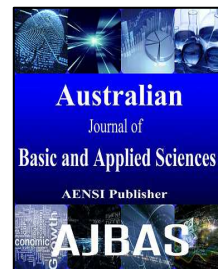




AUSTRALIAN JOURNAL OF BASIC AND APPLIED SCIENCES

ISSN:1991-8178 EISSN: 2309-8414
Journal home page: www.ajbasweb.com



Congenital heart disease in Saudi Arabia: the role of molecular genetics with a focus on Down syndrome

¹Abdulhadi H. Al-Mazroea, ²Sahar AF. Hammoudah, ³Lama M. El-Attar, ¹S. Justin Carlus, ^{1,4}Yousef Almohammadi, ¹Khalid M Al-Harbi

¹Cardiogenetic Team, Department of Pediatrics, College of Medicine, Taibah University

²Faculty of Medicine, Tanta University, Egypt

³Medical Research Institute, Alexandria University, Egypt

⁴Security Forces Medical Centre, Al-Madinah, Saudi Arabia

Address For Correspondence:

Dr. Khalid M Al-Harbi, Cardiogenetic Team, Department of Pediatrics, College of Medicine, Taibah University, Al-Madinah, Kingdom of Saudi Arabia

Mobile: 00966550118844; E-mail: drkharbi@yahoo.com

ARTICLE INFO

Article history:

Received 3 March 2016; accepted 2

May 2016; published 26 May 2016

Keywords:

Congenital heart diseases, Down syndrome, Molecular genetics, Saudi Arabia.

ABSTRACT

Background: Genetic and congenital disorders are the main causes of infant and child morbidity, mortality, and, disability in Arab populations. Congenital heart diseases (CHDs) are a major category of birth defects, reported in about 6–13 per 1000 live births and implicated in early childhood mortality. Materials and Methods: We had searched MEDLINE databases, clinical-science journals and reports from the earlier reviews. We used the search term “Congenital heart diseases”, “Down syndrome”, “Molecular genetics”. Results: CHDs are an important health problem in Saudi Arabia, exacerbated by widespread consanguinity. CHDs occur in about 50% of neonates born with Down Syndrome (DS) and almost 40% of DS survivors. In exploring the genes and molecular genetic pathways involved in heart development, some of which overlap with DS, we illustrate the genetic basis of CHDs. Unidentified genetic variations might contribute to this complex genetic disorder. Conclusions: We highlight that CHDs are a major health problem in Saudi Arabia and emphasize the role of molecular genetics in the pathogenesis of CHDs in children with DS.

INTRODUCTION

Globally, congenital heart disease (CHD) is a major type of birth defect and an important cause of neonatal death (Al-Gazali, L., *et al.*, 2006). Cardiac septation defects are the commonest form of CHD, occurring in approximately 50% of all cases (Pierpont, M.E *et al.*, 2007). Septation defects include atrial septal defect (ASD), ventricular septal defect (VSD), and atrioventricular septal defect (AVSD), which are strongly associated with some Mendelian syndromes (Wolf, M., C.T. Basson, 2010; Freeman, S.B., *et al.*, 2008).

Prevalence of CHD:

The worldwide prevalence of CHD is estimated to be 6–13 per 1000 live births (Ishikawa, T., *et al.*, 2001; Khoshnood, B. *et al.*, 2012). There are significant differences in prevalence based on geography: according to the American Heart Association (Go, A.S., *et al.*, 2013), Asia has the highest prevalence of CHD at 9.3 per 1000 live births, while in Europe it is 6.9 and in North America 8.2 per 1000 live births. Wu *et al.* (2010), however, reported in their population-based study that Taiwan may have the highest CHD prevalence at 13.1 per 1000 live births.

Open Access Journal

Published BY AENSI Publication

© 2016 AENSI Publisher All rights reserved

This work is licensed under the Creative Commons Attribution International License (CC BY).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

To Cite This Article: Abdulhadi H. Al-Mazroea, Sahar AF. Hammoudah, Lama M. El-Attar, S. Justin Carlus, Yousef Almohammadi, Khalid M Al-Harbi., Congenital heart disease in Saudi Arabia: the role of molecular genetics with a focus on Down syndrome. *Aust. J. Basic & Appl. Sci.*, 10(9): 98-109, 2016

The prevalence of CHD is thought to be high in Arab populations due to high rate of consanguineous marriages (25-60%, particularly first cousin marriages) (El Mouzan, M.I. *et al.*, 2008; Bener, A. *et al.*, 1996; al-Gazali, L.I. *et al.*, 1997). Furthermore, diabetes and obesity, which are known to increase the frequency of CHD in neonates, are common in Arab populations (Waller, D.K., *et al.*, 2007; Lisowski, L.A. *et al.*, 2010). To date, there has been no comprehensive study of CHD prevalence in Arab countries. However, some countries including Jordan, Kuwait, Saudi Arabia, Qatar, and Lebanon have instigated pediatric cardiac service plans, so quantitative data on CHD prevalence are likely to be reported soon.

Although CHD is a significant health problem in Saudi Arabia, only a limited number of studies have examined the issue. CHD prevalence in Saudi Arabia is reported to be 2.1 per 1000 live births, with VSD the most common type (Alqurashi, M. *et al.*, 2007). Tribal groups and families descended from constrained ancestors may accumulate genetic and congenital diseases. Tribal prevalence varies across Saudi Arabia, and it is likely that the incidence of CHD may vary accordingly (Alqurashi, M. *et al.*, 2007; Ferencz, C. *et al.*, 1989).

In 2007, CHD prevalence in the Madinah region of Saudi Arabia was reported to be 2.1/1000 live births (Fida, N.M. *et al.*, 2007; Alnajjar, A.A. *et al.*, 2009), similar to an earlier study by Baht *et al.* (1997). A total of 4348 follow-up (n=2301) and new cases (n=2047) referred to the Pediatric Cardiology Unit, Maternity and Children Hospital, Madina (MCH) were investigated by Alnajjar *et al.* in a cross-sectional study (Alnajjar, A.A. *et al.*, 2009) between January 2007 and June 2008. Of the newly-diagnosed cases, 38.8% had heart disease, of which 88% were CHD, giving rise to a CHD prevalence of about 3.48/1000 live births. VSD (34.5%) was the commonest CHD, followed by ASD (8.9%). CHD appears to represent a major health problem in Madinah, with the prevalence reported by Alnajjar *et al.* (2009) underestimating the problem due to being limited only to cases referred to MCH.

Prevalence of Down syndrome (DS):

Down syndrome (DS; OMIM #190685) is a chromosomal disorder that was first described by Down in 1866 (Down, J.L., 1995). DS is characterized by a specific phenotypic combination that includes mental retardation and characteristic facies. DS is one of the most common chromosomal abnormalities in live born children, and it is considered the main cause of mild to moderate mental retardation in children.

The global prevalence of DS is reported to be 1 in 650 to 1 in 1000 live births (Gardner, R.J.M. S.G, 2004). According to the National Down Syndrome Society, about 6000 babies are born with DS each year in the United States (US). There are over 400,000 individuals with DS in the US (National Down Syndrome Society, 2015).

DS prevalence varies considerably between countries depending on maternal age at the time of conception, the availability of prenatal diagnostics, the percentage of selective termination of pregnancy, and the method used to ascertain data. The only definite risk factor for DS is maternal age at conception, the risk of DS increasing with maternal age (Mikkelsen, M., 1977).

Cytogenetics of Down syndrome:

Most individuals with DS have three copies of chromosome 21 (trisomy 21); only 5% of patients have translocation of chromosome 21 to one of the acrocentric chromosomes, mostly chromosome 14 or another copy of chromosome (Zhu, J.L., *et al.*, 2013). A very few cases of DS have detectable trisomy and normal mosaicism (Altug-Teber, O. *et al.*, 2007).

Survival of Down syndrome children:

Zhu *et al.* (2013) reported that mortality and morbidity is higher in individuals with DS than in the normal population, although recent DS birth cohorts have lower mortality rates than older birth cohorts. This might be due to improvements in health care, diagnostic procedures, and early surgical interventions (Zhu, J.L. *et al.*, 2013; Thuline, H.C., S.M. Pueschel, 1982).

Prevalence of DS among Arab populations:

Several reports from different Arab countries have reported the prevalence of DS (Niazi, M.A. *et al.*, 1995; Amir, I.M., T.K. A-, A. Al-Harbi; Abeliovich, D.A.Y.,; Farag, T.I. *et al.*, 1988; Al-Naggar, R.L.M.S. *et al.*, 1999; Alhasari, S.M., 2010; al-Arrayed, S.S., 1999; Al-Arrayed, S.S.H.A., 2006; Wahab, A.A., *et al.*, 2006; Verma, I.C. *et al.*, 1990; Mokhtar, M.M., M. Abdel-Fattah, 2002; Chelli, D. *et al.*, 2008). These are summarized in (Table 1). The prevalence is relatively high in some Arab countries, perhaps due to the limited availability of prenatal diagnostic services and the prohibition of termination of pregnancy in Islamic societies.

Prevalence of CHD in DS children:

Chromosomal abnormalities account for about 10% of cases of CHD, with trisomy 21 the most common anomaly seen in patients with CHD (Roos-Hesselink, J.W. *et al.*, 2005; Genetics, AAoPCo. 2001). CHD is reported in about 40% of children with DS and is a frequent cause of early mortality in this population (van der Linde, D. *et al.*, 2011). AVSDs are the commonest CHD in DS, occurring in 1 in 5 live births compared to 1 in

10,000 live births in the general population (Reller, M.D. *et al.*, 2008). The worldwide frequency of CHD in DS children is shown in Table 2.

Prevalence of CHD in DS children in Saudi Arabia:

In view of common consanguinity in Saudi Arabia, several studies have attempted to explain its association with CHD, its severity, and DS. CHD was reported to be common and severe in DS offspring of consanguineous marriages (Amir, I.M., T.K. A-, A. Al-Harbi; el-Hazmi, M.A. *et al.*, 1995), although this association was not found in another study (El Mouzan, M.I. *et al.*, 2008).

In a three-year retrospective database study of cases of severe CHD in Al-Qassim province, Al-Mesned *et al.* (2012) observed that 15% of patients had a syndrome, the most common being DS. Furthermore, in a study conducted in western Saudi Arabia, Al-Aama *et al.* (2012) examined 130 DS cases diagnosed between 2007 and 2011. The incidence of CHD in these cases was reported to be 86.8%, the majority (77%) suffering from combined cardiac defects and isolated defects occurring in about 23% of cases. In descending order, the most frequently detected CHDs, either combined or isolated, were: patent ductus arteriosus (PDA; 47.8%), ASD (41.3%), trivial tricuspid regurgitation (TTR; 33.7%), VSD (29.3%), and patent foramen ovale (PFO; 28.3%). They concluded that the incidence of CHD in western Saudi Arabia was particularly high compared to other regions of the country (Al-Aama, J.Y. *et al.*, 2012). In addition to the high rate of consanguinity (56%), the tribal structure and large family size may also have contributed (Al-Owain, M. *et al.*, 2012). This population, therefore, is unique for investigating the molecular and genetic relationship between CHDs and DS with a focus on gene/gene interactions and/or DS susceptibility to CHD. The frequencies of various sub-types of CHD in DS patients in different regions of Saudi Arabia are shown in Table 3

Role of molecular genetics in the pathogenesis of CHD in DS:

Genes within the Down syndrome critical region:

Fuentes *et al.* (1995) cloned the Down syndrome critical region 1 (*DSCR1*) gene (regulator of calcineurin1; *RCAN1*; *602917). *DSCR1* is highly expressed in the brain and heart, supporting its role as a candidate in the pathogenesis of DS, especially with respect to mental retardation and/or cardiac defects. Nakamura *et al.* (1997) identified *DSCR4* (*604829) and *DSCR3* (*605298) in the 1.6 Mb DSCR. *DSCR4* is mainly expressed in the placenta, while Vidal-Taboada *et al.* (1998) identified *DSCR2* (*605296) in DSCR 2 between DNA marker D21S55 and MX1.

Molecular genetics of cardiac development:

Although the anatomy of cardiac development *in utero* is well established, the genes that control this developmental pathway are not fully understood. A full understanding of the genetic control of cardiac development would clarify CHD pathogenesis and help in the development of novel diagnostic and therapeutic approaches, such as gene therapy or genetic reprogramming of non-cardiac to cardiac cells (Srivastava, D., E.N. Olson, 2000).

Heart development is initiated by specific signaling molecules and facilitated by tissue-specific transcription factors. The formation of cardiomyocytes from mesodermal stem cells and the activation of genes responsible for cardiac contractility and morphogenesis are controlled by complicated genetic pathways (Bruneau, B.G., 2008; Hyun, C., L. Lavulo, 2006). Soon after gastrulation, the anterior lateral mesoderm gives rise to cardiomyocytes (Gilbert, S.F., 2000; Garcia-Martinez, V., G.C. Schoenwolf, 1993), which are produced in response to specific protein factors such as bone morphogenetic proteins (BMPs) secreted from adjacent endoderm. These cells group into two masses to form the cardiogenic mesoderm, which later differentiates into myocardium, endocardium, and smooth muscle cells to form the heart (Alsan, B.H., T.M. Schultheiss, 2002; Tirosch-Finkel, L. *et al.*, 2010). Cardiogenic signals activate *NKX2-5*, which activates transcription factors including those of the GATA and MEF2 families to activate other heart-specific genes (Linask, K.K., J.W. Lash, 1993; Linask, K.K., *et al.*, 1997).

Looping of the heart tube occurs at the fifth week of gestation under the control of *NKX2-5*-induced transcription factors: the linear heart tube undergoes rightward twisting, which is essential for correct alignment of the right and left ventricles and for proper configuration of the cardiac chambers (Toko, H. *et al.*, 2002; Akazawa, H., I. Komuro, 2005). The exact molecular pathways that control cardiac looping have yet to be fully defined. *HAND1* and *HAND2* are expressed in the left and right ventricles, respectively (Biben, C., R.P. Harvey, 1997). In addition, the left ventricle shows specific expression of paired-like homeodomain transcription factor 2 (*PITX2*) and cardiomyopathy-associated protein 1 (*CMYA1*, alternative symbol *XIN*). *PITX2* is a transcription factor thought to regulate extracellular matrix protein expression, while *CMYA1* initiates cytoskeletal changes to permit heart tube looping (Tsuda, T. *et al.*, 1996). Without restricted Hand protein expression, looping fails and the ventricles are not formed (Srivastava, D. *et al.*, 1995). Some of the candidate genes involved in the molecular pathways that contribute to cardiac development are summarized in Table 4.

At approximately the seventh week of gestation, the myocardium extends ventrally from the roof of the heart into the atrium and dorsally from the base into the ventricular space. This generates the muscular portions of the atrial and ventricular septa, respectively, and the beginnings of four distinct chambers (Bruneau, B.G. *et al.*, 1999; Bao, Z.Z. *et al.*, 1999). The muscular septa, however, are insufficient to completely construct the walls that separate the right and left chambers, which is completed by the endocardium located in the center of the heart (Markwald, R.R. *et al.*, 1977; Huang, J.B. *et al.*, 2010). Candidate genes likely to contribute to CHD in DS are shown in Table 4.

Gene mutations associated with ASD in DS:

CRELD1 (*607170) mutations are reported to be a risk factor for AVSD in normal subjects (Huang, J.B. *et al.*, 2010; Robinson, S.W. *et al.*, 2003). *CRELD1* gene mutations were analyzed in 39 individuals with DS and complete AVSD (Li, H., *et al.*, 2012), the results suggesting that *CRELD1* defects might be related to the pathogenesis of AVSD in the context of DS. Robinson *et al.* (2003) used a candidate gene approach to compare DS cases with AVSD with DS cases with a structurally normal heart. They reported a statistically significant increase in known harmful variants in AVSD-DS cases compared to normal control DS. The highest probability variants were in six genes: *COL6A1* (*120220), *COL6A2* (*120240), *CRELD1* (*606217), *FBLN2* (*135821), *FRZB* (*605083), and *GATA5* (*611496) (Maslen, C.L., 2004; Davies, G.E. *et al.*, 1995; Dey, A. *et al.*, 2013; Starnes, S.L. *et al.*, 2000; Tsuda, T. *et al.*, 2001; Clowes, C. *et al.*, 2014).

Salinae *et al.* (2013) performed chromosome 21-specific association analysis and showed that rs2832616 and rs1943950 are CHD risk alleles. Using copy number variant (CNV) analyses, three CNV regions associated with AVSD risk were detected: two of the regions were located within the previously identified CHD region on chromosome 21, one of the CNVs mapped near *RIPK4*, and the second included *ZBTB21* (previously *ZNF295*), highlighting the potential role of these genes in the pathogenesis of CHD in DS (Sailani, M.R. *et al.*, 2013).

Role of folate deficiency in CHDs in DS:

Folate pathway genes might also be involved in the pathogenesis of CHD in DS cases. Foliates are essential nutrients for one-carbon atom biosynthetic and epigenetic processes. After ingestion and intestinal absorption, folate is reduced and methylated in the liver to form 5-methyltetrahydrofolate (5-methyl-THF). 5-methyl-THF is then released via the circulation for cellular uptake so that it can be used for DNA and RNA precursor synthesis or for conversion of homocysteine (Hcy) to methionine (Botto, L.D. *et al.*, 2003; Huhta, J.C. *et al.*, 2005). Methionine can be used to form the main DNA methylating agent S-adenosylmethionine (SAM) (van Beynum, I.M. *et al.*, 2007; Locke, A.E. *et al.*, 2010).

An association between the folate pathway and CHD has been established in molecular studies. Genetic variations in folate pathway genes and, consequently, folate deficiency are common in CHD and DS (Sailani, M.R. *et al.*, 2012; Botto, L.D. *et al.*, 2003; Huhta, J.C. *et al.*, 2005). Variations in folate pathway enzymes and their encoding genes play a pivotal role in the pathogenesis of congenital/genetic birth defects (Bailey, L.B., R.J. Berry, 2005; Boyles, A.L. *et al.*, 2008; Eskes, T.K., 2006; Patterson, D., 2008; van der Linden, I.J. *et al.*, 2006). Pre-conception maternal folate supplementation is protective against non-syndromic CHD (Pei, L. *et al.*, 2006; EL-Gharib, M.N.M.A., S.A.F. Hammoudah, 2012; Shaw, G.M. *et al.*, 2002), and DS individuals are reported to have abnormal folate metabolism, providing a potential link between altered DNA or epigenetic effects and the etiology of DS-associated CHDs (Chadefaux, B. *et al.*, 1985; Pogribna, M. *et al.*, 2001; Coppedè, F., *et al.*, 2013).

Cystathionine- β -synthase (CBS) plays a central role in regulating folate metabolism by converting homocysteine into cystathionine, while SLC19A1 is the primary transporter of 5-methyltetrahydrofolate into and out of the cytoplasm (Chadefaux, B. *et al.*, 1985). The folate pathway is illustrated in Figure 1. Overexpression of CBS, which occurs in DS, produces functional folate deficiency (Pogribna, M. *et al.*, 2001). Furthermore, in DS cases, many folate pathway enzymes, such as those regulating homocysteine, methionine, SAM, and SAH, are functionally abnormal (Mamasoula, C. *et al.*, 2013). This results in impaired DNA and RNA synthesis that might alter cellular proliferation in the developing heart (Ahuja, P. *et al.*, 2007). This hypothesis is supported by experimental evidence in mice fed folate-deficient diets, who have an increased incidence of heart malformations due to defects in cellular proliferation⁹⁴. Folate pathway genes related to DS are shown in Figure 1 and Table 5.

Table 1: Reported prevalence of Down syndrome in Arab populations

Country/city	Prevalence/1000	Reference
Saudi Arabia	1.80	26
Saudi Arabia	2.34	27
Negev Desert Bedouin	2.90	28
Kuwait	3.50	29
Kuwait	2.90	30
Oman	2.59	31
Bahrain	0.9	32

Bahrain	1.2	33
Qatar	1.95	34
Dubai, UAE	3.13	35
Libya	1.93	36
Egypt	1.00	37
Tunisia	0.98	38

Table 2: Worldwide frequency of CHD in Down syndrome children

Country	Frequency (%)	Commonest CHD subtype	Reference
Libya	45.0	ASD	96
Guatemala	54.1	PDA	97,98
Pakistan	56.4	VSD	98
USA	44.0	AVSD	99
Mexico	58.0	IASD	100
Turkey	40.0	AVSD	101
Egypt	40.0	Not reported	102
Malaysia	49.2	Not reported	103
Brazilian	70.0	ASD	104
Brazilian	46.8	IASD	105
Saudi Arabia	61.3	VSD	106
Saudi Arabia	49.0	Not reported	107
Saudi Arabia	86.6	PDA	45
Nigeria	77.1	AVSD	108
Kurdistan -Iraq	53.0	VSD	109
Oman	60.0	AVSD	110
Nepal	80.0	VSD	111
India	65.5	VSD	112
Holland	43.0	AVSD	113
Sudan	48.0	AVSD	114

Table 3: Frequency of various subtypes of CHD in DS patients in different regions of Saudi Arabia

	Abbag 2006 Aseer ¹⁰⁶		Al-Jarallah 2009 Riyadh ¹⁰⁷		Al-Aama 2012 Jeddah ⁴⁵	
	n=57 (%)		n=54 (%)		n=92 (%)*	
Congenital heart defect						
Patent ductus arteriosus	8	14.0	4	7.4	44	47.8
Atria septal defect	12	21.1	14	26.0	38	41.3
Trivial tricuspid regurgitation					31	33.7
Ventricular septal defect	19	33.3	23	42.6	27	29.3
Patent foramen ovale					26	28.3
Atrioventricular septal defect	13	22.8	8	14.8	11	12.0
Mitral regurgitation					10	10.8
Pulmonary hypertension					9	9.7
Pericardial effusion					6	6.5
Dilated atrium/ventricle					5	5.4
Tetralogy of Fallot	3	5.3	2	3.7	2	1.5
Pulmonary stenosis	1	1.8	1	1.9	2	1.5
Hypertrophic cardiomyopathy			1	1.9	2	1.5
Right sided aortic arch					1	0.77
Aneurysmal interatrial septum					1	0.77
Tricuspid atresia	1	1.8				
Bicuspid aortic valve			1	1.9		

Table 4: Genes involved in molecular pathways contributing to cardiac development and candidates for CHD in DS

Gene	Gene name	Cytogenetic location	Function of encoded protein	Reference number
ALK2 (AVCR1)	Activin A receptor, type I	2q23-q24	Transcription factor	115
BMP2	Bone morphogenetic protein 2	20p12	Transforming growth factor	56,115
BMP4	Bone morphogenetic protein 4	14q22-q23	Transforming growth factor	56
BMP5	Bone morphogenetic protein 5	6p12.1	Transforming growth factor	56
CITED2	Chp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain 2.	6q23.3	Transcription factor	116,117
COL6A1	Collagen, type VI, alpha 1	21q22.3	Extracellular matrix protein	71,72
COL6A2	Collagen, type VI, alpha 2	21q22.3	Extracellular matrix protein	72
COL6A3	Collagen, type VI, alpha 3	2q37	Extracellular matrix protein	72
COL18A1	Collagen, type XVIII, alpha 1	21q22.3	Extracellular matrix protein	72
CRELD1	Cysteine-rich with EGF-like	3p25.3	Signaling protein	68

	<i>domain 1</i>			
<i>CRELD2</i>	Cysteine-rich with EGF-like domain 2	<u>22q13.33</u>	Signaling protein	68
<i>CTGF</i>	Connective tissue growth factor	<u>6q23.1</u>	Connective tissue growth factor	118
<i>FGF-2</i>	Fibroblast growth factor-2	<u>4q27-q28</u>	cell surface receptor signaling	73
<i>FBLN2</i>	Fibulin 2	<u>3p25.1</u>		74
<i>FRZB</i>	Frizzled-related protein	<u>2q32.1</u>	A modulator of Wnt signaling.	119
<i>GATA4</i>	GATA binding protein 4	<u>8p22-23</u>	Transcription factor	75
<i>GATA6</i>	GATA binding protein 6	<u>18q11.1</u>	Transcription factor	120
<i>HEY2</i>	Hairy/enhancer-of-split related to YRPW motif 2	<u>6q21</u>	Transcription factor	120
<i>SHH</i>	Sonic hedgehog gene	<u>7q36.3</u>	Signaling protein	121
<i>MYH6</i>	Myosin heavy chain 6	<u>14q12</u>	Sarcomeric protein	122
<i>MYH7</i>	Myosin heavy chain 7	<u>14q12</u>	Sarcomeric protein	122
<i>NKX2-5</i>	NK2 Transcription factor related, locus 5	<u>5q34</u>	Transcription factor	59-61,63
<i>NOTCH 1</i>	Notch (<i>Drosophila</i>) homolog 1 translocation-associated	<u>9q34.3</u>	Membrane ligand receptor	123
<i>ROCK1</i>	Rho-associated, coiled-coil containing protein kinase 1	<u>18q11.1</u>	Transcription factor	124
<i>TBX1</i>	T-box 1	<u>22q11.21</u>	Transcription factor	125
<i>TBX5</i>	T-box 5	<u>12q24.1</u>	Transcription factor	64
<i>TBX20</i>	T-box 20	<u>7p14.3</u>	Transcription factor	126
<i>VTN</i>	Vitronectin	<u>17q11.2</u>	multifunctional protein	127
<i>WNT9A</i>	Wingless-type MMTV integration site family member9A	<u>1q42.13</u>	Signaling protein	128
<i>ZIC3</i>	Zic family member 3	<u>Xq26.2</u>	Transcription factor	129

Table 5: Some folate pathway genes related to DS

Gene	Gene name	Cytogenetic location	Variation in DS	Reference Re
<i>MTHFR</i>	5,10-methylenetetrahydro-folate reductase.	<u>1p36.22</u>	677C> T 1298 A>C	⁷⁹
<i>5-MTR</i>	5-methylenetetrahydrofolate homo cysteine S-methyltransferase	<u>1q43</u>	2756 A>G	⁸⁰
<i>MTRR</i>	Methionine synthase reductase	<u>5p15.31</u>	66 A>G	⁸¹
<i>CBS</i>	Cystathionine synthase	<u>21q22.3</u>	844ins68	⁸⁹
<i>SLC19 (RFC1)</i>	Solute carrier family-19 folate, folate transporter	<u>21q22.3</u>	80 A>G	⁸⁸

Figure 1. The folate pathway and genetic variations in folate pathway genes. Adapted from Locke *et al.* (2010).

Conclusions:

Review of the published data over the past two decades indicates that genes mapped to a small region of chromosome 21 are expressed in the heart. These genes are potential candidates that might contribute to the pathogenesis of CHD in DS.

Recent advances in genomic technologies such as SNP arrays, next-generation sequencing (NGS; either for whole genome sequencing (WGS), whole exome sequencing, or targeted exome sequencing and copy number variations) are likely to speed up the identification of the genetic causes of CHD in children with DS. The exact genetic, epigenetic, and/or environmental causes of CHD in children with DS are still unclear, but molecular genetics studies will certainly yield valuable information for the development of novel diagnostic tools and therapeutic targets.

Understanding the molecular basis of CHD in children with DS will provide new insights into the etiology of the disease and an avenue for developing preventive measures and treatment options. A long-term goal of these studies will be to manipulate these pathways to decrease the severity and incidence of the diseases, similar to the current use of folate therapy to manage neural tube defects. One recent successful example of this approach was the use of N-acetylcysteine during gestation to significantly decrease the incidence of CHD induced by pre-gestational diabetes mellitus (Moazzen, H. *et al.*, 2014).

Due to the unique characteristics of the population, molecular genetic studies on Saudi DS patients with and without CHD are likely to generate particularly useful information to help identify the genetic variations that contribute to CHD.

REFERENCES

- Al-Gazali, L., H. Hamamy, S. Al-Arrayad, 2006. Genetic disorders in the Arab world. *BMJ.*, 333: 831-834.
- Pierpont, M.E., C.T. Basson, D.W. Benson, B.D. Gelb, T.M. Giglia, E. Goldmuntz, G. McGee, C.A. Sable, D. Srivastava, C.L. Webb, 2007. American Heart Association Congenital Cardiac Defects Committee CuoCDitY. Genetic basis for congenital heart defects: current knowledge: a scientific statement from the American Heart Association Congenital Cardiac Defects Committee, Council on Cardiovascular Disease in the Young: endorsed by the American Academy of Pediatrics. *Circulation*, 115: 3015-3038.
- Wolf, M., C.T. Basson, 2010. The molecular genetics of congenital heart disease: a review of recent developments. *Curr Opin Cardiol.*, 25: 192-197.
- Freeman, S.B., L.H. Bean, E.G. Allen, S.W. Tinker, A.E. Locke, C. Druschel, C.A. Hobbs, P.A. Romitti, M.H. Royle, C.P. Torfs, K.J. Dooley, S.L. Sherman, 2008. Ethnicity, sex, and the incidence of congenital heart defects: a report from the National Down Syndrome Project. *Genet Med.*, 10: 173-180.
- Ishikawa, T., S. Iwashima, A. Ohishi, Y. Nakagawa, T. Ohzeki, 2011. Prevalence of congenital heart disease assessed by echocardiography in 2067 consecutive newborns. *Acta Paediatr.*, 100: e55-60.
- Khoshnood, B., N. Lelong, L. Houyel, A.C. Thieulin, J.M. Jouannic, S. Magnier, A.L. Delezoide, J.F. Magny, C. Rambaud, D. Bonnet, F. Goffinet, E.S. Group, 2012. Prevalence, timing of diagnosis and mortality of newborns with congenital heart defects: a population-based study. *Heart.*, 98: 1667-1673.
- Go, A.S., D. Mozaffarian, V.L. Roger, E.J. Benjamin, J.D. Berry, W.B. Borden, D.M. Bravata, S. Dai, E.S. Ford, C.S. Fox, S. Franco, H.J. Fullerton, C. Gillespie, S.M. Hailpern, J.A. Heit, V.J. Howard, M.D. Huffman, B.M. Kissela, S.J. Kittner, D.T. Lackland, J.H. Lichtman, L.D. Lisabeth, D. Magid, G.M. Marcus, A. Marelli, D.B. Matchar, D.K. McGuire, E.R. Mohler, C.S. Moy, M.E. Mussolino, G. Nichol, N.P. Paynter, P.J. Schreiner, P.D. Sorlie, J. Stein, T.N. Turan, S.S. Virani, N.D. Wong, D. Woo, M.B. Turner, 2013. Subcommittee AHASCaSS. Heart disease and stroke statistics--2013 update: a report from the American Heart Association. *Circulation*, 127: e6-e245.
- Wu, M.H., H.C. Chen, C.W. Lu, J.K. Wang, S.C. Huang, S.K. Huang, 2010. Prevalence of congenital heart disease at live birth in Taiwan. *J Pediatr*, 156: 782-785.
- El Mouzan, M.I., A.A. Al Salloum, A.S. Al Herbish, M.M. Qurachi, A.A. Al Omar, 2008. Consanguinity and major genetic disorders in Saudi children: a community-based cross-sectional study. *Ann Saudi Med.*, 28: 169-173.
- Bener, A., Y.M. Abdulrazzaq, L.I. al-Gazali, R. Micallef, A.I. al-Khayat, T. Gaber, 1996. Consanguinity and associated socio-demographic factors in the United Arab Emirates. *Hum Hered.*, 46: 256-264.
- al-Gazali, L.I., A. Bener, Y.M. Abdulrazzaq, R. Micallef, A.I. al-Khayat, T. Gaber, 1997. Consanguineous marriages in the United Arab Emirates. *J Biosoc Sci.*, 29: 491-497.
- Waller, D.K., G.M. Shaw, S.A. Rasmussen, C.A. Hobbs, M.A. Canfield, A.M. Siega-Riz, M.S. Gallaway, A. Correa, 2007. Study NBDP. Prepregnancy obesity as a risk factor for structural birth defects. *Arch Pediatr Adolesc Med.*, 161: 745-750.
- Lisowski, L.A., P.M. Verheijen, J.A. Copel, C.S. Kleinman, S. Wassink, G.H. Visser, E.J. Meijboom, 2010. Congenital heart disease in pregnancies complicated by maternal diabetes mellitus. An international clinical collaboration, literature review, and meta-analysis. *Herz*, 35: 19-26.
- Alqurashi, M., M. El Mouzan, A. Al Herbish, A. Al Salloum, A. Al Omer, 2007. Symptomatic congenital heart disease in the Saudi Children and Adolescents Project. *Ann Saudi Med.*, 27: 442-444.
- Ferencz, C., J.A. Boughman, C.A. Neill, J.I. Brenner, L.W. Perry, 1989. Congenital cardiovascular malformations: questions on inheritance. Baltimore-Washington Infant Study Group. *J Am Coll Cardiol.*, 14: 756-763.
- Fida, N.M., J. Al-Aama, W. Nichols, M. Alqahtani, 2007. A prospective study of congenital malformations among live born neonates at a University Hospital in Western Saudi Arabia. *Saudi Med J.*, 28: 1367-1373.
- Alnajjar, A.A., M.F. Morsy, I.S. Almuzainy, A.A. Sheikh, 2009. Pediatric heart diseases in Madina, Saudi Arabia. Current status and future expectations. *Saudi Med J.*, 30: 1186-1191.
- Baht, B.A.S.A., A. Khan, M.A.I. Dad, 1997. Pattern of congenital heart disease among children in Madina Munawara. *Journal of the Saudi Heart Association.*, 9: 16-19.
- Down, J.L., 1995. Observations on an ethnic classification of idiots. 1866. *Ment Retard*, 33: 54-56.
- Gardner, R.J.M. S.G., 2004. ed *Chromosome Abnormalities and Genetic Counseling*. 3rd ed. Oxford Oxford University Press.
- National Down Syndrome Society, 2015. <http://www.ndss.org/Down-Syndrome/Down-Syndrome-Facts/>. Accessed.

- Mikkelsen, M., 1977. Down syndrome: cytogenetical epidemiology. *Hereditas*, 86: 45-50.
- Zhu, J.L., H. Hasle, A. Correa, D. Schendel, J.M. Friedman, J. Olsen, S.A. Rasmussen, 2013. Survival among people with Down syndrome: a nationwide population-based study in Denmark. *Genet Med.*, 15: 64-69.
- Altug-Teber, O., M. Bonin, M. Walter, U.A. Mau-Holzmann, A. Dufke, H. Stappert, I. Tekesin, H. Heilbronner, K. Nieselt, O. Riess, 2007. Specific transcriptional changes in human fetuses with autosomal trisomies. *Cytogenet Genome Res.*, 119: 171-184.
- Thuline, H.C., S.M. Pueschel, 1982. Cytogenetics in Down syndrome. In: Pueschel SM, Rynders JE, eds. *Down Syndrome. Advances in Biomedicine and the Behavioral Sciences*. Cambridge: Ware Press, :133.
- Niazi, M.A., A.S. al-Mazyad, M.A. al-Husain, S.M. al-Mofada, F.A. al-Zamil, T.Y. Khashoggi, Y.A. al-Eissa, 1995. Down's syndrome in Saudi Arabia: incidence and cytogenetics. *Hum Hered*, 45: 65-69.
- Amir, I.M., T.K. A-, A. Al-Harbi, Cytogenetics and prevalence of Down syndrome: a study from Saudi Arabia [abstract 1204].
- Abeliovich, D.A.Y., Sarov B. Down syndrome in Negev region.
- Farag, T.I., S.A. Al-Awadi, S.A. Al-Othman, T.S. Sundareshan, D.S. Krishna Murthy, R. Usha, S.A. Madg, R. Uma, 1988. Down syndrome and trisomy 18 in the Bedouins. *Am J Med Genet*, 29: 943-944.
- Al-Naggar, R.L.M.S., S.M. Tayel, T.I. Farag, S.A. Al-Awadi, M.M. Al-Ghanim, S.J. Abulhasan, M.A. Sabry, L. Bastaki, 1999. Profile of chromosomal abnormalities in the Al Jahara region of Kuwait. *Med Princ Pract.*, 8: 167-172.
- Alhasari, S.M., 2010. Down Syndrome in Oman: Etiology, Prevalence and Potential Risk Factors. A Cytogenetic, Molecular Genetic and Epidemiological Study, Ph.D. thesis, Institute of Human genetics, Charite University, Berlin, Germany.
- al-Arrayed, S.S., 1999. Review of the spectrum of genetic diseases in Bahrain. *East Mediterr Health J.*, 5: 1114-1120.
- Al-Arrayed, S.S.H.A., 2006. Premarital counseling services. *J Health social Environ Issues*, 6(2).
- Wahab, A.A., A. Bener, A.S. Teebi, 2006. The incidence patterns of Down syndrome in Qatar. *Clin Genet*, 69: 360-362.
- Murthy, S.K., A.K. Malhotra, S. Mani, M.E. Shara, E.E. Al-Rowaished, S. Naveed, A.I. Alkhayat, M.T. Alali, 2007. Incidence of Down syndrome in Dubai, UAE. *Med Princ Pract*, 16: 25-28.
- Verma, I.C., A.R. Mathews, A. Faquih, A.A. el-Zouki, G.R. Malik, F. Mohammed, 1990. Cytogenetic analysis of Down syndrome in Libya. *Indian J Pediatr*, 57: 245-248.
- Mokhtar, M.M., M. Abdel-Fattah, 2002. Major birth defects among infants with Down's syndrome in Alexandria, Egypt (1995-2000). *J Trop Pediatr*, 48: 247-249.
- Chelli, D., K. Dimassi, M. Chaabouni, M. Ben Saad, H. Mssaed, F. Bchir, B. Zouaoui, E. Sfar, S. Gaigi, H. Chelli, B. Chanoufi, 2008. [Prenatal diagnosis of trisomy 21: the Tunisian experience]. *Sante*, 18: 199-203.
- Roos-Hesselink, J.W., W.S. Kerstjens-Frederikse, F.J. Meijboom, P.G. Pieper, 2005. Inheritance of congenital heart disease. *Neth Heart J.*, 13: 88-91.
- Genetics, AAoPCo. 2001. American Academy of Pediatrics: Health supervision for children with Down syndrome. *Pediatrics.*, 107: 442-449.
- van der Linde, D., E.E. Konings, M.A. Slager, M. Witsenburg, W.A. Helbing, J.J. Takkenberg, J.W. Roos-Hesselink 2011. Birth prevalence of congenital heart disease worldwide: a systematic review and meta-analysis. *J Am Coll Cardiol.*, 58: 2241-2247.
- Reller, M.D., M.J. Strickland, T. Riehle-Colarusso, W.T. Mahle, A. Correa, 2008. Prevalence of congenital heart defects in metropolitan Atlanta, 1998-2005. *J Pediatr.*, 153: 807-813.
- el-Hazmi, M.A., A.R. al-Swailem, A.S. Warsy, A.M. al-Swailem, R. Sulaimani, A.A. al-Meshari, 1995. Consanguinity among the Saudi Arabian population. *J Med Genet.*, 32: 623-626.
- Al-Mesned, A., A.A. Al Akhflash, M. Sayed, 2012. Incidence of severe congenital heart disease at the province of Al-Qassim, Saudi Arabia. *Congenit Heart Dis.*, 7: 277-282.
- Al-Aama, J.Y., N.S. Bondagji, A.A. El-Harouni, 2012. Congenital heart defects in Down syndrome patients from western Saudi Arabia. *Saudi Med J.*, 33: 1211-1215.
- Al-Owain, M., H. Al-Zaidan, Z. Al-Hassnan, 2012. Map of autosomal recessive genetic disorders in Saudi Arabia: concepts and future directions. *Am J Med Genet A.*, 158A: 2629-2640.
- Fuentes, J.J., M.A. Pritchard, A.M. Planas, A. Bosch, I. Ferrer, X. Estivill, 1995. A new human gene from the Down syndrome critical region encodes a proline-rich protein highly expressed in fetal brain and heart. *Hum Mol Genet.*, 4: 1935-1944.
- Nakamura, A., M. Hattori, Y. Sakaki, 1997. A novel gene isolated from human placenta located in Down syndrome critical region on chromosome 21. *DNA Res.*, 4: 321-324.
- Vidal-Taboada, J.M., S. Sanz, A. Egeo, P. Scartezzini, R. Oliva, 1998. Identification and characterization of a new gene from human chromosome 21 between markers D21S343 and D21S268 encoding a leucine-rich protein. *Biochem Biophys Res Commun*, 250: 547-554.
- Srivastava, D., E.N. Olson, 2000. A genetic blueprint for cardiac development. *Nature.*, 407: 221-226.

- Bruneau, B.G., 2008. The developmental genetics of congenital heart disease. *Nature.*, 451: 943-948.
- Hyun, C., L. Lavulo, 2006. Congenital heart diseases in small animals: part I. Genetic pathways and potential candidate genes. *Vet J.*, 171: 245-255.
- Gilbert, S.F., 2000. *Developmental biology*. 6th ed. 2000, Sunderland (MA): Sinauer Associates. xviii, p: 749.
- Garcia-Martinez, V., G.C. Schoenwolf, 1993. Primitive-streak origin of the cardiovascular system in avian embryos. *Dev Biol.*, 159: 706-719.
- Alsan, B.H., T.M. Schultheiss, 2002. Regulation of avian cardiogenesis by Fgf8 signaling. *Development*, 129: 1935-1943.
- Tirosh-Finkel, L., A. Zeisel, M. Brodt-Ivenshitz, A. Shamai, Z. Yao, R. Seger, E. Domany, E. Tzahor, 2010. BMP-mediated inhibition of FGF signaling promotes cardiomyocyte differentiation of anterior heart field progenitors. *Development*, 137: 2989-3000.
- Linask, K.K., J.W. Lash, 1993. Early heart development: dynamics of endocardial cell sorting suggests a common origin with cardiomyocytes. *Dev Dyn*, 196: 62-69.
- Linask, K.K., K.A. Knudsen, Y.H. Gui, 1997. N-cadherin-catenin interaction: necessary component of cardiac cell compartmentalization during early vertebrate heart development. *Dev Biol.*, 185: 148-164.
- Toko, H., W. Zhu, E. Takimoto, I. Shiojima, Y. Hiroi, Y. Zou, T. Oka, H. Akazawa, M. Mizukami, M. Sakamoto, F. Terasaki, Y. Kitaura, H. Takano, T. Nagai, R. Nagai, I. Komuro, 2002. Csx/Nkx2-5 is required for homeostasis and survival of cardiac myocytes in the adult heart. *J Biol Chem.*, 277: 24735-24743.
- Akazawa, H., I. Komuro, 2005. Cardiac transcription factor Csx/Nkx2-5: Its role in cardiac development and diseases. *Pharmacol Ther.*, 107: 252-268.
- Biben, C., R.P. Harvey, 1997. Homeodomain factor Nkx2-5 controls left/right asymmetric expression of bHLH gene *eHand* during murine heart development. *Genes Dev.*, 11: 1357-1369.
- Tsuda, T., N. Philp, M.H. Zile, K.K. Linask, 1996. Left-right asymmetric localization of flectin in the extracellular matrix during heart looping. *Dev Biol.*, 173: 39-50.
- Srivastava, D., P. Cserjesi, E.N. Olson, 1995. A subclass of bHLH proteins required for cardiac morphogenesis. *Science*, 270: 1995-1999.
- Bruneau, B.G., M. Logan, N. Davis, T. Levi, Tabin, J.G. Seidman, C.E. Seidman, 1999. Chamber-specific cardiac expression of *Tbx5* and heart defects in Holt-Oram syndrome. *Dev Biol.*, 211: 100-108.
- Bao, Z.Z., B.G. Bruneau, J.G. Seidman, C.E. Seidman, C.L. Cepko, 1999. Regulation of chamber-specific gene expression in the developing heart by *Irx4*. *Science*, 283: 1161-1164.
- Markwald, R.R., T.P. Fitzharris, F.J. Manasek, 1977. Structural development of endocardial cushions. *Am J Anat.*, 148: 85-119.
- Huang, J.B., Y.L. Liu, X.D. Lv, 2010. Pathogenic mechanisms of congenital heart disease. *Fetal Pediatr Pathol.*, 29: 359-372.
- Maslen, C.L., 2004. Molecular genetics of atrioventricular septal defects. *Curr Opin Cardiol.*, 19: 205-210.
- Li, H., S. Cherry, D. Klinedinst, V. DeLeon, J. Redig, B. Reshey, M.T. Chin, S.L. Sherman, C.L. Maslen, R.H. Reeves, 2012. Genetic modifiers predisposing to congenital heart disease in the sensitized Down syndrome population. *Circ Cardiovasc Genet.*, 5: 301-308.
- Robinson, S.W., C.D. Morris, E. Goldmuntz, M.D. Reller, M.A. Jones, R.D. Steiner, C.L. Maslen, 2003. Missense mutations in *CRELD1* are associated with cardiac atrioventricular septal defects. *Am J Hum Genet.*, 72: 1047-1052.
- Davies, G.E., C.M. Howard, M.J. Farrer, M.M. Coleman, L.B. Bennett, L.M. Cullen, R.K. Wyse, J. Burn, R. Williamson, A.M. Kessling, 1995. Genetic variation in the *COL6A1* region is associated with congenital heart defects in trisomy 21 (Down's syndrome). *Ann Hum Genet.*, 59: 253-269.
- Dey, A., K. Bhowmik, A. Chatterjee, P.B. Chakrabarty, S. Sinha, K. Mukhopadhyay, 2013. Down Syndrome Related Muscle Hypotonia: Association with *COL6A3* Functional SNP rs2270669. *Front Genet.*, 4: 57.
- Starnes, S.L., B.W. Duncan, J.M. Kneebone, G.L. Rosenthal, T.K. Jones, R.G. Grifka, F. Cecchin, D.J. Owens, C. Fearneyhough, F.M. Lupinetti, 2000. Vascular endothelial growth factor and basic fibroblast growth factor in children with cyanotic congenital heart disease. *J Thorac Cardiovasc Surg.*, 119: 534-539.
- Tsuda, T., H. Wang, R. Timpl, M.L. Chu, 2001. Fibulin-2 expression marks transformed mesenchymal cells in developing cardiac valves, aortic arch vessels, and coronary vessels. *Dev Dyn.*, 222: 89-100.
- Clowes, C., M.G. Boylan, L.A. Ridge, E. Barnes, J.A. Wright, K.E. Hentges, 2014. The functional diversity of essential genes required for mammalian cardiac development. *Genesis.*, 52: 713-737.
- Sailani, M.R., P. Makrythanasis, A. Valsesia, F.A. Santoni, S. Deutsch, K. Popadin, C. Borel, E. Migliavacca, A.J. Sharp, G. Duriaux Sail, E. Falconnet, K. Rabionet, C. Serra-Juhé, S. Vicari, D. Laux, Y. Grattau, G. Dembour, A. Megarbane, R. Touraine, S. Stora, S. Kitsiou, H. Fryssira, C. Chatzisevastou-Loukidou, E. Kanavakis, G. Merla, D. Bonnet, L.A. Pérez-Jurado, X. Estivill, J.M. Delabar, S.E. Antonarakis,

2013. The complex SNP and CNV genetic architecture of the increased risk of congenital heart defects in Down syndrome. *Genome Res.*, 23: 1410-1421.

Botto, L.D., J. Mulinare, J.D. Erickson, 2003. Do multivitamin or folic acid supplements reduce the risk for congenital heart defects? Evidence and gaps. *Am J Med Genet A.*, 121A: 95-101.

Huhta, J.C., J.A. Hernandez-Robles, 2005. Homocysteine, folate, and congenital heart defects. *Fetal Pediatr Pathol.*, 24: 71-79.

van Beynum, I.M., M. den Heijer, H.J. Blom, L. Kapusta, 2007. The MTHFR 677C->T polymorphism and the risk of congenital heart defects: a literature review and meta-analysis. *QJM.*, 100: 743-753.

Locke, A.E., K.J. Dooley, S.W. Tinker, S.Y. Cheong, E. Feingold, E.G. Allen, S.B. Freeman, C.P. Torfs, C.L. Cua, M.P. Epstein, M.C. Wu, Lin, G. Capone, S.L. Sherman, L.J. Bean, 2010. Variation in folate pathway genes contributes to risk of congenital heart defects among individuals with Down syndrome. *Genet Epidemiol.*, 34: 613-623.

Bailey, L.B., R.J. Berry, 2005. Folic acid supplementation and the occurrence of congenital heart defects, orofacial clefts, multiple births, and miscarriage. *Am J Clin Nutr.*, 81: 1213S-1217S.

Boyles, A.L., A.J. Wilcox, J.A. Taylor, K. Meyer, A. Fredriksen, P.M. Ueland, C.A. Drevon, S.E. Vollset, R.T. Lie, 2008. Folate and one-carbon metabolism gene polymorphisms and their associations with oral facial clefts. *Am J Med Genet A.*, 146A: 440-449.

Eskes, T.K., 2006. Abnormal folate metabolism in mothers with Down syndrome offspring: review of the literature. *Eur J Obstet Gynecol Reprod Biol.*, 124: 130-133.

Patterson, D., 2008. Folate metabolism and the risk of Down syndrome. *Downs Syndr Res Pract.*, 12: 93-97.

van der Linden, I.J., L.A. Afman, S.G. Heil, H.J. Blom, 2006. Genetic variation in genes of folate metabolism and neural-tube defect risk. *Proc Nutr Soc.*, 65: 204-215.

Pei, L., H. Zhu, J. Zhu, A. Ren, R.H. Finnell, Z. Li, 2006. Genetic variation of infant reduced folate carrier (A80G) and risk of orofacial defects and congenital heart defects in China. *Ann Epidemiol.*, 16: 352-356.

EL-Gharib, M.N.M.A., S.A.F. Hammoudah, 2012. Maternal MTHFR gene polymorphisms and the risk of Down syndrome offspring. *Int J Med Biomed Res.*, 1(3):199-205.

Shaw, G.M., E.J. Lammer, H. Zhu, M.W. Baker, E. Neri, R.H. Finnell, 2002. Maternal periconceptional vitamin use, genetic variation of infant reduced folate carrier (A80G), and risk of spina bifida. *Am J Med Genet.*, 108: 1-6.

Chadefaux, B., M.O. Rethoré, O. Raoul, I. Ceballos, M. Poissonnier, S. Gilgenkranz, D. Allard, 1985. Cystathionine beta synthase: gene dosage effect in trisomy 21. *Biochem Biophys Res Commun.*, 128: 40-44.

Pogribna, M., S. Melnyk, I. Pogribny, A. Chango, P. Yi, S.J. James, 2001. Homocysteine metabolism in children with Down syndrome: in vitro modulation. *Am J Hum Genet.*, 69: 88-95.

Coppede, F., V. Lorenzoni, L. Migliore, 2013. The reduced folate carrier (RFC-1) 80A>G polymorphism and maternal risk of having a child with Down syndrome: a meta-analysis. *Nutrients.*, 5: 2551-2563.

Mamasoula, C., R.R. Prentice, T. Pierscionek, F. Pangilinan, J.L. Mills, C. Druschel, K. Pass, M.W. Russell, D. Hall, A. Töpf, D.L. Brown, D. Zelenika, J. Bentham, C. Cosgrove, S. Bhattacharya, J.G. Riveron, K. Setchfield, J.D. Brook, F.A. Bu'Lock, C. Thornborough, T.J. Rahman, J.P. Doza, H.L. Tan, J. O'Sullivan, A.G. Stuart, G. Blue, D. Winlaw, A.V. Postma, B.J. Mulder, A.H. Zwinderman, K. van Engelen, A.F. Moorman, A. Rauch, M. Gewillig, J. Breckpot, K. Devriendt, G.M. Lathrop, M. Farrall, J.A. Goodship, H.J. Cordell, L.C. Brody, B.D. Keavney, 2013. Association between C677T polymorphism of methylene tetrahydrofolate reductase and congenital heart disease: meta-analysis of 7697 cases and 13,125 controls. *Circ Cardiovasc Genet.*, 6: 347-353.

Ahuja, P., P. Sdek, W.R. MacLellan, 2007. Cardiac myocyte cell cycle control in development, disease, and regeneration. *Physiol Rev.*, 87: 521-544.

Li, D., R. Rozen, 2006. Maternal folate deficiency affects proliferation, but not apoptosis, in embryonic mouse heart. *J Nutr.*, 136: 1774-1778.

Moazzen, H., X. Lu, N.L. Ma, T.J. Velenosi, B.L. Urquhart, L.J. Wisse, A.C. Gittenberger-de Groot, Q. Feng, 2014. N-Acetylcysteine prevents congenital heart defects induced by pregestational diabetes. *Cardiovasc Diabetol.*, 13: 46.

Elmagrpy, Z., A. Rayani, A. Shah, E. Habas, E.H. Aburawi, 2011. Down syndrome and congenital heart disease: why the regional difference as observed in the Libyan experience? *Cardiovasc J Afr.*, 22: 306-309.

Vida, V.L., J. Barnoya, L.A. Larrazabal, G. Gaitan, F. de Maria Garcia, A.R. Castañeda, 2005. Congenital cardiac disease in children with Down's syndrome in Guatemala. *Cardiol Young.*, 15: 286-290.

Khan, I.M.T., 2012. Frequency and pattern of congenital heart defects in children with Down's syndrome. *Gomal J Med Sci.*, 10: 24-31.

Freeman, S.B., L.F. Taft, K.J. Dooley, K. Allran, S.L. Sherman, T.J. Hassold, M.J. Khoury, D.M. Saker, 1998. Population-based study of congenital heart defects in Down syndrome. *Am J Med Genet.*, 80: 213-217.

de Rubens Figueroa, J., B. del Pozzo Magaña, J.L. Pablos Hach, C. Calderón Jiménez, R. Castrejón Urbina, 2003. [Heart malformations in children with Down syndrome]. *Rev Esp Cardiol.*, 56: 894-899.

Nisli, K., N. Oner, S. Candan, H. Kayserili, T. Tansel, E. Tireli, B. Karaman, R.E. Omeroglu, A. Dindar, U. Aydogan, S. Başaran, T. Ertugrul, 2008. Congenital heart disease in children with Down's syndrome: Turkish experience of 13 years. *Acta Cardiol.*, 63: 585-589.

Afifi, H.H., A.A. Abdel Azeem, H.T. El-Bassyouni, M.E. Gheith, A. Rizk, J.B. Bateman, 2013. Distinct ocular expression in infants and children with Down syndrome in Cairo, Egypt: myopia and heart disease. *JAMA Ophthalmol.*, 131: 1057-1066.

Azman, B.Z., R. Ankathil, I. Siti Mariam, M.A. Suhaida, M. Norhashimah, A.B. Tarmizi, M.A. Nor Atifah, T.P. Kannan, B.A. Zilfalil, 2007. Cytogenetic and clinical profile of Down syndrome in Northeast Malaysia. *Singapore Med J.*, 48: 550-554.

Faria, P.F., J.A. Nicolau, M.Z. Melek, S. de Oliveira Ne, B.E. Bermudez, R.M. Nisihara, 2014. Association between congenital heart defects and severe infections in children with Down syndrome. *Rev Port Cardiol.*, 33: 15-18.

Vilas Boas, L.T., E.P. Albernaz, R.G. Costa, 2009. Prevalence of congenital heart defects in patients with Down syndrome in the municipality of Pelotas, Brazil. *J Pediatr (Rio J)* 85: 403-407.

Abbag, F.I., 2006. Congenital heart diseases and other major anomalies in patients with Down syndrome. *Saudi Med J.*, 27: 219-222.

Al-Jarallah, A.S., 2009. Down's syndrome and the pattern of congenital heart disease in a community with high parental consanguinity. *Med Sci Monit.*, 15: CR409-412.

Asani, M.A.I, U. Also, 2013. Pattern of congenital heart diseases among children with Down syndrome seen in Aminu Kano Teaching Hospital, Kano, Nigeria. *Niger J Basic Clin Sci.*, 10: 57-9.

AA., 2011. Congenital heart disease in Down syndrome: experience of Kurdistan of Iraq. *Duhok Med J*, 5(2): 24-33.

Venugopalan, P., A.K. Agarwal, 2003. Spectrum of congenital heart defects associated with Down Syndrome in high consanguineous Omani population. *Indian Pediatr.*, 40: 398-403.

Shrestha M SU., 2013. Down Syndrome and Congenital Heart Disease: Single centre, Prospective Study. *Nepal Journal of Medical Sciences*, 2(2): 96-101.

Podder, G.A.A., A. Halder, J. Banerjee, 2012. Madhusnata D. Assessment of Down syndrome patients in west Bengal India. *Paci J Med Sci.*, 10(2): 28- 35.

Lo, N.S., P.M. Leung, K.C. Lau, C.Y. Yeung, 1989. Congenital cardiovascular malformations in Chinese children with Down's syndrome. *Chin Med J (Engl.)*, 102: 382-386.

Ali, S.K., 2009. Cardiac abnormalities of Sudanese patients with Down's syndrome and their short-term outcome. *Cardiovasc J Afr.*, 20: 112-115.

Pfarr, N., C. Fischer, N. Ehlken, T. Becker-Grünig, V. López-González, M. Gorenflo, A. Hager, K. Hinderhofer, O. Miera, C. Nagel, D. Schranz, E. Grünig, 2013. Hemodynamic and genetic analysis in children with idiopathic, heritable, and congenital heart disease associated pulmonary arterial hypertension. *Respir Res.*, 14: 3.

Liu, Y., F. Wang, Y. Wu, S. Tan, Q. Wen, J. Wang, X. Zhu, X. Wang, C. Li, X. Ma, H. Pan, 2014. Variations of CITED2 are associated with congenital heart disease (CHD) in Chinese population. *PLoS One* 9: e98157.

Xu, M., X. Wu, Y. Li, X. Yang, J. Hu, M. Zheng, J. Tian, 2014. CITED2 mutation and methylation in children with congenital heart disease. *J Biomed Sci.*, 21: 7.

Szabó, Z., J. Magga, T. Alakoski, J. Ulvila, J. Piuholo, L. Vainio, K.I. Kivirikko, O. Vuolteenaho, H. Ruskoaho, K.E. Lipson, P. Signore, R. Kerkelä, 2014. Connective tissue growth factor inhibition attenuates left ventricular remodeling and dysfunction in pressure overload-induced heart failure. *Hypertension.*, 63: 1235-1240.

Ackerman, C., A.E. Locke, E. Feingold, B. Reshey, K. Espana, J. Thusberg, S. Mooney, L.J. Bean, K.J. Dooley, C.L. Cua, R.H. Reeves, S.L. Sherman, C.L. Maslen, 2012. An excess of deleterious variants in VEGF-A pathway genes in Down-syndrome-associated atrioventricular septal defects. *Am J Hum Genet.*, 91: 646-659.

Watanabe, T., N. Koibuchi, M.T. Chin, 2010. Transcription factor CHF1/Hey2 regulates coronary vascular maturation. *Mech Dev.*, 127: 418-427.

Carbe, C.J., L. Cheng, S. Addya, J.I. Gold, E. Gao, W.J. Koch, N.A. Riobo, 2014. Gi proteins mediate activation of the canonical hedgehog pathway in the myocardium. *Am J Physiol Heart Circ Physiol.*, 307: H66-72.

Ma, X., R.S. Adelstein, 2012. In vivo studies on nonmuscle myosin II expression and function in heart development. *Front Biosci (Landmark Ed)* 17: 545-555.

Penton, A.L., L.D. Leonard, N.B. Spinner, 2012. Notch signaling in human development and disease. *Semin Cell Dev Biol.*, 23: 450-457.

Shi, J., L. Wei, 2013. Rho kinases in cardiovascular physiology and pathophysiology: the effect of fasudil. *J Cardiovasc Pharmacol.*, 62: 341-354.

Gao, S., X. Li, B.A. Amendt, 2013. Understanding the role of Tbx1 as a candidate gene for 22q11.2 deletion syndrome. *Curr Allergy Asthma Rep.*, 13: 613-621.

Singh, R., A. Kispert, 2010. Tbx20, Smads, and the atrioventricular canal. *Trends Cardiovasc Med.*, 20: 109-114.

Latif, N., P. Sarathchandra, P.M. Taylor, J. Antoniow, M.H. Yacoub, 2005. Localization and pattern of expression of extracellular matrix components in human heart valves. *J Heart Valve Dis.*, 14: 218-227.

Katoh, M., 2002. Molecular cloning and expression of mouse Wnt14, and structural comparison between mouse Wnt14-Wnt3a gene cluster and human WNT14-WNT3A gene cluster. *Int J Mol Med.*, 9: 221-227.

Cowan, J., M. Tariq, S.M. Ware, 2014. Genetic and functional analyses of ZIC3 variants in congenital heart disease. *Hum Mutat.*, 35: 66-75.