Section-I

REVIEW OF LITERATURE ON CONGENITAL HEART DISEASE
REVIEW OF LITERATURE

In total population, it has been estimated that 4-6% of the population is suffering with genetic defects (Gardner and Sultherland, 1996). One of the most common defects includes the abnormalities of the heart which are congenital heart disease, congestive heart failures, cardiomyopathies and cardiovascular abnormalities (Carlson, 1994). Congenital heart disease is the most common form of human birth defects accounting for about 30% of the total anomalies (http://www.fi.edu/biosci/develop/develop.html) which is found to affect nearly 1% of newborns, and their frequency in spontaneously aborted pregnancies is estimated to be tenfold higher (Behrman et. al., 2000). The heart is the first organ to form and function in the embryo which is completely formed by the end of eight weeks into pregnancy. All subsequent events depend on the ability of the heart to match its output with the fetus demands for oxygen and nutrients (Cruz Robles et. al., 2005). It is the hardest working muscle in the human body, which can be described as a pump made up of muscle tissue that contracts regularly and continuously pumping blood to the body. As the body develops, the heart grows at the same rate as the fist. Any deviation from the normal course of development leads to Congenital Heart Disease (CHD) (Carlson, 1994).

Historical Account

Harvey (1628) described for the first time how the two circulations pulmonary and systemic work together. Stenson (1665) described the cardiac pathology of a stillborn fetus with multiple congenital anomalies including the
cardiac lesion, which is now recognized as Tetralogy of Fallot. Sandifort (1777), for the first time, described the clinical symptoms of a young child whom he called “blue boy” who appeared normal at birth, but later confirmed as a congenital defect based on the post mortem study which revealed patent foramen ovale, a small pulmonary artery with a blocked pulmonary valve, and a ventricular communication between the two ventricles. Further, Bouillard (1840) described ventricular septal defect and single ventricle heart. From the 19th century, various detailed descriptions of CHD began to be published, and the first book dealing with the full spectrum of CHD was published by Peacock (1858) leading to today’s understanding of the pathophysiology of numerous lesions which comprise CHD. The anatomic lesion, now called Tetralogy of Fallot, was named after Etienne-Louis Fallot (Fallot, 1888) who, gave a detailed description of the defect that had been well-described 200 years earlier (Noonan, 2004). Descriptive studies on CHD gave rise to Pediatric Cardiology in the early 1900s. By 1961, Pediatric Cardiology became the first subspecialty board in pediatrics. The last 15 years has added exciting basic research discoveries which are elucidating the cause of cardiac defects with hope for prevention in the future (Noonan, 2004).

**Heart Development**

The development of the primitive heart in the fetus begins at third week of gestation from the mesodermal germ layer through several phases. It is believed that the cardiomyocytes originate in the anterior lateral mesoderm soon after
gastulation. The heart is a cluster of angiogenic cells that are located in the cardiogenic plate, which is present at the cranial end of the embryo (Fig. 1.1A). Angiogenic cell clusters, which lie in a horseshoe shape configuration in the plate, unite together to form two endocardial tubes. These tubes are then forced into the thoracic region due to cephalic and lateral foldings, where they fuse together forming a single endocardial tube. At approximately 21 days, they are completely fused. The newly formed heart tube (Fig. 1.1B) is divided into 7 parts. They are: sinus venosus consisting of right and left horns; paired primitive atria; atrioventricular sulcus divides the atria and the primitive ventricle; primitive ventricle expands to become the left ventricle; interventricular sulcus divides the primitive ventricle and the bulbus cordis; bulbus cordis is further divided into - bulbus cordis, conus cordis and truncus arteriosus; and aortic sac (Carlson, 1994).

The heart tube begins to grow rapidly forcing it to bend upon itself resulting in bulboventricular loop. During the next phases, septa begins to grow in the atria, ventricle and bulbus cordis to form right and left atria, right and left ventricles and two great vessels, the pulmonary artery and aorta. By the end of the 8th week, partitioning is completed and the fetal heart has formed (Fig. 1.2). In fetal condition, the lungs are not used as a result of which the blood is shunted back and forth between the right and left auricles through the foramen ovale. A connection between the pulmonary artery and the aorta called as ductus arteriosus, also bipasses pulmonary circulation in the fetal heart (Carlson, 1994).
Heart development is a dynamic process involving a series of collective molecular and morphogenetic events. The complexity inherent in heart development is reflected on the expression of myriads of genes (Srivastava, 2001). Cardiomyocytes are produced in response to protein factors including bone morphogenetic proteins (BMP), which are secreted from adjacent endoderm. Cardiogenic signals activate expression of the homeobox gene, tinman, in flies and the related gene, NKX2.5, in vertebrates, the earliest molecular markers of the cardiac lineage. NKX2.5 cooperates with Zinc finger transcription factors of the GATA family which directly activates cardiac gene expression. These in turn regulate various other transcription factors that control myocyte differentiation (Fig. 1.3) (Srivastava and Olson, 2000).

The normal heart is composed of four chambers and is surrounded by a space called the pericardial cavity. It is formed by the pericardium, a closed double-layered sac that surrounds the heart and anchors it within the mediastinum. From the inside, the heart wall is composed of three layers of tissues: epicardium, myocardium and endocardium. The epicardium, also called as the visceral pericardium, is a thin serous membrane forming the smooth outer surface of the heart. The thick middle layer of the heart, the myocardium is composed of cardiac muscle cells and is responsible for the ability of the heart to contract. The smooth inner surface of the heart chambers is the endocardium, which consists of simple squamous epithelium covering a layer of connective tissue. The two atria collects blood returning from the veins, the contraction of the atria forces the blood into the ventricles. The right atrium has two major
openings, called inferior and superior vena cava, collecting the blood from various parts of the body. In addition, the coronary sinus enters the right atrium from the wall of the heart. The left atrium has four openings that receive the four pulmonary veins from the lungs. The two atria are separated from each other by the interatrial septum. The ventricles are the major pumping chambers of the heart. They eject the blood into the arteries and force it to flow through the circulatory system, each ventricle harboring one large outflow root located superiorly near the midline of the heart. The right ventricle opens into the pulmonary trunk, while the left ventricle ejects into the aorta. The two ventricles are separated from each other by the interventricular septum. The atrioventricular valve separating the right atrium and the right ventricle is called “tricuspid valve”, and the one located between the left atrium and the left ventricle is “mitral valve”. These valves allow blood flow from the atria into the ventricles, but prevent the blood from running back into the atria. The aorta and the pulmonary trunk possess aortic and pulmonary semilunar valves, preventing the back flow into the ventricles (Fig. 1.4) (Tortora and Anagnostakos, 1999).

CONGENITAL HEART DISEASE (CHD)

Congenital heart disease is a collection of various defects which can be collectively defined as the malformations of the heart or the large blood vessels associated with the heart. These defects can involve the walls and valves of the heart; the arteries and veins associated with the heart. They also vary widely in
complexity, from a simple hole in the wall between two heart chambers to a complicated set of malformations (Carlson, 1994; Srivastava, 2001).

It is the leading cause of birth defect-related deaths in the first year of life. Of the dozens of heart defects, some are mild and may need minimal or no medical treatment even through adulthood, while others are life threatening, either immediately to the newborn or over a time period. In most patients, CHD occurs as an isolated malformation, but about 33% have associated anomalies (Frias, 1993).

**Incidence and Epidemiology of CHD**

During earlier centuries, CHD was believed to be a rare curiosity. Population based studies on the prevalence of CHD have been carried out as early as 1950s (Mitchell et al., 1971). The prevalence of CHD worldwide was found to range from as low as 1.0 per 1000 livebirths to as high as 150 per 1000 livebirths (Table 1.1) (Mitchell et al., 1971; Kenna et al., 1975; Dickinson et al., 1981; Fixler et al., 1990; Sung et al., 1991; Meberg et al., 1994; Stumpflen et al., 1996; Hassan et al., 1997; Robida et al., 1997; Bitar et al., 1999; Samanek et al., 1999; Subramanyan et al., 2000; Bassili and Mokhtar, 2000; Alabdulgader, 2001; Begic et al., 2003; Bolisetty et al., 2004; Abu-Sulaiman et al., 2004; Oloron et al., 2005). Whereas, in India, it was found to range between 2.2 to 50.89 per 1000 livebirths (Table 1.2) (Manchandha and Sachdev, 1962; Gupta et al., 1968; Bidwai et al., 1971; Pai and Varkey, 1974; Verma et al., 1979; Shrestha and Padmavathi, 1980; Vashishtha et al., 1993; Khalil et al., 1993).
1994; Thakur et. al., 1995; Chadha et. al., 2001; Tank et. al., 2004). The most frequent type of CHD worldwide was found to be VSD followed by ASD, TOF and PDA (Table 1.3) (Gupta et. al., 1968; Pai and Varkey, 1974; Suresh et. al., 1995; Subramanyan et. al., 2000; Bassili and Mokhtar, 2000; Alabdulgader, 2001; Wickramasinghe et. al., 2001; Venugopalan and Agarwal, 2002; Begic et. al., 2003; Tank et. al., 2004).

**Diagnosis of CHD**

CHD is usually present in infancy as, i) Symptomatically with cyanosis, congestive heart failure, shock, arrhythmias, ii) Asymptomatically with heart murmur and iii) Others which includes different respiratory tract infections and growth failure. There are several different types of cardiac testing that can provide a large amount of information about the defects. Diagnosis of these defects in many cases can be made by only one type of test whereas; in others, combining the results of two or more tests may yield greater precision in the diagnosis. Different methods of diagnosis include careful physical and cardiovascular examination, chest radiography, electrocardiography, echocardiography, cardiac catheterization, cine-angiography and cardiac MRI (http://www.americanassociation.com/The Merck manual of Diagnosis/html).

**Classification of CHD**

about 85% of the types of CHD are common, whereas, about 15% of them are rare (Fig. 1.5). They are described as follows:

1) **Ventricular Septal Defect (VSD):** This is the most common type of CHD, which is called as a hole in the heart that exists between the lower chambers of the heart which occurs when the septum, the muscular wall separating the right and left ventricles, fails to fully form. The hole allows oxygen-rich blood to leak from the left ventricle into the right ventricle, instead of moving into the aorta and on to the body. This leads to too much blood flow to the lungs. As the heart has to pump extra blood and is overworked it might transform the septum into a honey combed Swiss cheese structure with sieve like fenestrations. It is classified into 2 main types according to their location, relative to the components of the septum. The common types are perimembranous and trabecular VSD.

2) **Atrial Septal Defect (ASD):** Similar to a VSD, ASD is a hole in different part of the atrial septum that occurs when the septum separating the right and left atria does not close properly. This allows blood from the left atrium to flow into the right atrium, instead of left ventricle and on to the aorta and rest of the body. It may be single or multiple and can be located anywhere in the atrial septum. Based on this, the ASD are classified into 3 major types: a) Ostium secundum (Fossa ovalis), b) Ostium primum and c) Sinus venosum.
3) **Patent Ductus Arteriosus (PDA):** Ductus arteriosus, the temporary duct connects the pulmonary artery and the aorta before birth. This allows blood to bypass the lungs because oxygen is delivered to the fetus through the placenta and the umbilical cord. The temporary vessel normally closes within a few hours or days of birth, since the lungs take over. If it remains open (patent), some blood that should circulate through the body is misdirected to the lungs.

4) **Pulmonary Stenosis (PS):** In this defect, the flow of blood from the right ventricle to the pulmonary artery is obstructed by narrowing of the pulmonary valve. When there is an obstruction (stenosis), the right ventricle must pump harder to get blood into the pulmonary artery. The defect may occur along with other defects, such as thickening of the muscle of the right ventricle immediately below the valve.

5) **Aortic Stenosis (AS):** This is a defect that narrows or obstructs the aortic valve opening, making it difficult for the heart to pump blood into aorta. Mild cases may not have symptoms initially, but they can worsen over time. Normally there are 3 leaflets or cups in a valve, but in a stenotic valve there is one (unicuspid) or two (bicuspid valve). Obstruction may be valvular, subvalvular (sub aortic) or supravalvular. This makes it hard for the heart to pump blood to the body.

6) **Tetralogy Of Fallot (TOF):** This is a combination of four congenital abnormalities. The defects are: (a) VSD that lets blood pass from the right
to the left ventricle without going through the lungs; (b) a narrowing (stenosis) at or just beneath the pulmonary valve which partially blocks the blood flow from the right side of the heart to the lungs; (c) the right ventricle is more muscular than normal and (d) the aorta lies directly over the VSD. Collectively, this results in cyanosis or blue baby, which may appear soon after birth, in infancy or later in childhood.

7) Coarctation Of Aorta (COA): This is a constricted segment of the aorta that obstructs blood flow to the lower part of the body and increases blood pressure above the constriction. Coarctation forces the heart to pump harder to get blood through the aorta and on to the rest of the body. It usually occurs as isolated defect, but may occur with a VSD, sub aortic stenosis or complex CHD.

8) Transposition of the Great Arteries (TGA): This is a defect in which the positions of the aorta and the pulmonary artery (the great arteries) are reversed (transposed). The aorta is connected to the right ventricle, while the pulmonary artery is connected to the left ventricle. This results in the right ventricle pumping oxygen poor blood to different parts of the body and the left ventricle pumping oxygen rich blood to the lungs. This creates a circulatory pattern that prevents nourishing oxygenated blood from reaching the body. This defect is commonly associated with VSD, PS, heart block and an Ebstein like malformation of the tricuspid valve, which
helps in communicating the oxygen rich blood to different parts of the body.

9) **Atrioventricular Septal Defect (AVSD):** This is a large hole in the center of the heart, where the walls between the upper chambers and the lower chambers meet. This is called as a complete AVSD. In case of partial AVSD, either the upper or the lower part of the septum is affected. In addition, tricuspid and mitral valves that normally separate the hearts upper and lower chambers are not formed as individual valves; instead, a single large valve is formed. This large opening in the center of the heart lets blood to flow in all direction inside the heart.

10) **Persistent Truncus Arteriosus:** This is a defect in which where pulmonary artery and aorta merge into single great vessel (truncus) arising from the right and left ventricles. In addition, there is usually a large VSD, essentially turning the right and left ventricles into a single chamber. This allows oxygenated and unoxygenated blood to mix.

11) **Tricuspid Artesia (TA):** In this defect, the valve between the right atrium and ventricle is missing. As a result, oxygen-poor blood is pumped into the body along with the oxygen-rich blood. This results in cyanosis or blue baby. This defect is found in association with ASD, VSD and PDA.

12) **Pulmonary Artesia (PA):** In this defect, no pulmonary valve exists, therefore blood cannot flow from the right ventricle into the pulmonary
artery and on to the lungs. The only way for the blood to reach the lungs is
the ductus arteriosus, which is found during the fetal condition, which
closes after birth. The mixing of oxygen rich blood and oxygen poor blood
results in cyanosis.

13) Total Anomalous Pulmonary Venous Connection (TAPVC): In this
defect, all the pulmonary veins drain into the right atrium instead of left
atrium, which brings the mixing of the blood. In addition to this, there is
also presence of ASD and VSD, which results in cyanosis. There are three
main types of TAPVC depending on where the pulmonary veins drain.
They are referred to as supracardiac, intracardiac, and infracardiac
defects.

14) Hypoplastic Left Heart Syndrome (HLHS): In this defect, the left side
of the heart is underdeveloped (hypoplastic), including the aorta, aortic
valve, left ventricle and mitral valve. As a result, the body does not receive
enough oxygenated blood.

15) Double Outlet Right Ventricle (DORV): This is a most uncommon defect
in which both the pulmonary artery and aorta arises from the right
ventricle, each with its own outflow tract and valve.

16) Single Ventricle (SV): This refers to a congenital malformation in which
two atria are related to one ventricle that qualifies as left, right or
indeterminate ventricle on purely morphologic ground.
17) **Ebsteins Anomaly (EA):** This is a defect of the tricuspid valve, which controls blood flow between the right atrium and ventricle. The valve is positioned lower than normal into the ventricle instead of remaining between the atrium and the ventricle. Consequently, the ventricle is too small and the atrium too large, and neither functions properly. The valve is also malformed, often allowing blood to leak from the ventricle into the atrium. This defect often occurs along with other heart defects, including patent foramen ovale, ASD or Wolff-Parkinson-White syndrome.

18) **Dextrocardia (heart on the right):** If the developing heart tube bends to the left instead of the right, then the heart is displaced to the right and develops in a mirror image of its normal state. This condition is called as situs inversus. In many cases dextrocardia heart functions normally unless there are no associated vascular abnormalities. In cases where, the heart is the only organ, which is transposed, known as isolated dextrocardia, there are usually other severe cardiac abnormalities.

19) **Interrupted Aortic Arch (IAA):** In this defect, part of the aorta is absent and this leads to severe obstruction to blood flow to the lower parts of the body. In the immediate newborn period blood flows through the ductus into the descending aorta and reaches the lower parts of the body. As the ductus closes after birth, blood pressure in the lower circulation becomes inadequate and severe symptoms develop.
20) **Mitral Valve Prolapse (MVP):** This is a defect in the mitral valve of the heart. It occurs when the valve does not close correctly, allowing backward leaking of blood in the heart.

**Etiology of CHD**

From the early centuries, embryologists and anatomists were interested in cardiac development. Little was known about the cause of cardiac malformations, which are usually sporadic without any identifiable cause. However, the causes for CHD can be categorized into three major groups such as, environmental factors (2%); chromosomal and single gene disorders (8-13%) and multiple factors (85-90%) (Payne et al., 1995). The recent exponential increase in the knowledge of genetics has revolutionized the understanding of CHD (Prasad and Chudley, 2002).

1) **Environmental factors**

It was not clear until 1960s, what role environmental teratogens played in causing CHD in children. Lenz and Knapp (1962) have reported the phocomelia and CHD caused by thalidomide. Jones and Smith (1973) brought attention to alcohol as a cause of fetal alcohol syndrome, which was often associated with CHD. Further studies on the influence of environmental teratogens contributing to CHD lead to the discovery of several factors which includes intake of certain drugs like accutane (acne medication), lithium (used to treat certain forms of mental illness), bendeelin and possibly certain anti-seizure medications during pregnancy.
(Zeirler and Rothman, 1985); infections such as rubella (Schlesinger et al., 1985; Gersony et al., 1993); abnormal levels of retinoic acid (Sinning, 1998) and smoking and consumption of alcohol (Chen et al., 1999). These factors are found to act either directly on the embryo or alter the placental function or molecular dynamics of the cell resulting in CHD.

2) Chromosomal and single gene disorders

The importance of genetic factors in the etiology of CHD is demonstrated by clinical, epidemiological, and embryological studies. Development of heart requires precise timing and migration to achieve correct function, which is facilitated by the involvement of large number of genes during development. The study of chromosomal disorders and autosomal dominant syndromes in the setting of CHD and genetic linkage analysis of rare pedigrees with milder forms of CHD has been informative, particularly in conjunction with functional studies in model organisms (Marino and Digilio, 2000).

Chromosomal disorders

The refinements in cytogenetic techniques have promoted progress in understanding the role played by chromosomal anomalies in the cause of CHD (Noonan, 1978). It was found that about 0.4 - 26.8% of all CHD are associated with several chromosomal anomalies (Naganuma et al., 1981; Ferencz et al., 1989; Roskes et al., 1990; Allan et al., 1991; Knight
et. al., 1999; Chaoui et. al., 1999; Grech et. al., 1999; Prasad and Chudley, 2002; Patel et. al., 2004; Ergun et. al., 2004; Riegel et. al., 2005; Soares et. al., 2005). Recent studies have stated that ~1/3rd of children with CHD are associated with noncardiac anomalies and ~10% of children have multiple anomalies (Noonan, 2004).

Aneuploidy associated with CHD

Aneuploidy is the condition of having less than or more than the normal diploid number of chromosomes (46,XX or 46,XY), and is the most frequently observed type of chromosomal abnormality in CHD (Paladini et. al., 2002; Wimalasundera and Gardiner, 2004).

1) Trisomy 21 (Down Syndrome): This chromosomal defect has the highest association with major heart abnormalities constituting to about 40% of the total trisomy 21 cases (Hook and Fabia, 1978). About 40 - 60% of trisomy 21 patients have CHD with varying degree of clinical features (Stoll et. al., 1998) with cardiac malformations as the principal cause of mortality in the first two years of life. The most common types of CHD found in these patients are the VSD, ASD, PDA and AVSD (Vida et. al., 2005).

2) Trisomy 18 (Edwards Syndrome): This is the second most common autosomal aneuploidy after Down syndrome. The common CHD found in these patients include VSD, AVSD, DORV and HLHS (Root and Carey, 1994).
3) **Trisomy 13 (Patau Syndrome):** This syndrome is associated with high morbidity and mortality. Many die in the neonatal period with this syndrome. The common CHD found in these patients include ASD, VSD, PDA and cardiac malpositions especially Dextrocardia (Musewe et. al., 1990).

4) **45 X (Turner Syndrome):** About 10% of girls with this syndrome have a clinically evident heart defect and a further 10% will display cardiac disease on echocardiography (Chu et. al., 1994). The common CHD found in these patients includes VSD, COA, bicuspid aortic valve; HLHS, MVP and idiopathic aortic root dilatation (Mazzanti and Cacciari, 1998).

5) **Tetrasomy 22q (cat eye syndrome):** The important features of this syndrome are iris colobomata, ear tags and imperforate anus. The CHD are found to be in 30% of the patients with TAPVC as the major defect (Paul et. al., 1991).

6) **Fragile-X Syndrome:** It is caused by a trinucleotide repeat expansion (CGG) in the fragile X mental retardation gene (*FMR1*) at Xq27.3 (Hagerman et. al., 2001). The CHD found in these patients is MVP, which can be seen in up to 50% of adult patients with Fragile X syndrome. There are also incidences of mild dilation of aortic root in adults (Prasad and Chudley, 2002).
**Structural chromosomal abnormalities associated with CHD**

1) **Chromosome deletion and duplication syndromes:**

Congenital heart lesions are common in most of the macrodeletion syndromes and the cardiac anomalies vary widely even in those with apparently identical deletion breakpoints. It includes 3q, 4q, 5p, 8p, 9p, 11q, 13q, 18p and 18q deletion syndromes (Prasad and Chudley, 2002). There are an equal number of duplication syndromes that also can be present with multiple congenital malformations and cardiac lesions such as 1p, 2p, 2q, 2p, 5p, 8p, 13q, 18p and 16q duplication syndromes (Prasad and Chudley, 2002). Many of the affected children might have a combination of deletions and duplications involving the respective chromosome segments that were involved in the rearrangement.

2) **22q11.2 Deletion Syndrome:** This is the second most common syndrome, after trisomy 21 associated with CHD. In 22q11 deletion syndrome, a smaller part is missing (deleted) from the long arm (q) at position 11.2 of only one of the chromosome, so it is a gene haploinsufficiency syndrome. It has been estimated that ~1 in every 4000 children are born with a 22q11.2 deletion (Amati et. al., 1995). It is sporadic in ~90% of the cases. This deletion is ~3Mb long in 90% of the patients and 1.5Mb in 10% of cases. It is estimated to encompass ~30 genes.
This particular syndrome is comprised of three major syndromes namely, DiGeorge Syndrome (DGS), Velo Cardio Facial Syndrome (VCFS) and Conotruncal Anomaly Face Syndrome (CTAFS). Clinically these syndromes have overlapping phenotypes. The landmarking features of DGS are heart abnormalities, hypocalcaemia, immune system disorders due to the small size or absence of the thymus and or parathyroid. The important features of VCFS are cleft palate, heart disease, learning disabilities and a characteristic facial appearance. The clinical features of CTAFS include hypertelorism, hypospadias, swallowing problems and noisy breathing. These findings were extremely variable, from severe to mild, in affected family members (Emanuel et. al., 2001).

**CATCH 22** is the medical acronym of 22q11 deletion syndrome which stands for Cardiac defect, Abnormal face, Thymic hypoplasia, Cleft palate, Hypocalcemia and 22q11.2 deletion. Individuals with this syndrome have a range of findings, including CHD particularly conotruncal malformations (TOF, IAA and TA), palatal abnormalities (velopharyngeal incompetence), submucosal cleft palate, as well as cleft palate and learning difficulties (Fig 1.6) (Chung et. al., 2001). Less than 1% of patients with clinical findings of the 22q11.2 deletion syndrome have a translocation between chromosome 22 and 11 (Driscoll et. al., 1991; Wilson et. al., 1992). Standard karyotypic
analysis, even with high-resolution banding techniques will only detect 10-20% of 22q11.2 deletions (Goldmutz et. al., 2001).

3) **1q21 Microdeletions**: Microdeletion on chromosome 1q21.1 spanning between 1.5 to 3Mb has been reported to be associated with CHD, particularly, anomalies of aortic arch (Christiansen et. al., 2004).

4) **Subtelomeric deletions**: In recent years, subtelomeric rearrangements have been identified as a major cause of mental retardation and/or malformation syndromes. So far, over 2500 subjects with mental retardation have been tested and reported of whom ~5% appeared to have a subtelomeric rearrangement (De Vries et. al., 2003). Besides mostly microscopically visible deletions including 4p, 5p, 9p, 13q and 18p syndromes, submicroscopic deletions of 1p, 2q and 22q are frequently observed (Johnson et. al., 1976; Battaglia et. al., 1999; De Vries et. al., 2003). Recent studies have indicated an association of subtelomeric deletion of chromosome 9 and CHD (Schimmenti et. al., 1994; Ayyash et. al., 1997; Iwakoshi et. al., 2004). However, the majority of these cases with subtelomeric deletions lack a characteristic phenotype (Seemanova, 2002; Stewart, 2004; Neas et. al., 2005).

5) **Translocations**: Apart from the aneuploidy and deletions, several balanced and unbalanced translocations have also been reported to be associated with various forms of CHD along with extracardiac
anomalies. Some of them are t(1;8), t(1;18), t(2;22), t(2;6), t(2;11),
t(4;21), t(6;7), t(6;8), t(8;12), t(9;13), t(10;21), t(11;22) and t(15;22)
(Knight et. al., 1999; Prasad and Chudley, 2002; Patel et. al., 2004;  
Ergun et. al., 2004; Riegel et. al., 2005).

**Single gene disorders**

1) **Noonan syndrome**: Noonan syndrome is a genetic condition that affects the heart, growth, blood clotting; mental and physical development. The disorder may be transmitted as an autosomal dominant trait. Genetic analysis suggests that the disorder may result from spontaneous genetic mutations of a gene located on chromosome 12q24. The cardiac disease seen in this syndrome includes PS (Burch et. al., 1993), ASD, PDA, VSD and asymmetric septal hypertrophy (Marino et. al., 1997).

2) **Kabuki syndrome**: This syndrome is characterized by distinct facial anomalies, variable degrees of mental retardation, CHD and skeletal malformation. The CHD includes ASD, VSD, TOF, PDA, TGA, SV with common atrium and right bundle branch block (Digilio et. al., 2001).

3) **Ellis- van Creveld syndrome**: It shows skeletal dysphasia characterized by short limbs, short ribs, postaxial polydactyl, dysplastic nails and teeth and cardiac (heart) malformations (Mc Kusick, 2000). The EvC syndrome is a rare autosomal recessive trait located on chromosome 4p16.1 and has been found to be due to mutations in EVC or in another gene dubbed EVC2. Affected individuals with
mutations in EVC and EVC2 have the typical spectrum of features and are indistinguishable. CHD occur in 60% of affected individuals that are primary atrial septation, single atrium and HLHS (Ruiz-Perez et. al., 2000).

4) **William syndrome:** This is a genetic disorder characterized by mild mental retardation, unique personality characteristics, unusual facial features, cardiovascular disease, hypercalcemia and hypercalciuria. Cardiac anomalies include supravalvar aortic stenosis. This syndrome is inherited in an autosomal dominant manner and is due to a microdeletion. The region deleted is from chromosome 7 (band 7q11) and includes the ELN (elastin) gene. The syndrome is not merely to the loss of ELN, but to contiguous gene deletion (Zalzstein et. al., 1991).

5) **Marfan syndrome:** This is a connective tissue disorder, affecting many structures, including the skeleton, lungs, eyes, heart and blood vessels. It is an autosomal dominant disorder that has been linked to the *FBN1* gene on chromosome 15q21.1. *FBN1* encodes a protein called fibrillin, which is essential for the formation of elastic fibres found in connective tissue. Without the structural support provided by fibrillin, many tissues are weakened, which can have severe consequences, for example, ruptures in the walls of major arteries. Mutation in this gene causes cardiac disease that includes dilation of ascending aorta,
MVP and dilation of pulmonary artery (Francke and Furthmayr, 1993; Srivastava and Olson, 2000).

6) **Long QT syndrome (LQT):** This is an abnormality of the hearts electrical system. The electrical problem is due to defects in heart muscle cell structures called ion channels. It is usually inherited as an autosomal dominant trait. In the case of LQT1, which has been mapped to chromosome 11, mutations lead to serious structural defects in the persons cardiac potassium channels that do not allow proper transmission of the electrical impulses throughout the heart. There also appear to be other genes, tentatively located on chromosomes 3, 6 and 11 whose mutated products may contribute to, or cause, LQT syndrome (Nagai and Hoshino, 1998).

7) **Eisenmenger's syndrome:** Eisenmenger's complex is a VSD combined with pulmonary high blood pressure, the passage of blood from the right side of the heart to the left and an enlarged right ventricle. It may also include a malpositioned aorta that receives blood from both the right and left ventricles (an overriding aorta) (Bouzas and Gatzoulis, 2005).

8) **Char syndrome:** A genetic disorder characterized by PDA and unusual facial features including a long philtrum, down-slanting palpebral fissures, and thick lips as well as incurring fifth fingers. The syndrome is inherited as an autosomal dominant trait. The gene responsible for this syndrome is **TFAP2B** which is located on chromosome 6p12. It is
a transcription factor expressed in neuroectoderm during embryonic
development (Satoda et. al., 2000).

Multifactorial disorders

About 90% of the CHD are caused due by multifactors which could be
the combined effect of one or more genes interacting with various
environmental risk factors (http://www.lpch.org/DiseaseHealthInfo/
HealthLibrary/cardiac/fcchd.html).

Gene mutations causing isolated CHD

The past 15 years has witnessed an explosion of knowledge about
genes involved in normal cardiac development as well as genetic mutations,
which result in cardiac malformations. The recent findings of candidate genes
responsible for CHD have provided new insights into the genetic basis of
heart malformations (Srivastava and Olson, 2000). Candidate genes that play
critical functions at specific stages of cardiac development are emerging from
studies in vertebrate and invertebrate model organisms, providing possible
cause for various forms of CHD in human beings (Epstein and Buck, 2000).
The recent discovery that dominant inherited transcription factor mutations
cause isolated CHD has brought direct medical relevance to the study of
heart development (Bruneau et. al., 2000). Homeobox-containing genes play critical roles in regulating tissue-specific
gene expression essential for tissue differentiation, as well as, determining
the temporal and spatial patterns of development (Shiojima et al., 1995). The most common genes, associated with CHD are as follows:

1) **CSX / NKX2.5**: This is the earliest molecule marker of the cardiac lineage in vertebrates, which is present on chromosome 5q34 - q35.3 in humans. It is a vertebrate homeobox gene with a sequence homology to the *Drosophila tinman*, which is required for the dorsal mesoderm specification. It has highly conserved regions of DNA binding, protein-protein interactions, nuclear translocation and regulation of other transcription factors (Azpiazu and Frasch, 1993; Bodmer, 1993). Heterozygous mutations in NKX2.5 were found to cause human CHD such as ASD, VSD with atrial ventricular block, TOF and tricuspid valve abnormalities (Schott et al., 1998; Benson et al., 1999; Hosoda et al., 1999; Goldmuntz et al., 2001; McElhinney et al., 2003).

2) **GATA4**: The heart disease critical region is a 10cM segment defined by markers distally, D8S1706 and proximally, D8S1759 on chromosome 8p22-p23.1. This region harbors GATA4, a transcription factor, which is a member of the GATA family zinc finger proteins (Garg et al., 2003). The GATA-binding proteins are a group of structurally related transcription factors that control gene expression and differentiation in a variety of cell types. The proteins in the GATA family recognize a specific consensus sequence (A/TGATAA/G) and is known as the “GATA” motif, which is an important cis-element in the promoters of
many genes. \textit{GATA4}, is expressed in adult vertebrate heart, gut epithelium, and gonads. During fetal development, \textit{GATA4} is expressed in yolk sac endoderm and cells involved in heart formation (Pikkarainen et. al., 2004). Promoter and enhancer studies suggested that this factor might regulate genes critical for myocardial differentiation and function, including troponin C, cardiac alpha-myosin heavy chain (MYH6), and brain-type natriuretic factor (Lepore et. al., 2006).

\textit{GATA4} is capable of synergizing with other transcription factors such as \textit{NKX2.5}, \textit{dHAND} and \textit{TBX5} to activate cardiac-specific gene expression (Basson et. al., 1997; Garg et. al., 2003). The mutation in \textit{GATA4} diminishes DNA-binding affinity and transcriptional activity. Durocher et. al., (1997) demonstrated that \textit{GATA4} and \textit{NKX2.5} specifically cooperate in activating atrial natriuretic factor (ANF) and other cardiac promoters, and physically interact both in vitro and in vivo. Garg et. al., (2003) found that \textit{GATA4} interacts with \textit{TBX5} and raised the possibility that \textit{GATA4}, \textit{NKX2.5} and \textit{TBX5} function in a complex to regulate a subset of genes required for cardiac septal formation. Mutations in this gene are also found to cause septal defects in humans.

3) \textbf{TBX5}: TBX5 is a T-box containing transcription factor expressed early in heart development and is located on 12q21.3-q22 (Srivastava, 2001). In humans, missense mutations, which disrupt the 5' end of the
T-Box, have more severe cardiac effects than missense mutations at 3’ end of the T-Box. The range of cardiac abnormalities include atrioventricular blocks, failure of septa formation, HLHS, AS, MVP, TOF, secundum ASD, VSD, AVSD, and TA (Srivastava, 1999). Holt-Oram syndrome is a developmental disorder affecting the heart and upper limbs caused by mutation in the TBX5 gene (Li et al., 1997; Yang et al., 2000).

Garg et al., (2003) demonstrated that GATA4 interacts with TBX5 and showed that a missense mutation in GATA4, G296S, abrogated this interaction. Conversely, interaction of GATA4 and TBX5 was disrupted by specific human TBX5 missense mutations that cause similar cardiac septal defects.

4) dHAND/eHAND: Human dHAND and eHAND are two related basic helix-loop-helix transcription factors that are expressed in a complementary fashion in the developing right and left ventricles, respectively. They are also expressed in the neural crest-derived cardiac outflow tract and aortic arch arteries. dHAND mutations exhibit hypoplasia of the right ventricle, branchial arches and aortic arch arteries (Srivastava, 1999). dHAND expression was observed in all four chambers of the healthy human heart. In contrast, eHAND was expressed in the right and left ventricles but down regulated in both atrial chambers (Natarajan et al., 2001).
5) *Iroquois homeobox gene 4 (IRX4)*: Members of the Iroquois complex in *Drosophila*, including the highly homologous homeobox genes caupolican, araucan and mirror, act as prepattern molecules in neurogenesis. Bosse *et. al.*, (1997) identified three members of the Iroquois homeobox gene family in mice and showed that they are involved in several embryonic developmental processes including anterior/posterior and dorsal/ventral patterning of specific regions of the central nervous system and regionalization of the otic vesicle, branchial epithelium and limbs. The *IRX4* gene appears to be an important mediator of ventricular differentiation during cardiac development (*Bruneau et. al.*, 2000). Human *IRX4* is present in the developing central nervous system, skin and vibrissae, but are predominantly expressed in the cardiac ventricles. This Homeobox gene is likely to be an important mediator of ventricular differentiation during cardiac development, which is downstream of *NKX2.5* and *dHAND* (*Bruneau et. al.*, 2000).

6) *JAGGED-1*: This is a Notch Ligand, present on chromosome 20p12 and is expressed in the developing right heart. Missense mutation (G274D) in *JAGGED-1* causes defect in all forms of TOF. Mutation in *JAGGED-1* has also been identified in patients with Alagille syndrome (*Li et. al.*, 1997).
Inheritance of CHD

Almost all CHD have complicated modes of inheritance, altering standard autosomal dominance and autosomal recessiveness (Hoffman, 1990). In the general population, about 1% of all children are born with CHD. However, the risk increases when either parent is affected, or when another sibling was born with the same disease. If one of the children is born with CHD, the chance that another child will be born with the disease ranges from 1.5 - 5%, depending on the type of CHD in the first child. If there are more than 2 children with CHD, then the risk increases to 5 - 10%, to have another child with CHD. If the mother is affected, the risk for a child to be born with CHD ranges from 2.5 - 18%, with an average risk of 6.7%, whereas if the father is affected, the risk for a child to be born with CHD ranges from 1.5 - 3%. If there is another child born with CHD in a family, it can be a different type of defect than seen in another affected member in the family (Roodpeyma et. al., 2002). Today, more children born with CHD survive to adulthood due to surgeries and interventions now available. Thus, individuals live to have children who may inherit a specific CHD that is why occurrence of CHD is on the rise and will continue until prevention on the molecular level is feasible.

Congenital heart disease in India

Unfortunately, as the researchers understanding of the science of congenital heart disease improve with time, the same in India has not kept pace. Despite a high incidence of congenital heart disease in India, there is a paucity of genetic data from our population. Myriads of castes, sub castes and tribes and
high degree of endogamy and consanguinity in various sects along with more than one billion populations, provide an excellent opportunity for a systematic study of such birth defect in India.

Mysore is a city of southern India southwest of Bangalore belonging to Karnataka. Inhabited before the 3rd century B.C., it was the center of a Muslim state after the late 16th century and was occupied by the British in 1831. Mysore is an administrative district of Karnataka with an area of 6,268 sq km, and a population of 26,24,911 (2001 census), an increase of 15.04% from 1991. Mysore hospitals have a birth rate of 30 to 50 babies everyday and the clinicians of major hospitals of Mysore have an opinion that prevalence of CHD is more among the livebirths.

This is the maiden study, which aims to understand the genetics of congenital heart disease in a group of subjects from Mysore, South India. Thus, based on the available literature studies; the author putforth the following questions to understand the most complex disease – Congenital Heart Disease in Mysore population.

- What is the prevalence of CHD?
- What is the inheritance pattern of CHD?
- Is consanguinity a causative factor for CHD?
- Are there any chromosomal anomalies associated with CHD?
- Is ASD caused by exon-3 missense mutation (G-A) of GATA4?

Author made an attempt to answer these questions in subsequent sections of the thesis.
### Table 1.1: Prevalence of congenital heart disease at global level from the year 1975-2005

<table>
<thead>
<tr>
<th>Country/City</th>
<th>Year</th>
<th>Frequency per 1000 livebirths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liverpool</td>
<td>1975</td>
<td>6.6</td>
</tr>
<tr>
<td>UK</td>
<td>1981</td>
<td>5.51</td>
</tr>
<tr>
<td>USA</td>
<td>1990</td>
<td>6.60</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>1991</td>
<td>6.35</td>
</tr>
<tr>
<td>Norway</td>
<td>1994</td>
<td>10.6</td>
</tr>
<tr>
<td>Austria</td>
<td>1996</td>
<td>6.90</td>
</tr>
<tr>
<td>Karachi</td>
<td>1997</td>
<td>4.0</td>
</tr>
<tr>
<td>Qatar</td>
<td>1997</td>
<td>12.23</td>
</tr>
<tr>
<td>Bohemia</td>
<td>1999</td>
<td>6.16</td>
</tr>
<tr>
<td>Lebanon</td>
<td>1999</td>
<td>11.5</td>
</tr>
<tr>
<td>Oman</td>
<td>2000</td>
<td>7.1</td>
</tr>
<tr>
<td>Egypt</td>
<td>2000</td>
<td>1.01</td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>2001</td>
<td>10.7</td>
</tr>
<tr>
<td>Bosnia-Herzegovina</td>
<td>2003</td>
<td>6.12</td>
</tr>
<tr>
<td>Central Australia</td>
<td>2004</td>
<td>17.5</td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>2004</td>
<td>150</td>
</tr>
<tr>
<td>Spain</td>
<td>2005</td>
<td>8.96</td>
</tr>
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</table>
Table 1.2: Prevalence of congenital heart disease in different regions of India from the year 1962-2004

<table>
<thead>
<tr>
<th>Region</th>
<th>Year</th>
<th>Frequency per 1000 livebirths</th>
</tr>
</thead>
<tbody>
<tr>
<td>North India (Punjab)</td>
<td>1962</td>
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</tr>
<tr>
<td>South India</td>
<td>1968</td>
<td>25.6</td>
</tr>
<tr>
<td>Chandigarh</td>
<td>1971</td>
<td>50.89</td>
</tr>
<tr>
<td>Jammu (J&amp; K)</td>
<td>1979</td>
<td>9.7</td>
</tr>
<tr>
<td>New Delhi</td>
<td>1980</td>
<td>3.2</td>
</tr>
<tr>
<td>Agra</td>
<td>1993</td>
<td>5.2</td>
</tr>
<tr>
<td>New Delhi</td>
<td>1994</td>
<td>3.9</td>
</tr>
<tr>
<td>Shimla</td>
<td>1995</td>
<td>2.25</td>
</tr>
<tr>
<td>New Delhi</td>
<td>2001</td>
<td>4.2</td>
</tr>
<tr>
<td>Mumbai</td>
<td>2004</td>
<td>16.5</td>
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</table>
Table 1.3: Prevalence of major types of congenital heart disease from the year 1971-2004

<table>
<thead>
<tr>
<th>Country/City (Year)</th>
<th>ASD</th>
<th>VSD</th>
<th>PDA</th>
<th>TOF</th>
<th>AVSD</th>
<th>AS</th>
<th>PS</th>
<th>COA</th>
<th>TGA</th>
<th>Dextro-cardia</th>
<th>Complex</th>
<th>Others</th>
</tr>
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<td>12.0</td>
<td>29.0</td>
<td>11.0</td>
<td>17.0</td>
<td>0</td>
<td>2.5</td>
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<td>2.0</td>
<td>4.5</td>
<td>0</td>
<td>10.0</td>
<td>15.0</td>
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<tr>
<td>UK (1981)</td>
<td>5.9</td>
<td>32.5</td>
<td>11.9</td>
<td>5.9</td>
<td>2.4</td>
<td>5.1</td>
<td>7.6</td>
<td>6.3</td>
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<td>0</td>
<td>8.9</td>
<td>8.5</td>
</tr>
<tr>
<td>USA (1990)</td>
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<td>32.1</td>
<td>8.3</td>
<td>3.8</td>
<td>3.8</td>
<td>8.6</td>
<td>6.7</td>
<td>2.6</td>
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<td>0</td>
<td>8.9</td>
<td>14.2</td>
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<tr>
<td>Bohemia (1999)</td>
<td>8.67</td>
<td>41.59</td>
<td>5.07</td>
<td>3.36</td>
<td>4.00</td>
<td>7.77</td>
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<td>5.29</td>
<td>5.39</td>
<td>0</td>
<td>12.1</td>
<td></td>
</tr>
<tr>
<td>Denmark (1980)</td>
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<td>24.0</td>
<td>12.6</td>
<td>5.8</td>
<td>2.6</td>
<td>4.7</td>
<td>5.9</td>
<td>7.0</td>
<td>4.8</td>
<td>0</td>
<td>9.1</td>
<td>14.1</td>
</tr>
<tr>
<td>Qatar (1997)</td>
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<td>5.1</td>
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<td>2.5</td>
<td>8.7</td>
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<td>18.6</td>
</tr>
<tr>
<td>Japan (1990)</td>
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<td>60.0</td>
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<td>5.8</td>
<td>1.8</td>
<td>1.0</td>
<td>9.6</td>
<td>2.7</td>
<td>2.2</td>
<td>0</td>
<td>0</td>
<td>9.5</td>
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<tr>
<td>Bangalore (1995)</td>
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<td>16.4</td>
<td>18.7</td>
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<td>0</td>
<td>8.5</td>
<td>1.4</td>
<td>0</td>
<td>0</td>
<td>2.9</td>
<td>9.8</td>
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<td>Mexico (1998)</td>
<td>24</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>16</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>28</td>
</tr>
<tr>
<td>India (1999)</td>
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<td>9</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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</tr>
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<td>Egypt (2000)</td>
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<td>35.3</td>
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<td>5.0</td>
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<td>3.9</td>
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<td>0</td>
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<td>Oman (2000)</td>
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<td>10.3</td>
<td>9.6</td>
<td>5.9</td>
<td>3.6</td>
<td>8.8</td>
<td>3.7</td>
<td>3.6</td>
<td>0</td>
<td>8.5</td>
<td>6.7</td>
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<tr>
<td>New Delhi (2001)</td>
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<td>0</td>
<td>4.0</td>
<td>4.0</td>
<td>0</td>
<td>0</td>
<td>2.0</td>
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<td>2.0</td>
</tr>
<tr>
<td>S. Arabia (2001)</td>
<td>11.5</td>
<td>39.5</td>
<td>8.6</td>
<td>4.2</td>
<td>3.5</td>
<td>3.5</td>
<td>8.9</td>
<td>2.7</td>
<td>1.9</td>
<td>0</td>
<td>0</td>
<td>15.7</td>
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<tr>
<td>India (2004)</td>
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<td>17.68</td>
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<td>0</td>
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<td>0</td>
<td>0.68</td>
<td>25.16</td>
</tr>
</tbody>
</table>
Figure 1.1: Embryo showing the presence of (A) Cardiogenic plate at 16th day of gestation and (B) Heart tube formation after 21 days of gestation.

http://www.meddean.luc.edu/lumen/MedEd/GrossAnatomy/thorax0/Heart_Development/HeartIndex.html
Figure 1.2: Diagrammatic representation of the fetal heart showing the foramen ovale and ductus arteriosus.
www.echocharity.org.uk/sonogposter.html
Figure 1.3: A genetic blueprint of heart development (Srivastava et. al., 2000).
Figure 1.4: Diagrammatic representation of a normal human heart.
www.musckids.com/health_library/cardiac/chd.htm
Figure 1.5: Diagrammatic representation of different types of congenital heart disease
http://www.mayoclinic.org/heart_disease/types.html
Figure 1.5 continued

Tricuspid Arteria
- ASD
- Tricuspid valves absent
- Small Right Ventricle

Pulmonary Arteria
- ASD
- Pulmonary valves absent
- Small Right Ventricle
- VSD

Total Anomalous Pulmonary Venous Connection
- Abnormal pulmonary vein
- Left pulmonary vein

Aortic Arch
- Apex of Heart
- Dextrocardia

Single Ventricle
- Aorta
- Pulmonary artery
- Right Atrium
- Left Atrium
- SV

Double Outlet Right Ventricle
- Both great arteries arise from RV
- VSD related to both Aorta and Pulmonary artery


Contd..
Figure 1.5 continued

Interrupted aortic arch

Mitral Valve Prolapse

http://www.achaheart.org/defects.php
Figure 1.6: Flow chart of the clinical phenotypes associated with 22q11.2 deletion syndrome (CATCH 22) (Chung et. al., 2001).