)	-0.1564	0.0519	-0.1553	0.1322
CC time at AVSD repair (min)	-0.0089	0.0069		
Cleft closure at AVSD repair	-0.7312	0.3239	-0.7657	0.3160
Postop LAVVR				
None-mild	Reference			
Mild-moderate	-0.4040	0.8621		
Moderate-severe	-0.6766	0.8191	-1.2016	0.3340
Postop mitral stenosis	-0.7978	0.4643		
Postop complete heart block	-1.3494	0.4730	-1.4466	0.4647

Note: AVSD=atrioventricular septal defect, AV=atrioventricular, preop=preoperative, LAVVR=left atrioventricular valve regurgitation, mod-severe=moderate to severe, CC time=cross-clamp time, postop=postoperative.

Table A9: Univariable and Multivariable Regression Analysis of Time to Replacement (ß Parameter Estimates and Standard Errors) (N=127)

	Univariable		*Multivariable*	
Characteristic	*ß estimate*	*SE (ß)*	*ß estimate*	*SE (ß)*
Male	0.0596	0.6469		
Down syndrome present	-1.6878	0.6124	-1.665	0.6245
Institutional surgical volume				
Small (<100 surgeries)	Reference			
Medium (101-199 surgeries)	0.3417	1.3080		
Large (200-299 surgeries)	1.7298	1.2447		
Very large (>300 surgeries)	0.1267	1.3807		
Complete AVSD morphology	-1.3493	1.3916		
Rastelli classification				
Type A	Reference			
Type B	-0.0076	1.3612		
Type C	-0.7311	1.1876		
Left AV valve cleft present	-0.1580	0.7926		
Preop LAVVR				
None-mild	Reference			
Mild-moderate	-1.6671	1.0370		
Moderate-severe	-1.7211	1.0667		
AVSD repair era				
1982-1989	Reference			
1990-1998	-2.0837	0.8545	-2.2360	0.9127
1999-2007	-3.5776	0.9239	-3.9651	0.9992
Type of AVSD repair				
One-patch	1.5722	1.2536		
Two-patch	1.2900	1.2318		
Modified one-patch	Reference			

Table A9 Continued

	Univariable		*Multivariable*	
Characteristic	*ß estimate*	*SE (ß)*	*ß estimate*	*SE (ß)*
Age at AVSD repair (yrs)	-0.1280	0.2376	-0.5237	0.6428
Weight at AVSD repair (kg)	-0.0064	0.0913	-0.0369	0.2500
CC time at AVSD repair (min)	-0.0398	0.0144		
Cleft closure at AVSD repair	-0.3254	0.6888		
Postop LAVVR				
None-mild	Reference			
Mild-moderate	-8.9057	3.4847		
Moderate-severe	-9.3842	3.3837		
Postop mitral stenosis	-2.1143	0.6905	-1.5010	0.6800
Postop complete heart block	-0.5229	0.7573		

Note: AVSD=atrioventricular septal defect, AV=atrioventricular, preop=preoperative, LAVVR=left atrioventricular valve regurgitation, mod-severe=moderate to severe, CC time =cross-clamp time, postop=postoperative.

Table A10: Demographic, Anatomic, and Operative Characteristics of the Repair Subgroup by Outcome

	Survived (N=228)		*In-Hospital Death (N=15)*		*p-value*
Characteristic	*N (%)*	*Median*	*N (%)*	*Median*	
Male	84 (39)		10 (38)		0.97
Down syndrome present	149 (69)		19 (73)		0.67
Institutional surgical volume					0.90
Small (<100 surgeries)	22 (10)		3 (12)		
Medium (101-199 surgeries)	87 (40)		10 (38)		
Large (200-299 surgeries)	72 (33)		10 (38)		
Very large (>300 surgeries)	35 (16)		3 (12)		
Complete AVSD morphology	194 (91)		26 (100)		0.24
Rastelli classification					0.65
Type A	55 (52)		9 (69)		
Type B	15 (14)		1 (8)		
Type C	36 (34)		3 (23)		
Left AV valve cleft present	164 (80)		18 (78)		0.79
Preop LAVVR					0.18
None-mild	108 (69)		17 (89)		
Mild-moderate	35 (22)		2 (11)		
Moderate-severe	14 (9)		0 (0)		
AVSD repair era					0.96
1982-1989	29 (13)		3 (12)		
1990-1998	103 (48)		13 (50)		
1990-2007	84 (39)		10 (38)		
Type of AVSD repair					0.05
One-patch	48 (23)		3 (12)		
Two-patch	127 (60)		22 (88)		
Modified one-patch	20 (9)		0 (0)		

Table A10 Continued

	Survived (N=228)		*In-Hospital Death (N=15)*		*p-value*
Characteristic	*N (%)*	*Median*	*N (%)*		
Age at AVSD repair (yrs)		0.44		0.34	0.13
Weight at AVSD repair (kg)		5.00		5.02	0.53
CC time at AVSD repair (min)		76		95	0.06
Cleft closure at AVSD repair	142 (69)		17 (71)		0.82
Postop LAVVR					0.97
None-mild	10 (5)		1 (4)		
Mild-moderate	54 (27)		7 (28)		
Moderate-severe	138 (68)		17 (68)		
Postop mitral stenosis	8 (4)		1 (4)		0.97
Postop complete heart block	12 (6)		1 (4)		0.71
Time to first reoperation (yrs)		1.13		0.09	**<0.0001**
Age at first reoperation (yrs)		1.62		0.42	**<0.0001**
Weight at first reoperation (kg)		8.45		4.90	**<0.0001**
CC time at first reoperation (min)		45		46	0.98

Note: AVSD=atrioventricular septal defect, AV=atrioventricular, preop=preoperative, LAVVR=left atrioventricular valve regurgitation, CC time=cross-clamp time, postop=postoperative.

Significant p-values displayed in bold.

Table A11: Demographic, Anatomic, and Operative Characteristics of the Replacement Subgroup by Outcome

	Survived (N=89)		*In-Hospital Death (N=38)*		*p-value*
Characteristic	*N (%)*	*Median*	*N (%)*		
Male	41 (46)		19 (56)		0.33
Down syndrome present	38 (43)		20 (53)		0.30
Institutional surgical volume					0.18
Small (<100 surgeries)	5 (6)		4 (11)		
Medium (101-199 surgeries)	21 (23)		13 (34)		
Large (200-299 surgeries)	49 (54)		13 (34)		
Very large (>300 surgeries)	15 (17)		8 (21)		
Complete AVSD morphology	80 (93)		36 (97)		0.35
Rastelli classification					0.30
Type A	29 (62)		22 (78)		
Type B	7 (15)		3 (11)		
Type C	11 (23)		3 (11)		
Left AV valve cleft present	59 (76)		25 (76)		0.98
Preop LAVVR					0.93
None-mild	48 (70)		19 (68)		
Mild-moderate	10 (15)		5 (18)		
Moderate-severe	10 (15)		4 (14)		
AVSD repair era					0.24
1982-1989	17 (19)		3 (8)		
1990-1998	47 (53)		21 (55)		
1990-2007	25 (28)		14 (37)		
Type of AVSD repair					0.41
One-patch	34 (40)		15 (40)		
Two-patch	42 (50)		21 (57)		
Modified one-patch	2 (2)		1 (3)		

Table A11 Continued

	Survived (N=89)		*In-Hospital Death (N=38)*		*p-value*
Characteristic	*N (%)*	*Median*	*N (%)*		
Age at AVSD repair (yrs)		0.45		0.36	**0.0267**
Weight at AVSD repair (kg)		5.00		4.67	0.08
CC time at AVSD repair (min)		76		95	0.15
Cleft closure at AVSD repair	57 (68)		20 (67)		0.90
Postop LAVVR					0.37
None-mild	1 (1)		0 (0)		
Mild-moderate	8 (10)		6 (19)		
Moderate-severe	72 (89)		26 (81)		
Postop mitral stenosis	29 (32)		6 (18)		0.13
Postop complete heart block	24 (27)		4 (11)		0.05
Time to first reoperation (yrs)		0.40		0.04	**0.0001**
Age at first reoperation (yrs)		1.32		0.43	**0.0001**
Weight at first reoperation (kg)		7.60		5.50	**0.0002**
CC time at first reoperation (min)		65		79	0.32
Time to replacement (yrs)		0.86		0.09	**<0.0001**
Valve size to body weight (mm/kg)		2.28		3.45	**<0.0001**

Note: AVSD=atrioventricular septal defect, AV=atrioventricular, preop=preoperative, LAVVR=left atrioventricular valve regurgitation, CC time=cross-clamp time, postop=postoperative.

Significant p-values displayed in bold.

Figure A8: Frequency of Replacements and Mortality Rate by Reoperation

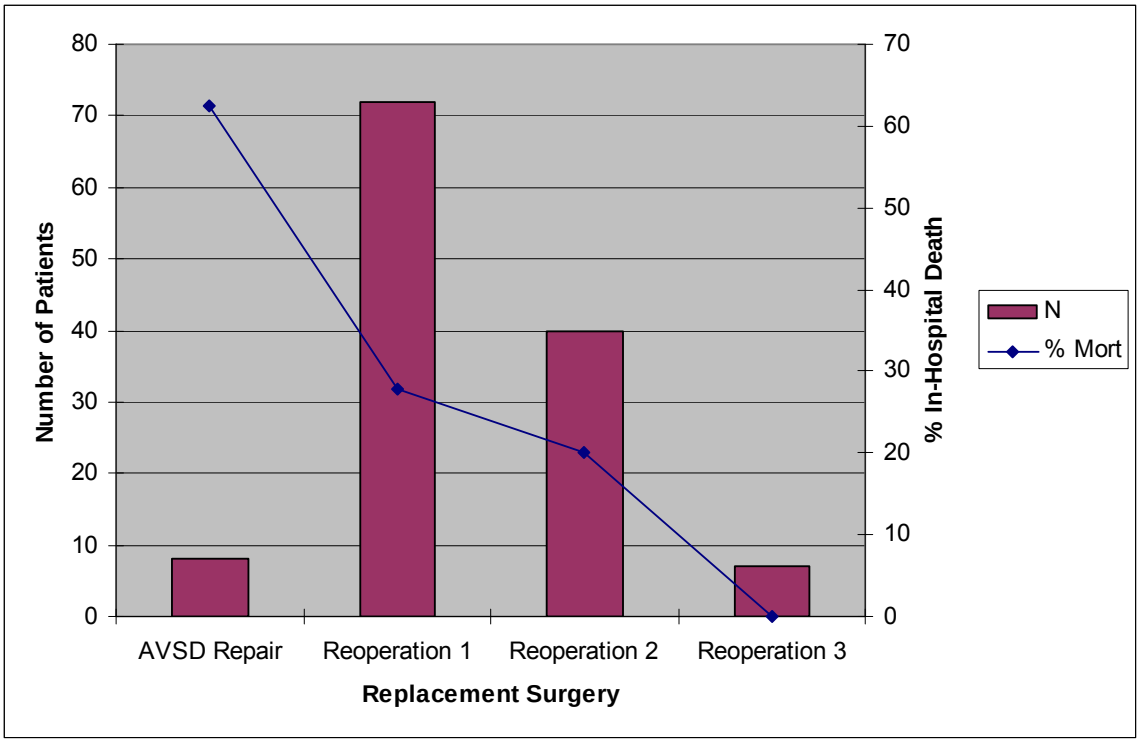


Figure A9: Frequency of Replacement and Mortality Rate by Time Interval from Primary AVSD Repair

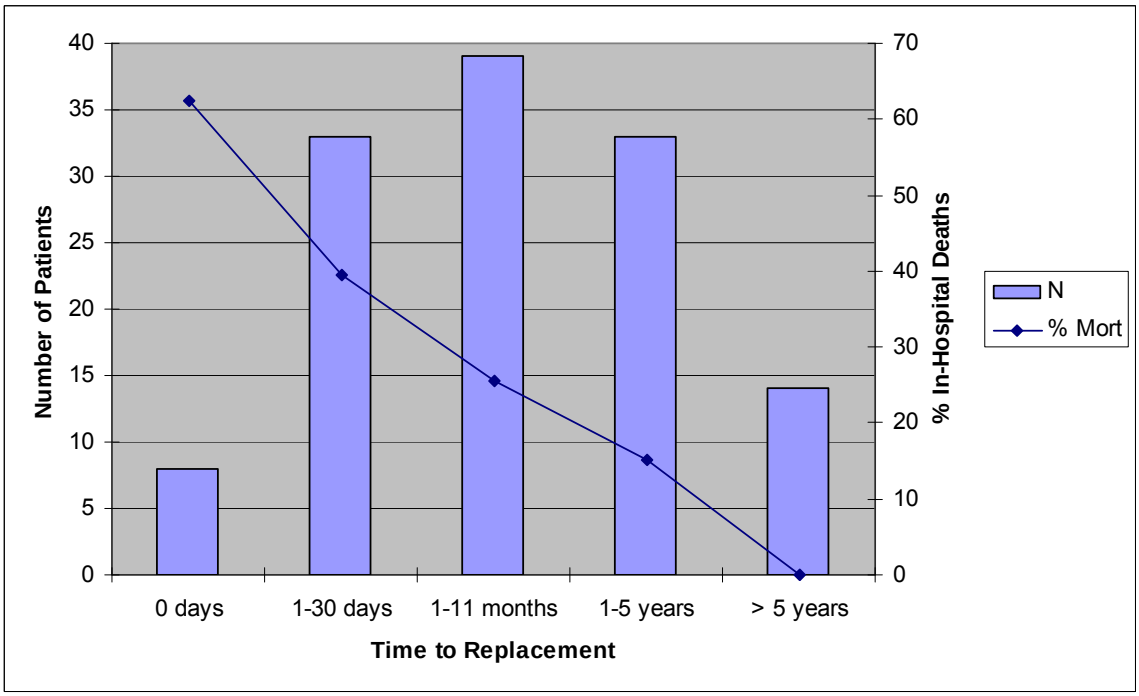


Table A12: Univariable and Multivariable Regression Analysis of Factors Associated with In-Hospital Death in the Reoperation Cohort (ß Parameter Estimates and Standard Errors) (N=370)

Characteristic	*Univariable*		*Multivariable*	
	ß estimate	*SE (ß)*	*ß estimate*	*SE (ß)*
Male	0.0340	0.2897		
Down syndrome present	0.3195	0.2878		
Institutional surgical volume				
Small (<100 surgeries)	Reference			
Medium (101-199 surgeries)	-0.2921	0.4717		
Large (200-299 surgeries)	-0.4206	0.4673		
Very large (>300 surgeries)	-0.3384	0.5287		
Complete AVSD morphology	1.3630	1.010		
Rastelli classification				
Type A	Reference			
Type B	0.4924	0.5362		
Type C	1.0509	0.5363		
Left AV valve cleft present	-0.2147	0.3380		
Preop LAVVR				
None-mild	Reference			
Mild-moderate	0.0115	0.4237		
Moderate-severe	0.1456	0.5361		
AVSD repair era				
1982-1989	Reference			
1990-1998	0.5086	0.4882	0.3054	0.4951
1999-2007	0.6701	0.5009	0.2995	0.5176
Type of AVSD repair				
1-patch	1.9233	1.0289		
2-patch	1.9983	1.0145		
Modified 1-patch	Reference			

Table A12 Continued

Characteristic	*Univariable*		*Multivariable*	
	ß estimate	*SE (ß)*	*ß estimate*	*SE (ß)*
Age at AVSD repair (yrs)	-0.3505	0.2713	-1.1557	0.4380
Weight at AVSD repair (kg)	-0.1184	0.0795		
CC time at AVSD repair (min)	-0.0123	0.0056		
Cleft closure at AVSD repair	-0.1136	0.3024		
Postop LAVVR				
None-mild	Reference			
Mild-moderate	-0.2573	0.3606		
Moderate-severe	-0.0047	0.3595		
Postop mitral stenosis	0.1920	0.4132		
Postop complete heart block	0.7973	0.5221		
Time to reoperation (yrs)	-1.3276	0.1317		
Age at reoperation (yrs)	-0.2937	0.1090	-0.2910	0.1290
Weight at reoperation (kg)	-0.0966	0.0396	-0.0976	0.0407
Replacement reoperation	1.1311	0.3154	1.1916	0.3237

Note: AVSD=atrioventricular septal defect, AV=atrioventricular, preop=preoperative, LAVVR=left atrioventricular valve regurgitation, CC time=cross-clamp time, postop=postoperative.

Table A13: Univariable and Multivariable Regression Analysis of Factors Associated with In-Hospital Death in the Replacement Subgroup (ß Parameter Estimates and Standard Errors) (N=127)

Characteristic	*Univariable*		*Multivariable*	
	ß estimate	*SE (ß)*	*ß estimate*	*SE (ß)*
Male	0.2462	03464		
Down syndrome present	0.2612	0.3251	0.7498	0.3369
Institutional surgical volume				
Small (<100 surgeries)	Reference			
Medium (101-199 surgeries)	0.2307	0.5725		
Large (200-299 surgeries)	0.9258	0.5738		
Very large (>300 surgeries)	0.3866	0.6135		
Complete AVSD morphology	0.7946	1.0138		
Rastelli classification				
Type A	Reference			
Type B	0.4558	0.6157		
Type C	0.8429	0.6166		
Left AV valve cleft present	0.1221	0.4069		
Preop LAVVR				
None-mild				
Mild-moderate	0.2090	0.5041		
Moderate-severe	0.2987	0.5522		
AVSD repair era				
1982-1989	Reference			
1990-1998	0.8954	0.6192	0.6068	0.6357
1999-2007	0.8153	0.6409	0.6149	0.6600
Type of AVSD repair				
1-patch	1.1519	1.0333		
2-patch	1.3021	1.0238		
Modified 1-patch	Reference			

Table A13 Continued

Characteristic	*Univariable*		*Multivariable*	
	ß estimate	*SE (ß)*	*ß estimate*	*SE (ß)*
Age at AVSD repair (yrs)	-0.2871	0.2704		
Weight at AVSD repair (kg)	-0.1143	0.0917		
CC time at AVSD repair (min)	-0.1522	0.0523		
Cleft closure at AVSD repair	0.1737	0.3647		
Postop LAVVR				
None-mild	Reference			
Mild-moderate	0.5061	0.4530		
Moderate-severe	0.3960	0.4531		
Postop mitral stenosis	0.7353	0.4500		
Postop complete heart block	1.0651	0.5290		
Time to reoperation (yrs)	-0.5441	0.2524		
Age at reoperation (yrs)	-0.4168	0.1669		
Weight at reoperation (kg)	-0.1773	0.0640		
Time to replacement (yrs)	-0.6746	0.2539		
Age at replacement (yrs)	-0.5105	0.1687	-0.1613	0.3979
Weight at replacement (kg)	-0.2237	0.0650	-0.1836	0.1629
Prosthetic valve size (mm)	-0.1490	0.0571		
Valve size to body weight ratio (mm/kg)	0.4758	0.1145	0.4894	0.1407

Note: AVSD=atrioventricular septal defect, AV=atrioventricular, preop=preoperative, LAVVR=left atrioventricular valve regurgitation, postop=postoperative.

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