MUTATIONAL SCREENING OF EXON 1 OF *SMAD7* IN MALAY PATIENTS WITH VENTRICULAR SEPTAL DEFECT

By

DR HASHIMA HASHIM

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Dr Hashima Hashim

MPath Medical Genetics

Human Genome Centre,

School of Medical Sciences,

Universiti Sains Malaysia Health Campus,

16150 Kubang Kerian, Kelantan, Malaysia

Introduction: Congenital heart disease (CHD) affects approximately 8 in every 1000 live births with ventricular septal defect (VSD) being the most common phenotype. VSD is thought to arise from genetics and environmental factors, however most of the causes remain unknown. It was hypothesized that *SMAD7* gene could influence the risk of VSD. *SMAD7* is a potent antagonist of TGF-β signalling pathways and has been found to be involved in embryonic cardiovascular development and function in mouse models. However, its role in the pathogenesis of VSD in human has yet to be fully understood. Therefore, *SMAD7* gene was examined in for its susceptibility to VSD in this study.

Objectives: The aims of this study were to screen and identify the genetic variants in exon 1 of *SMAD7* gene and to determine the association of the genetic variants with VSD in Malay population.

Patients and Methods: Peripheral blood samples were collected from 30 non-syndromic Malay patients with a clinical diagnosis of VSD. From these samples, genomic DNAs were extracted before subjected to PCR amplifications using 2 sets of designed

primers encompassing the exon 1 of *SMAD7*. Subsequently, re-sequencing was conducted to identify and characterize the genetic variants in *SMAD7* gene. Observed genetic variants were then genotyped in 30 healthy controls using the re-sequencing technique.

Results: A total of 2 genetic variants were identified in both cases and controls, comprising one common upstream gene sequence, rs7236774 and one rare synonymous sequence, rs368427729. Further analysis on these two genetic variants did not show any statistically significance association with the risk of developing VSD.

Conclusion: This case-control study has indicated that the exon 1 of *SMAD7* was not associated with VSD in Malay population. However, these findings could have been limited by small sample size. Therefore, further study in a larger cohort is warranted to yield a concrete evidence of this association.

Supervisor: **Dr Tan Huay Lin**

Co-Supervisor: **Associate Professor Colonel (Retired) Dr Wan Pauzi Wan Ibrahim**

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LIST OF SYMBOLS AND ABBREVIATIONS

µl	Microlitre
^{o}C	Degree Celcius
A_{260}/ A_{280}	Ratio of 260 absorbance over 280 absorbance
BLASTn	Standard Nucleotide Basic Local Alignment Search Tool
BMP	Bone Morphogenetic Protein
bp	Base pairs
CHD	Congenital Heart Defect/Disease
CI	Confidence Interval
CLT	Central Limit Theorem
ddH_2O	Deionized distilled water
DNA	DeoxyriboNucleic Acid
dNTPs	DiNucleotide TriPhosphate
EDTA	EthyleneDiamineTetraAcetic Acid
GWAS	Genome Wide Association Studies

HWE	Hardy-Weinberg Equilibrium
ICD-10	International Statistical Classification of Diseases and Related Health Problems, 10th Revision
MADH	Mothers Against Decapentaplegic, Drosophila, Homolog
MAF	Minor Allele Frequency
$MgCl_2$	Magnesium Chloride
MH1	Mad Homology 1
MH2	Mad Homology 2
ml	Millilitre
mM	Milimolar
mRNA	Messenger Ribonucleic Acid
NA	Not Applicable/ Not Available
NCBI	National Centre for Biotechnology Information
ng/µl	Nanogram per microlitre
nm	Nanometer
OR	Odds Ratio
PCR	Polymerase Chain Reaction
rpm	Round per minute
SD	Standard Deviation

SMAD	Small Mother Against Decapentaplegic
SNP	Single Nucleotide Polymorphism
SPSS	Statistical Package for Social Sciences
SSXS	Ser-Ser-X-Ser motifs
Taq	*Thermophilus aquaticus*
TBE	Tris Boric EDTA
TGF-β	Transforming Growth Factor-β
Tm	Melting Temperature
UV	Ultraviolet
V	Voltage
VSD	Ventricular Septal Defect

ABSTRAK

SARINGAN MUTASI EXON 1 GEN *SMAD7* DALAM KALANGAN PESAKIT MELAYU DENGAN KECACATAN SEPTAL VENTRIKEL

Penyakit jantung kongenital (CHD) menyerang kira-kira 8 orang dalam setiap 1000 kelahiran hidup dengan kecacatan septal ventrikel (VSD) sebagai fenotip yang paling kerap berlaku. Faktor-faktor genetik dan persekitaran dianggap sebagai punca terjadinya penyakit VSD, namun sebahagian besar daripada punca-puncanya masih belum diketahui. Hipotesis mengatakan bahawa gen *SMAD7* boleh mempengaruhi risiko berlakunya penyakit VSD. *SMAD7* adalah antagonis kepada laluan isyarat TGF-β dan ia juga dilaporkan terlibat dalam proses pembentukan kardiovaskular embrio dalam model tetikus. Walau bagaimanapun, peranannya dalam patogenesis penyakit VSD dalam manusia masih belum difahami secara jelas. Oleh itu, kajian ini dijalankan bagi menguji kecenderungan gen *SMAD7* terhadap penyakit VSD.

Satu kajian kes-kawalan telah dijalankan bagi mengenalpasti hubung kait antara *SMAD7* dengan penyakit VSD dalam kalangan penduduk Melayu. Exon 1 daripada *SMAD7* yang mengekod domain fungsian MH1 telah disusun semula melibatkan 30

pesakit VSD bukan sindromik dan 30 individu kawalan. Satu jujukan di bahagian pangkal gen yang biasa berlaku, iaitu rs7236774 dan satu urutan sinonim yang jarang berlaku, iaitu rs368427729 telah dikenal pasti di dalam kedua-dua kumpulan kes dan kawalan. Analisis lanjut mendapati, kedua-dua variasi tidak menunjukkan mana-mana hubungkait statistik yang signifikan dengan risiko mendapat VSD.

Kesimpulannya, kajian ini telah menunjukkan bahawa exon 1 daripada gen *SMAD7* tidak mempunyai kaitan dengan VSD dalam populasi Melayu. Walau bagaimanapun, hasil kajian ini mungkin dipengaruhi oleh saiz sampel yang kecil. Justeru, kajian lanjut dalam saiz sampel yang lebih besar adalah amat perlu untuk mengkaji hubungan gen dengan penyakit ini bagi mendapatkan bukti yang lebih kukuh.

ABSTRACT

MUTATIONAL SCREENING OF EXON 1 OF *SMAD7* IN MALAY PATIENTS WITH VENTRICULAR SEPTAL DEFECT

Congenital heart disease (CHD) affects approximately 8 in every 1000 live births with ventricular septal defect (VSD) being the most common phenotype. VSD is thought to arise from genetics and environmental factors, however most of the causes remain unknown. It was hypothesized that *SMAD7* gene could influence the risk of VSD. *SMAD7* is a potent antagonist of TGF-β signalling pathways and has been found to be involved in embryonic cardiovascular development in mouse models. However, its role in the pathogenesis of VSD in human has yet to be fully understood. Therefore, *SMAD7* gene was examined in for its susceptibility to VSD in this study.

A case-control study was conducted to examine whether *SMAD7* is associated with VSD in Malay population. Exon 1 of *SMAD7* which encodes the functional MH1 domain was re-sequenced in 30 non-syndromic VSD patients and 30 control individuals. One common upstream gene sequence, rs7236774 and one rare synonymous sequence, rs368427729 were observed in both cases and controls. Further

analysis on these two variations did not show any statistically significance association with the risk of developing VSD.

In conclusion, this study has indicated that the exon 1 of *SMAD7* was not associated with VSD in Malay population. However, these findings could have been limited by small sample size. Therefore, further study in a larger cohort is warranted to yield a concrete evidence of this association.

CHAPTER 1

INTRODUCTION

1.1 Introduction and overview

"The heart is the youngest, most diverse, most fluid, most changeable, most versatile part of creation"

Johann Wolfgang Von Goethe (Williams, 1998)

The heart is the first functional organ to form during embryogenesis in order to provide adequate nutrients and oxygen to the growing organ systems in a developing foetus. In relation to other organs; the heart is unique because it can perform its function throughout development. As early as 3 weeks of gestation, the heart is already functional although it is far from fully developed (Moore and Persaud, 1998). Several major processes such as heart tube formation, looping of the heart, chamber formation, valve development and coronary vasculogenesis are necessary for the development of heart in its mature form. The whole process involves a precisely orchestrated series of molecular and morphogenetic events and it takes about 11 weeks for it to be fully

formed (Iaizzo, 2009). Any subtle changes of this process can lead to serious consequences in the form of congenital heart disease (CHD).

CHD is one of the leading causes of childhood mortality and morbidity worldwide (Richards and Garg, 2010). Despite advances in the patient management, understanding of the aetiopathogenesis of CHD remains incomplete. Advances have been made in understanding the molecular basis of CHD, including the identification of signalling molecules, transcriptional regulators and structural genes. Animal models have significantly aided in identifying the molecular mechanisms of CHD, however more human-based research is needed to better understand the pathogenesis of human CHD. Understanding the molecular aspects of heart development will help to identify the risk of having CHD with screening tests, improve clinical diagnosis with diagnostic tests, provide accurate genetic counselling and identify potential therapeutic interventions.

1.2 Cardiogenesis

The cardiovascular system is the first functional major system in the embryo. The primordial heart and vascular system develop in the cardiogenic region of the mesoderm from week 3 of gestation (**Figure 1.1**).

1.2.1 Primordial heart tube

Beginning at day 13 of human development, a primitive streak starts to develop. Three primary germ layers; ectoderm, mesoderm and endoderm are formed around day 15 of gestation. The primordium of the heart is mainly derived from the splanchnic mesoderm which appears around day 18 of embryonic development. Primitive streak cells migrate to an anterior and lateral position and then fuse to form a cardiac crescent. Lateral folding of the embryo causes the cells of the crescent to coalesce along the ventral midline to form a single primitive heart tube. As the folding process continues, the heart tube comes to lie ventrally to the foregut. The heart tube continues to develop to form five distinct regions; truncus arteriosus, bulbous cordis, primitive ventricle, primitive atrium and sinus venosus. This is followed by the heart looping process whereby the quick development of bulbous cordis and ventricle are causing the heart tube to bend upon itself and to form a U-shaped bulboventricular loop. As a result, the atrium and sinus venosus end up lying dorsal to the truncus arteriosus, bulbous cordis and ventricle. The whole process completes by day 28 of embryonic development (Moore and Persaud, 1998).

1.2.2 Partitioning of primordial heart and formation of heart chambers

This process involves formation of the atrioventricular (AV) canal, primordial atrium and primordial ventricle; which occur concurrently from the end of the fourth week till late fifth week.

At the end of the fourth week, two endocardial cushions appear from the dorsal and ventral walls of AV canal. These endocardial cushions fuse and form right and left AV canals, partially separating the atrium from the ventricle.

Septum primum appears at the end of the fourth week and grows towards the fusing endocardial cushions, thereby partially dividing the primordial atrium into right and left atria. Foramen primum resulted from this process, which is located at the area between the endocardial cushion and the free edge of the septum primum. The extensions of the endocardial cushions grow along the margin of the septum primum and eventually close the ostium primum. Coalescence of these perforations results in formation of the septum secundum. This septum grows towards the endocardial cushion, gradually overlapping the septum primum causing the formation of the foramen ovale. The foramen ovale will only close after birth, resulting in a complete partition of the atria.

A muscular interventricular (IV) septum is also developed at the end of fourth week, located in the floor of ventricle near its apex. While growing, a gap is formed between the IV septum and the endocardial cushion, called the IV foramen. The IV foramen usually closes by the end of the seventh week as a result of fusion of bulbar ridges and

the endocardial cushion. This fusion also results in the formation of the membranous part of the IV septum. The whole process causes the division of the primordial ventricle into right and left ventricles, which is completed by the end of week 7 (Moore and Persaud, 1998).

1.2.3 Partitioning of the bulbous cordis and truncus arteriosus

Early in the fifth week of development, ridges develop in the truncus arteriosus and are continuous with the bulbar ridges. Both bulbar and truncal ridges undergo 180 degree spiralling motion before fusing, resulting in the formation of a spiral aorticopulmonary septum. This septum eventually creates the aorta and the pulmonary trunk. The whole process completes by the end of week 7 (Moore and Persaud, 1998).

1.2.4 Development of heart valves

Semilunar valves begin to form at late fifth to early sixth week of development, just before the partitioning of truncus arteriosus completes. The semilunar valves develop from three swellings of subendocardial tissue located around the openings of the aorta and pulmonary trunk. These swellings eventually reshape to form three thin-walled cusps. The atrioventricular valves also formed in the similar manner as aforementioned. These valves develop from swellings of the tissue around the AV canals (Moore and Persaud, 1998).

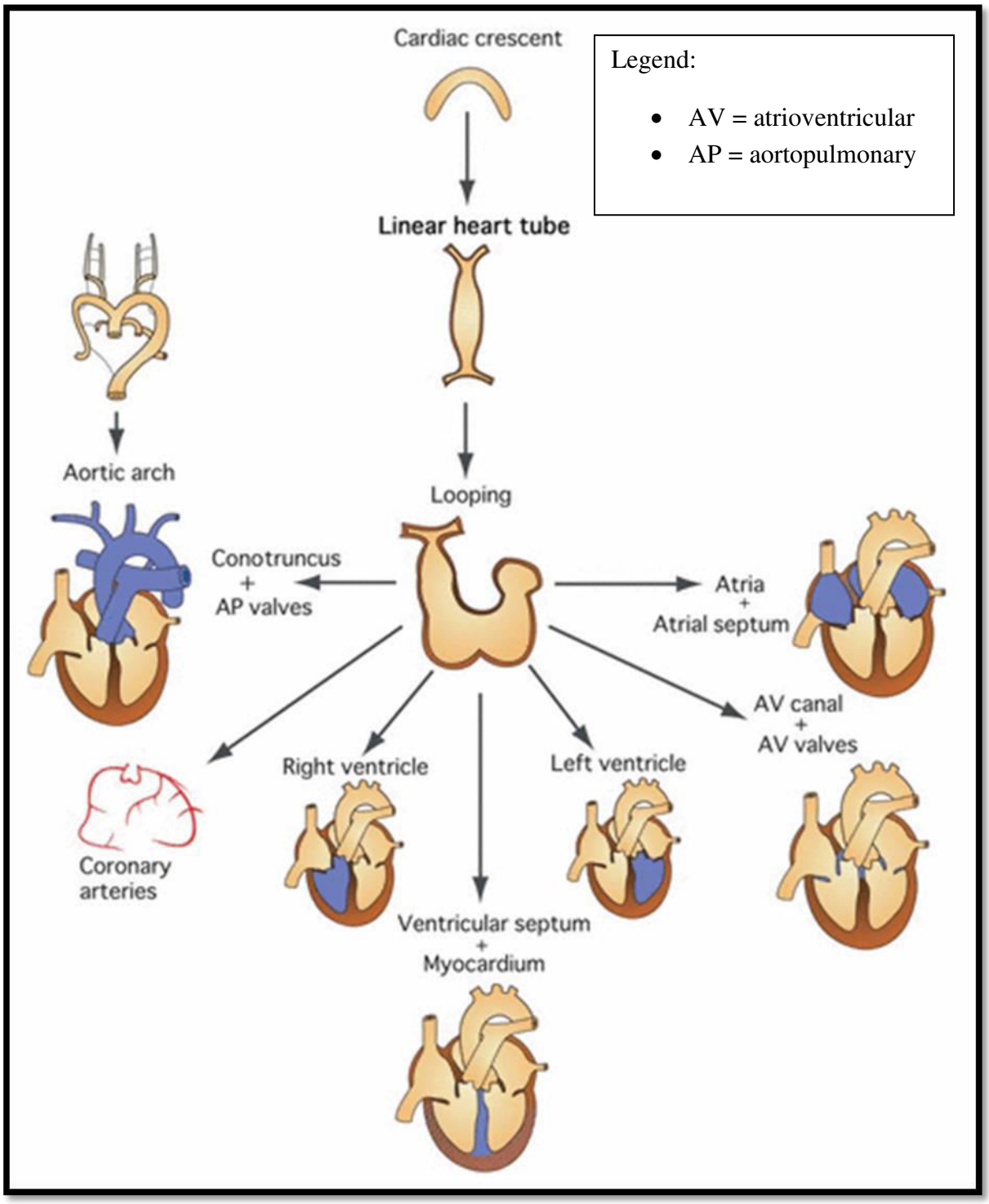

Figure 1.1 Cardiovascular developments

Adapted from Yamagishi et al. (2009)

1.3 Congenital Heart Disease (CHD)

Congenital (*con*, together; *genitus*, born) heart disease is defined as a gross structural abnormality of the heart or intrathoracic great vessels that is actually or potentially of functional significance (Mitchell *et al.*, 1971).

1.3.1 Epidemiology of CHD

CHD is the most frequently occurring birth defect, comprising approximately 40% of all congenital defects (Romano-Zelekha *et al.*, 2001). It is the leading non-infectious cause of worldwide neonatal and infants mortality. The reported birth prevalence of CHD is approximately 8 in 1000 live births worldwide (Bernier *et al.*, 2010). Geographical distributions influence the birth prevalence of CHD. Compared to other continents, Asia is reported to show the highest CHD birth prevalence with 9.3 per 1000 live births, followed by Europe with 8.2 per 1000 live births. Africa shows the lowest birth prevalence of CHD, with only 1.9 per 1000 live births (van der Linde *et al.*, 2011).

The estimated frequency of severe heart defects in live born infants is 0.6% (Hoffman and Kaplan, 2002; Pradat *et al.*, 2003); however, the incidence is higher if less serious and mild defects are included which account for nearly 0.9% of live born infants (Hoffman and Kaplan, 2002; Tutar *et al.*, 2005). For instance, the incidence of bicuspid arterial valve (BAV), which often goes undetected until late middle age; is nearly 2% (Tutar *et al.*, 2005). To top it all, the percentage would be even higher if the proportion of pregnancies which do not continue to term were included, as there is a 10-fold higher rate of CHD in foetuses compared to live born infants (Salvador *et al.*, 2005).

According to Hoffman and Kaplan (2002), the estimated rate of severe cardiovascular malformations has not differed much from one study to the other over the last 50 years. However, there is an apparent increase in the frequency over the last two decades, attributed largely by the increased use of echocardiography (Hoffman and Kaplan, 2002; Bosi *et al.*, 2003). The higher prevalence may be caused by changes in diagnostic techniques and screening modalities rather than a true increase (van der Linde *et al.*, 2011). Prior to the echocardiography era, diagnosis of CHD was dependent on physical examination, conventional x-rays, surgical findings, death certificates and autopsy findings. Medical advancement and evolution not only improved diagnostic approach, but also played a role in the outcome and survival of patients with CHD. Before the introduction of cardiac surgery in 1939, fewer than 25% of infants born with complex CHD survived past their first year of life. However, nowadays more than 90% of such infants can survive into adulthood (Warnes, 2005).

1.3.1 (a) Epidemiology of CHD in Malaysia

To date, there are only limited studies in Malaysia describing the epidemiology, clinical presentation and outcome of CHD; despite the significant role of CHD in causing mortality. According to the Ministry of Health, there are no local data on the incidence of congenital heart defects in Malaysia. Based on the international, particularly Asian prevalence of 8 – 9.3 in 1000 live births, with a birth rate of approximately 500,000 per annum in Malaysia; it is estimated that about 4,000 to 4650 infants will be born with congenital heart defects every year. About one third of these patients have simple lesions and will not require any intervention while another two thirds will require cardiac intervention or surgery.

1.3.2 Classification of CHD

There are several ways to classify CHD, typically by using a physiological or anatomical approach. The physiological approach divides CHD into either cyanotic or acyanotic lesions. Cyanosis occurs when there is more than 5 g/dl of deoxygenated venous blood. Blood is often shunted to the left heart (right-to-left shunt), systemically reducing arterial O_2 saturation. One of the most frequent cyanotic heart diseases is Tetralogy of Fallot. In the case of acyanotic heart lesion, oxygenated blood from the left heart shunts to the right, creating the left-to-right shunt. Ventricular septal defect, atrial septal defect and patent ductus arteriosus fall into this category. The anatomical approach divides CHD into either simple or complex diseases. One of the preferred methods of classifying CHD is by combining both anatomical and physiological approach, as shown in **Table 1.1**.

Apart from the aforementioned methods, another preferred method is by classifying CHD based on ICD classification, as shown in **Table 1.2**. ICD, an acronym for International Classification of Diseases, is a set of codes used by healthcare personnel to indicate diagnosis for all patients encounters; based World Health Organization (WHO). In ICD-10 (International Statistical Classification of Diseases and Related Health Problems, 10th Revision), CHD is grouped as 'Congenital malformations, deformations and chromosomal abnormalities' in chapter XVII, Q20-Q24.

Table 1.1 Classification of CHD, *Adapted from Wu and Child (2004)*

Types of CHD
Left to right shunt
Atrial septal defect (ASD)
Ventricular septal defect (VSD)
Atrioventricular septal defect (AVSD)
Patent ductus arteriosus (PDA)
Partial anomalous pulmonary venous drainage
Outflow obstruction
Bicuspid aortic valve
Coarctation of the aorta
Pulmonary stenosis
Hypoplastic left heart syndrome
Cyanosis and decreased pulmonary blood flow
Tetralogy of Fallot
Tricuspid atresia
Pulmonary atresia
Hypoplasia of right ventricle
Ebstein's anomaly
Cyanosis and increased pulmonary blood flow
Transposition of great arteries
Double outlet ventricle
Double inlet ventricle
Truncus arteriosus
Total anomalous pulmonary venous drainage
Cyanosis and increased pulmonary vascular resistance
VSD with Eisenmenger syndrome
ASD with Eisenmenger syndrome
PDA with Eisenmenger syndrome
Anomalies of major blood vessels
Congenitally corrected transposition of the great arteries
Coronary artery anomalies

Table 1.2 ICD-10 classification of CHD *(Source: WHO)*

Code	Description
Q20	**Congenital malformations of cardiac chambers and connections**
	Common arterial trunk: Persistent truncus arteriosus
	Double outlet right ventricle: Taussig-Bing syndrome
	Double outlet left ventricle
	Discordant ventriculoarterial connection: Transposition of great vessels
	Double inlet ventricle
	Discordant atrioventricular connection: Corrected transposition
	Isomerism of atrial appendages
Q21	**Congenital malformations of cardiac septa**
	Ventricular septal defect
	Atrial septal defect
	Atrioventricular septal defect
	Tetralogy of Fallot
	Aortopulmonary septal defect
	Other congenital malformation of of cardia septa: Eisenmenger's syndrome
Q22	**Congenital malformations of pulmonary and tricuspid valves**
	Pulmonary valve atresia
	Congenital pulmonary valve stenosis
	Congenital pulmonary valve insufficiency
	Other congenital malformations of pulmonary valve
	Congenital tricuspid stenosis: Tricuspid atresia
	Ebstein's anomaly
	Hypoplastic right heart syndrome
	Other congenital malformations of tricuspid valve
	Congenital malformation of tricuspid valve, unspecified
Q23	**Congenital malformations of aortic and mitral valves**
	Congenital stenosis of aortic valve
	Congenital insufficiency of aortic valve
	Congenital mitral stenosis
	Congenital mitral insufficiency
	Hypoplastic left heart syndrome
	Other congenital malformations of aortic and mitral valves
	Congenital malformation of aortic and mitral valves, unspecified
Q24	**Other congenital malformations of heart**
	Dextrocardia, Laevocardia
	Cor triatriatum
	Pulmonary infundibular stenosis
	Congenital subaortic stenosis
	Malformation of coronary vessels
	Congenital heart block
	Other specified congenital malformations of heart
	Congenital malformation of heart, unspecified

1.3.3 Aetiology of CHD

CHD is thought to be resulting from multiple factors that cause disruptions at various points along the cardiac developmental pathway. Some genetic defects and certain environmental factors have been identified contributing to the abnormal formation of the heart. While genetic and environmental causes of CHD are well recognized, the majority of the causes of CHD remain unknown. Evidence from familial aggregation studies, chromosomal studies and animal studies indicate that CHD is caused by 'multifactorial inheritance', which is due to a combination of both genetic and environmental factors, where the environmental factor interacts with a genetic predisposition in an individual (Nora, 1968; Gelb, 2004; Garg, 2006). It is estimated that more than 85% of CHDs result from a complex interaction between foetal – maternal exposures and genetic susceptibilities (Botto and Correa, 2003). Even in families with multiple affected individuals, environmental factors may be needed for the development of CHD.

1.3.3 (a) Environmental factors

Environmental factors of CHD are broadly meant to include all factors external to the genetic architecture of the mother and the developing foetuses. Potential environmental agents include infections, chemical and pharmacological exposures, physical factors as well as maternal nutrition. Human exposure to environmental agents during the three months before pregnancy and weeks 2 through 7 of gestation are most likely to result in cardiac structural abnormalities, therefore; this segment is focused on factors that influence cardiac development during this period (Jenkins *et al.*, 2007). **Table 1.3** summarized the potential environmental factors associated with CHD.

These factors may exert effect directly on the foetus or indirectly via the mother. However, the exact mechanisms by which these environmental agents disrupt embryogenesis and cause defects still remain to be elucidated. Depending on the maternal or foetal genotype, environmental exposure may differently affect the heart development; for example, exposure to the different level of the environmental factor might result in different phenotype. Furthermore, it is likely that some genetic polymorphisms are more environmentally sensitive resulting phenotypic heterogeneity (Wyszynski *et al.*, 2010).

Table 1.3 Environmental factors and associated CHD

Adapted from Jenkins et al. (2007) and Mahler and Butcher (2011)

Factors	Associated defects
Maternal Illness	
Pregestational diabetes	Conotruncal defects Laterality and looping Transposition of the great arteries Septal defects Hypoplastic left heart syndrome Outflow tract defects Patent ductus arteriosus
Phenylketonuria	Tetralogy of Fallot Coarctation of the aorta
Febrile illness	Conotruncal defects Right-sided obstructive defects Tricuspid atresia Left-sided obstructive defects Coarctation of the aorta Ventricular septal defect
Maternal Infections	
Influenzae	Transposition of the great arteries Right-sided obstructive defects Left-sided obstructive defects Coarctation of the aorta Ventricular septal defect
Rubella	Patent ductus arteriosus Peripheral pulmonary artery stenosis Atrial septal defect Ventricular septal defect
Maternal therapeutic drug exposure	
Indomethacin tocolysis	Patent ductus arteriosus
Ibuprofen	Transposition of the great arteries Ventricular septal defect Bicuspid aortic valve
Anticonvulsants	Any defects
Thalidomide	Any defects
Maternal non-therapeutic drug exposure	
Vitamin A	Outflow tract defects Cranial neural crest defects (cardiac and noncardiac) Pulmonic stenosis
Recreational drugs (Marijuana and cocaine)	Atrial septal defect Ventricular septal defect Pulmonary stenosis Ebstein's anomaly
Others	
Organic solvents	Any defects

1.3.3 (b) Genetic factors

CHD may occur as an isolated defect (non-syndromic) or be associated with other birth defects and dysmorphism (syndromic). Isolated defects are the most prevalent form of CHD. While most CHD patients do not have other defects, studies have shown that it can occur in the setting of other multiple congenital anomalies, developmental abnormalities or growth abnormalities and be a part of a syndrome (Bernstein, 2004; Pierpont *et al.*, 2007). The pathogenesis of and risk factors for CHD in a syndromic child are likely to be different from those in a child without accompanying syndrome. A number of studies have considered infants with a syndrome to be a highly susceptible population, providing insight into the risk factors of heart defects (Kerstann *et al.*, 2004).

Genetic factors of CHD can be classified according to the underlying genetic mechanism. Syndromic CHD can be due to chromosomal, single gene or unknown causes (Fahed and Nemer, 2012), whereas non-syndromic CHD is more likely to be caused by single gene mutation, genomic imbalances and complex inheritance (Wyszynski *et al.*, 2010). Approximately 20% of CHD is associated with a chromosomal abnormality, a congenital syndrome or a single gene mutation with Mendelian inheritance pattern (Pierpont *et al.*, 2007; Warnes *et al.*, 2008). However, for the other 80% of non-syndromic and sporadic CHD cases, the underlying genetic mechanisms are still poorly understood.

1.3.3 (b) (i) Chromosomal abnormality and CHD

CHD of chromosome abnormality can either be due to chromosome dosage disorders, large chromosomal deletions or small micro-deletions (Fahed and Nemer, 2012). A recent population study by Hartman *et al.* (2011) has identified chromosome abnormalities in 12.3% of infants with CHD. The most likely chromosome abnormality-associated CHDs are interrupted aortic arch (69.2%), atrioventricular septal defect (67.2%) and double-outlet right ventricle (33.3%). (Hartman *et al.*, 2011).

Approximately 30% of children with a chromosomal abnormality have CHD (Pierpont *et al.*, 2007). The most common chromosomal abnormalities are trisomy 21, trisomy 18, 22q11.2 deletion and trisomy 13. **Table 1.4** listed some chromosomal abnormalities with the corresponding CHD.

Table 1.4 Chromosomal abnormalities and CHD

Adapted from (Richards and Garg, 2010)

Diseases of chromosomal abnormalities	Types of CHD
Trisomy 21 (Down Syndrome)	Atrial septal defect Ventricular septal defect Atrioventricular septal defect Tetralogy of Fallot
Trisomy 18 (Edward Syndrome)	Atrial septal defect Ventricular septal defect Patent ductus arteriosus Tetralogy of Fallot Double outlet right ventricle Coarctation of aorta Bicuspid aortic valve
Trisomy 13 (Patau Syndrome)	Atrial septal defect Ventricular septal defect Patent ductus arteriosus Hypoplastic left heart syndrome
Monosomy X (Turner Syndrome)	Coarctation of aorta Bicuspid aortic valve Aortic stenosis Hypoplastic left heart syndrome
47, XXY (Klinefelter Syndrome)	Patent ductus arteriosus Atrial septal defect Mitral valve prolapsed
22q11.2 deletion (DiGeorge Syndrome)	Interrupted aortic arch type B Aortic arch anomalies Truncus arteriosus Tetralogy of Fallot
7q11.23 deletion (Wlliams-Beuren Syndrome)	Supravalvular aortic stenosis Peripheral pulmonic stenosis

1.3.3.(b) (ii) Single gene mutation and CHD

The majority of CHD cases is thought to occur due to a gene mutation; suggested by early observation of Mendelian inheritance in familial CHD and the involvement of micro and macro deletions in chromosomal regions resulting in CHD with other manifestation. Advancement in gene sequencing and other techniques have helped to identify molecular genetic causes of CHD. Many genes have been found to be involved in the complex process of heart development in animal models, however few have shown an association with CHD in humans.

Some of the identified genes are associated with non-syndromic CHD and others with syndromic CHD (Wessels and Willems, 2010; Fahed and Nemer, 2012). In syndromic and non-syndromic cases, CHD can occur due to mutations in more than one gene (genetic heterogeneity) and mutations in a single gene can result in variable CHDs (phenotypic heterogeneity). These genes encode for agents of the heart developmental programme involving numerous pathways with cross communications, ligand-receptor interactions, complex signal transductions, and a network of transcription factors that regulate the expression of cardiac specific genes. Any disruption of these genes will result in cardiac defects. **Table 1.5** and **Table 1.6** show the association of single gene defects with syndromic and non-syndromic CHD respectively. Of all genes identified, three categories of genes comprise the majority of the known genetic causes of CHD. Those groups are GATA transcription factors (*GATA4, GATA5*, and *GATA6*), Homebox transcription factors (*NKX2-5* and *NKX2-6*), and T-box transcription factors (*TBX1, TBX5*, and *TBX20*) (Fahed and Nemer, 2012). Genes from these categories interact to regulate the expression of downstream genes involved in heart development.

Table 1.5 Single gene defects and syndromic CHD

Adapted from Pierpont et al. (2007) and Richards and Garg (2010)

Syndromes	Types of CHD	Causal gene(s)
Noonan syndrome	Pulmonic valvular stenosis	*PTPN11*
	Atrioventricular septal defect	*KRAS*
	Hypertrophic cardiomyopathy	*RAF1*
	Coarctation of aorta	*SOS1*
	Tetralogy of Fallot	
Alagille syndrome	Pulmonic valvular stenosis	*JAG1*
	Tetralogy of Fallot	*NOTCH2*
	Atrial septal defect	
	Peripheral pulmonary stenosis	
Holt-Oram syndrome	All septal defects	*TBX5*
	Progressive atrioventricular conduction system disease	
Char syndrome	Patent ductus arteriosus	*TFAP2b*
CHARGE syndrome	Atrial septal defect	*CHD7*
	Ventricular septal defect	*SEMA3E*
	Atrioventricular canal defects	
	Tetralogy of Fallot	
	Coarctation of aorta	
	Patent ductus arteriosus	

Table 1.6 Single gene defects and non-syndromic CHD

Adapted from Garg (2006) and Fahed and Nemer (2012)

Causal gene(s)	Types of CHD
NKX2.5	Atrial septal defect Atrioventricular conduction delay Tetralogy of Fallot Tricuspid valve abnormalities
GATA4	Atrial septal defect Ventricular septal defect
MYH6	Atrial septal defect Hypertrophic cardiomyopathy
BMPR2	Cardiac septation defects associated with pulmonary hypertension
CRELD1, ALK2	Endocardial cushion defects
NOTCH1	Bicuspid aortic valve Early valve calcification
PROSIT-240	Transposition of the great arteries

1.4 Ventricular septal defect (VSD) and *SMAD7* gene

1.4.1 VSD

VSD is the most common congenital heart defect at birth (Hoffman and Kaplan, 2002) accounting for 25% of all congenital heart defects and occurring in approximately 3.0 to 3.5 infants per 1000 live births (Warnes *et al.*, 2008). Previous studies have reported that there is a high incidence of spontaneous closure of small VSDs, therefore the incidence is much less in older infants and in adults (Du *et al.*, 1998).

VSD is classified in regards to the anatomic landmarks of the ventricular septum. The ventricular septum is a curvilinear, three-dimensional partition that divides the heart into five components: membranous, infundibular (or subarterial), inlet, muscular (or trabecular) and the atrioventricular septum (**Figure 1.2**). Incomplete fusion or deficit growth of any of these components will lead to VSD. Based on the Congenital Heart Surgery Nomenclature and Database Project (Jacobs *et al.*, 2000), VSD is classified into multiple, type 1, type 2, type 3, type 4, Gerbode type and secondary VSD; as shown as in **Table 1.7**.

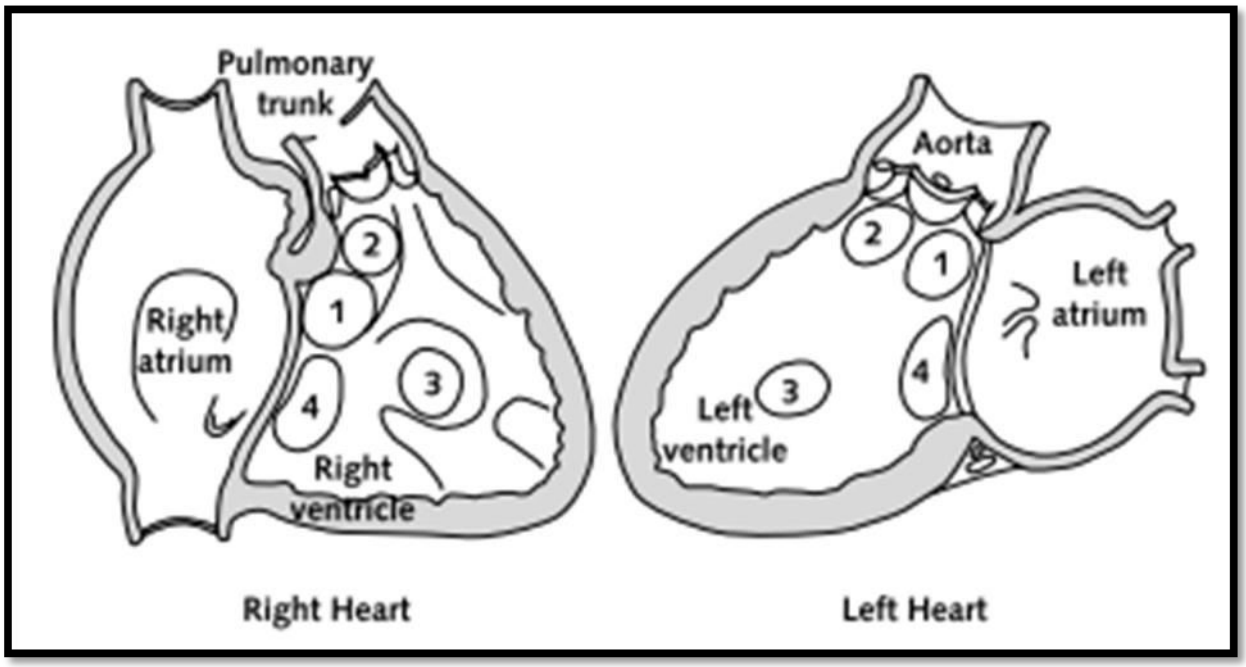

Figure 1.2 Positions of different ventricular septal defect

Adapted from Ammash and Warnes (2001)

Legends:

1 membranous

2 subarterial or supracristal

3 muscular or trabecular

4 inlet or canal

Table 1.7 Classification of VSD, *based on the Congenital Heart Surgery Nomenclature and Database Project (Jacobs et al., 2000)*

VSD subtypes	Synonyms
Type 1	Conal septal defect
	Subarterial
	Supracristal
	Subpulmonary
	Infundibular
Type 2	Perimembranous
	Paramembranous
	Conoventricular
Type 3	Inlet
	Atrioventricular canal type
Type 4	Muscular
Gerbode type	Atrioventricular

Of all subtypes of VSD, membranous type is the most commonly seen defect, account for 80% of the cases. It is located inferior to the aortic valve and bordering the septal leaflet of the tricuspid valve. It can extend into the adjacent muscular septum, termed perimembranous VSD; which often associated with aneurysm of the septal leaflet of the triscuspid valve and aortic regurgitation (Warnes, 2009).

Infundibular VSD, which account for 5-8% of isolated VSDs in the United States but 30% in Southeast Asia; is a defect that lies beneath the pulmonic valve and communicates with the right ventricle outflow tract above the supraventricular crest. It is associated with aortic regurgitation secondary to the prolapse of the right aortic cusp. Aortic regurgitation can help to reduce the size of the defect but no spontaneous closure is reported (Warnes, 2009).

Inlet VSD lies beneath both atrioventricular valve and extends to the septal leaflet of the tricuspid valve. Although it is in close proximity with the atrioventricular valves, they are not associated with defects of the mitral or tricuspid regurgitation. About 8-10% of VSDs are of this type. Inlet VSD is often seen in Down syndrome patients (Warnes, 2009).

Muscular VSD is entirely bounded by the muscular septum and represents 5-20% of all defects. The defect can either be small or large, single or multiple. The term Swiss-cheese septum has been used to describe multiple muscular VSDs (Warnes, 2009).