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

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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.

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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; *RCAN1I*; *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; Tirosh-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*., 2010l 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. Folates 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 proliferation [94]. 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

Congenital heart defect	*Abbag 2006 Aseer* [106] *n=57 (%)*		*Al-Jarallah 2009 Riyadh* [107] *n=54 (%)*		*Al-Aama 2012 Jeddah* [45] *n=92 (%)**	
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	*Cbp/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]

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

Table 5: Some folate pathway genes related to DS

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

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