

Genetic and epigenetic mechanisms in the development of congenital heart diseases

Yue Wu,¹ Xiaosi Jin,² Yuhao Zhang,³ Jing Zheng,¹ Rulai Yang¹

To cite: Wu Y, Jin X, Zhang Y, et al. Genetic and epigenetic mechanisms in the development of congenital heart diseases. *World Jnl Ped Surgery* 2021;4:e000196. doi:10.1136/wjps-2020-000196

► Additional supplemental material is published online only. To view, please visit the journal online (<http://dx.doi.org/10.1136/wjps-2020-000196>).

YW and XJ contributed equally.

Received 8 August 2020
Revised 28 March 2021
Accepted 30 March 2021



© Author(s) (or their employer(s)) 2021. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

¹Department of Genetic and Metabolic Diseases, The Children's Hospital, Zhejiang University School of Medicine, National Clinical Research Center for Child Health, Hangzhou, China

²Department of Obstetrics, Hangzhou Women's Hospital, Hangzhou First People's Hospital Qianjiang New City Campus, Hangzhou, China

³Cardiovascular Key Lab of Zhejiang Province, Department of Cardiology, The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China

Correspondence to

Dr Rulai Yang; chsczx@zju.edu.cn

ABSTRACT

Congenital heart disease (CHD) is the most common of congenital cardiovascular malformations associated with birth defects, and it results in significant morbidity and mortality worldwide. The classification of CHD is still elusive owing to the complex pathogenesis of CHD. Advances in molecular medicine have revealed the genetic basis of some heart anomalies. Genes associated with CHD might be modulated by various epigenetic factors. Thus, the genetic and epigenetic factors are gradually accepted as important triggers in the pathogenesis of CHD. However, few literatures have comprehensively elaborated the genetic and epigenetic mechanisms of CHD. This review focuses on the etiology of CHD from genetics and epigenetics to discuss the role of these factors in the development of CHD. The interactions between genetic and epigenetic in the pathogenesis of CHD are also elaborated. Chromosome abnormalities and gene mutations in genetics, and DNA methylations, histone modifications and on-coding RNAs in epigenetics are summarized in detail. We hope the summative knowledge of these etiologies may be useful for improved diagnosis and further elucidation of CHD so that morbidity and mortality of children with CHD can be reduced in the near future.

INTRODUCTION

Congenital heart disease (CHD) is a group of disorders attributed by abnormalities in fetal heart and large vessels that lead to actual or potential impairment of cardiac function in infants. CHD is the most common type of congenital defect worldwide and is the most common and the most life-threatening class of birth defects in infants, affecting approximately 1% live births annually worldwide.¹ Epidemiological investigations indicate that the overall incidence of CHD varies across countries and continents, and the prevalence of CHD in Asia is higher than that in North America.² Despite benefits from the remarkable progress in therapeutic strategies of surgery and catheter intervention, CHD is still the principal source of mortality in infants. However, owing to medical, surgical and technological evolutions during the past decades, more than 90% of CHD infants now survive to adulthood.³ Improvement in

surgical intervention techniques and peri-operative care has dramatically changed the management of these populations with CHD. However, CHD is still a bothersome question owing to its undesirable outcomes and expensive healthcare costs, which bring substantial physiological, emotional and socioeconomic challenges to patients, families and society.

According to the final anatomical and pathophysiological complexities, CHD can be classified as mild, moderate or severe. Detailed classification is shown in [box 1](#).⁴ The prognosis, morbidity and mortality vary with the severity of the anomalies. Despite the rapid advances in medical care and detection technology, the etiology of most CHD remains poorly understood. It is therefore imperative to improve our understanding of the disease mechanisms to reduce the frequent occurrence of CHD. During the past decades a consensus has emerged that both genetic (eg, chromosomal abnormalities, smaller copy number variants and point mutations) and environmental (extrinsic factors, such as teratogen exposure and nutrient deficiencies; intrinsic factors, including maternal disease, illness and viral infection)⁵ factors are related to the occurrence of CHD. Progress in molecular genetic diagnosis has provided a valuable opportunity to investigate the genetic factors of CHD. Furthermore, a multitude of animal models (eg, mouse, zebrafish, frog and fruit fly) have witnessed the significant effects of genetic etiology of CHD. These in vivo studies on animal models, in turn, have resulted in the identification of numerous structural genes, transcriptional regulators and signaling molecules that are critical for normal cardiac morphogenesis.⁶

To our knowledge, although numerous literatures have discussed the genetic mechanisms of CHD, few have comprehensively elaborated the genetic and epigenetic mechanisms of CHD. In this review, we focus on CHD origin from the etiology of genetics and epigenetics. Chromosomal abnormalities

Box 1 Classification of congenital heart disease (*the copyright can be viewed in the online supplemental file*)

Mild:

- ▶ Isolated congenital aortic valve disease and bicuspid aortic disease.
- ▶ Isolated congenital mitral valve disease (except parachute valve and cleft leaflet).
- ▶ Mild isolated PS (infundibular, valvular and supra-valvular).
- ▶ Isolated small ASD, VSD or PDA.
- ▶ Repaired secundum ASD, sinus venosus defect, VSD or PDA without residuae or sequellae, such as chamber enlargement, ventricular dysfunction or elevated pulmonary artery pressure.

Moderate (repaired or unrepaired where not specified; alphabetical order):

- ▶ Anomalous pulmonary venous connection (partial or total).
- ▶ Anomalous coronary artery arising from the PA.
- ▶ Anomalous coronary artery arising from the opposite sinus.
- ▶ AS subvalvular or supra-valvular.
- ▶ AVSD, partial or complete, including primum ASD (excluding pulmonary vascular disease).
- ▶ Coarctation of the aorta.
- ▶ Double-chambered right ventricle.
- ▶ Ebstein anomaly.
- ▶ Marfan syndrome and related HTAD and Turner syndrome.
- ▶ PDA, moderate or large unrepaired (excluding pulmonary vascular disease).
- ▶ PPS.
- ▶ PS (infundibular, valvular and supra-valvular), moderate or severe.
- ▶ Sinus of Valsalva aneurysm/fistula.
- ▶ Sinus venosus defect.
- ▶ TOF repaired.
- ▶ Transposition of the great arteries after arterial switch operation.
- ▶ VSD with associated abnormalities (excluding pulmonary vascular disease) and/or moderate or greater shunt.

Severe (repaired or unrepaired where not specified; alphabetical order):

- ▶ Any CHD (repaired or unrepaired) associated with pulmonary vascular disease (including Eisenmenger syndrome).
- ▶ Any cyanotic CHD (unoperated or palliated).
- ▶ Double-outlet ventricle.
- ▶ Fontan circulation.
- ▶ IAA.
- ▶ Pulmonary atresia (all forms).
- ▶ Transposition of the great arteries (except for patients with arterial switch operation).
- ▶ Univentricular heart (including double inlet left/right ventricle, tricuspid/mitral atresia, hypoplastic left heart syndrome and any other anatomic abnormality with a functionally single ventricle).
- ▶ Truncus arteriosus.
- ▶ Other complex abnormalities of atrioventricular and ventriculoarterial connection (ie, crisscross heart, heterotaxy syndromes and ventricular inversion).

AS, aortic stenosis; ASD, atrial septal defect; AVSD, atrioventricular septal defect; CHD, congenital heart disease; HTAD, heritable thoracic aortic disease; IAA, interrupted aortic arch; PA, pulmonary artery; PDA, patent ductus arteriosus; PPS, peripheral pulmonary stenosis; PS, pulmonary stenosis; TOF, tetralogy of Fallot; VSD, ventricular septal defect.

and gene mutations in genetics, and DNA methylations, histone modifications and on-coding RNAs in epigenetics are summarized in detail. Moreover, we expect that

rapidly emerging data could provide a further understanding of genetics and epigenetics in the development of CHD and also a basis for further exploring the early diagnosis and individualized therapy of CHD.

CHROMOSOME ABNORMALITIES UNDERLYING CHD

Chromosome abnormalities refer to abnormal chromosome numbers and structural aberrations including aneuploidies and copy number variations (CNVs), respectively. Conventional chromosome anomalies associated with CHD were identified half a century ago. A study by Pierpont *et al*⁷ suggested that ~30% of children with a chromosomal abnormality would suffer from CHD. In the following section, we summarize the involvement of chromosomal aneuploidies and CNVs in CHD in detail.

Aneuploidy in CHD

Chromosomal aneuploidy is the earliest recognized genetic cause of CHD and accounts for a great proportion of CHD (table 1). Approximately 50% of individuals born with trisomy 21 have the phenotypes of CHD, ranging from atrial septal defect (ASD)/ventricular septal defect (VSD) to atrioventricular canal lesions.^{1 2} The prevalence of CHD in newborns with trisomy 13 and trisomy 18 increases to 80%, and the major phenotypes of CHD are heterotaxy, laterality and septal defects.^{8 9} CHD is observed in approximately 33% of females with Turner syndrome or monosomy X, and the cardiac malformations are usually diagnosed as VSD, coarctation of aorta (CoA), bicuspid aortic valve and hypoplastic left heart.¹ Although abnormal X chromosome numbers are rare, they also could result in CHD. For example, males with Klinefelter syndrome or 47, XXY have a 50% chance of CHD with the phenotypes of patent ductus arteriosus (PDA) and ASD.⁷ Moreover, sporadic 49, XXXXX cases with the phenotypes of ASD and vascular malformations have also been reported.¹⁰ At present, chromosomal G-banded karyotype analysis has been applied to detect the anomalies of chromosomes in spite of its limitation for the restrictive base resolution in exploring the tiny abnormalities of chromosomes.

CNVs in CHD

Conventional chromosomal microscopy in clinic could only detect the alterations of structure, numbers of chromosomes and abnormalities of large fragments, causing incomplete diagnosis of CHD. Numerous detection technologies include fluorescent in situ hybridization and multiplex ligation-dependent amplification. Chromosomal microarrays have been applied to explore the submicroscopic chromosomal anomalies and to elucidate the pathogenic mechanisms of CHD, which may become a better approach for diagnosis.

CNVs refer to structural aberrations consisting of deletions or duplications, which are too small to be detected by routine karyotype analysis. A few CNVs can alter one or more contiguous genes and then inappropriately

Table 1 Common congenital heart disease resulting from chromosomal abnormalities

Disorder	Causative gene(s)	Locus	Inheritance	Clinical features	Associated cardiac anomalies	Reference(s)
Chromosomal aneuploidy associated with CHD						
Trisomy 21 (Down syndrome)	Unknown	Chr21	Error in meiosis	Distinctive facial features, mental retardation, hypotonia, conductive hearing loss; CHD.	AVSD, ASD, VSD and TOF.	1 2
Trisomy 18 (Edward syndrome)	Unknown	Chr18	Error in meiosis	Severe mental retardation, biliary atresia, hypotonia, distinctive facial features, distinctively clenched fingers, poor survival and CHD.	ASD, VSD, PDA, TOF, BAV and CoA.	8 9
Trisomy 13 (Patau syndrome)	Unknown	Chr13	Error in meiosis	Microcephaly, orofacial clefts, severe mental retardation, postaxial polydactyly, omphalocele, microphthalmia, poor survival and CHD.	ASD, VSD, PDA and HLHS.	8
Monosomy X (Turner syndrome)	Unknown	ChrX	Error in meiosis	Short stature, webbed neck, primary amenorrhea, lymphedema and CHD.	CoA, HLHS, BAV and AS.	1
47, XXY (Klinefelter syndrome)	Unknown	ChrX	Error in meiosis	Developmental delay, tall stature, hypoplastic testes, delayed puberty and CHD.	PDA, ASD and mitral valve prolapses.	7
Copy number variations associated with CHD						
1q21.1 deletion	GJA5, BCL9, CHD1L, FMO5 and ACP6	1q21.1	De novo, AD, N/A	Mild to moderate mental retardation, microcephaly, cataracts and CHD.	TOF, VSD, AS and CoA.	24-26
4p16.3 deletion (Wolf-Hirschhorn syndrome)	WHSC1 and FGFR1	4p16.3	De novo and N/A	Distinctive facial features, neurological and growth delay, seizures and CHD.	Mild septal defects and arterial ductus persistency.	27
4q22.1 deletion	PPM1K	4q22.1	De novo and AD	Dementia, Lewy body, Parkinson diseases and CHD.	TOF.	26 28
7q11.23 deletion (Williams-Beuren syndrome)	ELN	7q11.23	De novo and AD (minority of cases)	Developmental delay, mental retardation; elfin facies, hypercalcemia, renal disorders, hearing loss and CHD.	PAS, PPS, AV and MV defects and SVAS.	16 17
8p23.1 deletion	GATA4	8p23.1	N/A	Hernia, Testicular anomalies, Congenital diaphragmatic 2, Ebstein anomaly, CHD.	ASD, AVSD, TOF, VSD and Ebstein anomalies.	18
9q34.3 deletion	NOTCH1 and EHM11	9q34.3	De novo and AD	Distinctive facial features and CHD.	HLHS, TOF and CoA.	26 29
11q23 deletion (Jacobsen syndrome)	ETS1	11q23	De novo and AD	Distinctive facial features, growth and psychomotor retardation, strabismus, thrombocytopenia, hammetoes and CHD.	HLHS and LVOT defects.	22 23

Continued

Table 1 Continued

Disorder	Causative gene(s)	Locus	Inheritance	Clinical features	Associated cardiac anomalies	Reference(s)
15q11.2 deletion	TUBGCP5, CYFIP1, NIPA2 and NIPA1	15q11.2	N/A	Delayed psychomotor development, speech delay, autism spectrum disorder, attention deficit-hyperactivity disorder, obsessive-compulsive disorder, possibly seizures and CHD.	ASD, VSD, CoA, TAPVD and complex left-sided.	28
22q11.2 deletion (DiGeorge Syndrome)	TBX1	22q11.2	De novo and AD (28% of cases).	Thymus and parathyroid aplasia or hypoplasia, immunodeficiency, hypocalcemia, distinctive facial features, OFT abnormalities and CHD, arteriosus.	TOF, IAA type B, VSD, TA, aortic arch anomalies and truncus arteriosus.	13 14
Distal 22q11.2 deletion	CRKL and ERK2/MAPK1	22q11.22	De novo and AR (minority of cases)	Distinctive facial features, psychiatric and cognitive deficits, sepsis and CHD.	Interrupted aortic arch and truncus arteriosus.	15

AD, autosomal dominant; AR, autosome recessive; AS, aortic stenosis; ASD, atrial septal defect; AV, aortic valve; AVSD, atrioventricular septal defect; BAV, bicuspid aortic valve; CHD, congenital heart disease; Chr, chromosome; CoA, coarctation of the aorta; HLHS, hypoplastic left heart syndrome; IAA, interrupted aortic arch; LVOT, left ventricular outflow tract; MV, mitral valve; N/A, not available; OFT, outflow tract; PAS, pulmonary artery stenosis; PDA, patent ductus arteriosus; PPS, peripheral pulmonic stenosis; SVAS, supravalvar aortic stenosis; TA, tricuspid atresia; TAPVD, total anomalous pulmonary venous drainage; TOF, tetralogy of Fallot; VSD, ventricular septal defect.

affect their expression, leading to the development of CHD.^{11 12} CNVs can occur de novo in sporadic cases, or they can be inherited familiarly causing complex congenital heart malformations. However, the underlying mechanisms of CNVs in CHD still need to be elucidated. One of the most common CNVs syndromes in CHD, 22q11.2 deletion syndrome (DiGeorge syndrome or velocardiofacial syndrome), is caused by a microscopic deletion on chromosome 22q11.2. Variable phenotypes, such as craniofacial abnormalities, neurocognitive disabilities, palate abnormalities, hypocalcemia and immunodeficiencies, can be observed in this syndrome. The main cardiac malformations of this syndrome contain VSD, arch abnormalities and tetralogy of Fallot (TOF).¹³ To date, more than 30 gene deletions have been identified in the 22q11.2 locus by sequencing. Deletions in the T-box transcription factor *TBX1* account for the major molecular basis of these cardiac malformations.¹⁴ Further work revealed that distal 22q11.2 deletion presented atypical clinical features of DiGeorge syndrome; therefore, this deletion was proposed as one of the etiologies of CHD.¹⁵ Williams-Beuren syndrome, which is characterized by supravalvar aortic stenosis (SVAS), peripheral pulmonary stenosis, coronary artery stenosis, pulmonary artery sling, developmental delays, typical elfin facies, infantile hypercalcemia and cognitive disability, has been reportedly caused by microdeletion of over 25 genes in the 7q11.23 region.¹⁶ It has been known that cardiovascular abnormalities, such as SVAS, can be induced by haploinsufficiency of the elastin gene (*ELN*).¹⁷

Some CNVs encompass previously identified CHD genes, or genes known to be implicated in heart development. For example, 8p deletion syndrome and mutations in *GATA4*, a cardiac transcription factor, are reported to be associated with CHD.¹⁸ The 8p23.1 delete region that overlaps with the locus of *GATA4* could elucidate this causal outcome. However, Kumar *et al* reported an interesting case of 8p23.3p23.1 deletion and 8p23.1p11.1 interstitial duplication syndrome that a male toddler with global developmental delay, dysmorphic facies, seizures and large doubly committed VSD occurred without the *GATA4* gene involvement.¹⁹ Duplications at the 8p23.1 locus have also been identified in CHD, including ASD, VSD and TOF.^{20 21} Other studies have confirmed that the CNVs at chromosome 11q23 were associated with Jacobsen syndrome.^{22 23} Moreover, several CNVs have been identified from larger cohorts of patients with CHD, including 1q21.1,²⁴⁻²⁶ 4p16.3,²⁷ 4q22.1,^{26 28} 9q34.3^{26 29} and 15q11.2.²⁸ All of these CNVs mentioned above are detailed in table 1 for their relationship to CHD in human.

GENE MUTATIONS UNDERLYING CHD

Numerous mutations are implicated in the development of CHD (table 2). Some mutations are identified in pedigrees of CHD, while others are initially observed in sporadic cases of CHD.² Currently, mutations in more

Table 2 Common congenital heart disease resulting from single gene defects

	Gene	Locus	Protein	Cardiac phenotype	OMIM
Gene associated with transcription factors of cardiac development	<i>GATA4</i>	8p23.1	GATA4 transcription factor	ASD, VSD, AVSD, PS, PAPVR and TOF.	600576
	<i>GATA5</i>	20q13.33	GATA5 transcription factor	Congenital bicuspid aortic valve and VSD.	617912
	<i>GATA6</i>	18q11.2	GATA6 transcription factor	ASD, VSD, AVSD, OFT defects, PDA, PS and TOF.	601656
	<i>NKX2.5</i>	5q35.1	Homeobox containing transcription factor	ASD, VSD, TOF, HLHS, CoA, TGA, DORV, IAA and OFT defects.	600584
	<i>NKX2.6</i>	8p21.2	Homeobox containing transcription factor	PTA and conotruncal heart malformations.	217095
	<i>TBX1</i>	22q11.2	T-Box 1 transcription factor	TOF.	602054
	<i>TBX5</i>	12q24.21	T-Box 5 transcription factor	ASD, VSD and AVSD.	601620
	<i>TBX20</i>	7p14.2	T-Box 20 transcription factor	ASD, VSD and MS.	611363
	<i>TFAP2β</i>	6p12.3	Transcription factor AP-2 beta	PDA.	601601
	<i>ZIC3</i>	Xq26.3	Zinc finger transcription factor	ASD, VSD, HLHS, DORV, PS, TGA, TAPVR, dextrocardia, L-R axis defects and heterotaxy.	300265
Gene associated with signaling pathways of cardiac development	<i>AXIN2</i>	17q24.1	Axin-related protein 2	Congenital valve defect.	/
	<i>BRAF</i>	7q34	Serine/threonine-protein kinase B-raf	ASD, and PAS.	164757
	<i>CBL</i>	11q23.3	E3 ubiquitin-protein ligase CBL	AVSD, HCM and PS.	613563
	<i>DLL4</i>	15q15.1	Delta-like protein 4	Left-sided obstructive lesions, septal and conotruncal defects and tricuspid atresia.	616589
	<i>FOXH1</i>	8q24.3	Forkhead box protein H1	TOF and TGA.	/
	<i>GALNT11</i>	7q36.1	Polypeptide N-acetylgalactosaminyltransferase 11	Heterotaxy.	/
	<i>GLI1</i>	12q13.3	Zinc finger protein GLI1	Abnormality of atrioventricular separation and cardiac OFT.	165220
	<i>HHEX</i>	10q23.33	Hematopoietically expressed homeobox	Ventricular aplasia, dense myocardial dysplasia and intracardiac membrane dysplasia.	/
	<i>HRAS</i>	11p15.5	GTPase HRas	PAS and tachycardia.	190020
	<i>JAG1</i>	20p12.2	Protein Jagged-1	PAS and TOF.	601920
	<i>KRAS</i>	12p12.1	GTPase KRas	ASD and PAS.	190070
	<i>MAML1</i>	5q35.3	Mastermind-like protein 1	Aortic valve disease.	/
	<i>MEK1</i>	15q22.31	Dual specificity mitogen-activated protein kinase kinase 1	ASD and PAS.	176872
	<i>MEK2</i>	19p13.3	Dual specificity mitogen-activated protein kinase kinase 2	ASD and PAS.	601263
	<i>NOTCH1</i>	9q34.3	Notch receptor 1	Aortic valve disease.	190198
	<i>NOTCH2</i>	1p12	Notch receptor 2	AS, TOF and PAS.	610205
	<i>NRAS</i>	1p13.2	GTPase NRas	HCM and PS.	164790
<i>PPP1CB</i>	2p23.2	Serine/threonine-protein phosphatase PP1-beta catalytic subunit	ASD, VSD, HCM, PAS and TOF.	617506	
<i>PTPN11</i>	12q24.13	Protein tyrosine phosphatase non-receptor type 11	ASD, VSD and PAS.	176876	

Continued

Table 2 Continued

	Gene	Locus	Protein	Cardiac phenotype	OMIM
	<i>RAF1</i>	3p25.2	RAF proto-oncogene serine/threonine-protein kinase	ASD and TOF.	164760
	<i>RIT1</i>	1q22	GTP-binding protein Rit1	VSD, TOF and PAS.	615355
	<i>SHOC2</i>	10q25.2	Leucine-rich repeat protein SHOC-2	ASD, VSD, HCM, PAS and TOF.	607721
	<i>SMAD6</i>	15q22.31	Mothers against decapentaplegic-related protein 6	AV disease.	602931
	<i>SOS1</i>	2p22.1	Son of sevenless homolog 1	ASD, VSD and TOF.	182530
	<i>SOS2</i>	14q21.3	Son of sevenless homolog 2	ASD, VSD and TOF.	616559
	<i>TGF-β1</i>	19q13.2	Transforming growth factor beta-1 proprotein	CoA, HLHS, BAV and AS.	/
Gene associated with structural proteins of cardiac development	<i>ACTC</i>	15q14	Alpha cardiac actin	ASD.	102540
	<i>ELN</i>	7q11.23	Elastin	AS, PAS, PS and SVAS.	130160
	<i>MYH6</i>	14q11.2	Alpha myosin heavy chain	AS, ASD, PFO, TA and TGA.	160710
	<i>MYH7</i>	14q11.2	Beta myosin heavy chain	ASD, NVM and Ebstein anomaly.	160760
	<i>MYH11</i>	16p13.11	Myosin heavy chain 11	Aortic aneurysm and PDA.	132900

AS, aortic stenosis; ASD, atrial septal defect; AV, aortic valve; AVSD, atrioventricular septal defect; BAV, bicuspid aortic valve; CoA, coarctation of the aorta; DORV, double outlet right ventricle; HCM, hypertrophic cardiomyopathy; HLHS, hypoplastic left heart syndrome; IAA, interrupted aortic arch; L-R, Left-right; MS, mitral stenosis; NVM, non-compaction of ventricular myocardium; OFT, outflow tract; OMIM, online mendelian inheritance in man; PAPVR, partial anomalous pulmonary venous return; PAS, pulmonary artery stenosis; PDA, patent ductus arteriosus; PFO, patent foramen ovale; PS, pulmonary (valve) stenosis; PTA, persistent truncus arteriosus; SVAS, supravalvar aortic stenosis; TA, tricuspid atresia; TAPVR, total anomalous pulmonary venous return; TGA, transposition of the great arteries; TOF, tetralogy of Fallot; VSD, ventricular septal defect.

than 50 genes have been found to be associated with CHD by the application of high-throughput sequencing of whole-genome and whole-exome, and many of these affected genes have been confirmed to be involved in transcriptional regulation, signal transduction and cardiac development.

Mutations of genes encoding transcription factors in CHD

Heart development is regulated by several transcriptional circuits that are members of a core group of transcription factors, including *NKX2.5*, *GATA4* and *TBX5*.³⁰⁻³¹ Therefore, transcription factors have been considered as the prime inducer of CHD. *NKX2.5* is the earliest known marker of myocardial progenitor cells in all species.³² The mechanisms of *NKX2.5* regulation and its interaction with other transcription factors in early cardiac development have been studied extensively. It has been found that mutations in *NKX2.5* could result in variable types of CHD, including ASD, VSD, TOF, hypoplastic left heart syndrome, CoA, transposition of the great arteries, double outlet right ventricle (DORV), interrupted aortic arch and cardiac outflow tract defects.³³ Furthermore, *NKX2.5* mutations are the most common cause of ASD in individuals with defects of conduction system.³⁴⁻³⁵

However, some individuals with CHD caused by the mutations of *NKX2.5* can manifest an individual phenotype of ASD and/or conduction defects.³⁶ To date, approximately 80 different mutations have been identified in

NKX2.5, including missense mutations [eg, c.44A>T (p.K15I), c.232A>G (p.N19S), c.673C>A (p.N188K) and c.1089A>G (p.S305G)], synonymous mutations [eg, c.543G>A (p.Q181Q), c.677A>G (p.E167E), c.902C>G (p.G242G) and c.1142A>G (p.R322R)] and nonsense mutations [eg, c.1149T>C (stop→Gln)].³³ The location of some of these mutations is depicted in figure 1A. Holt-Oram syndrome, which is characterized by CHD (eg, ASD, VSD and atrioventricular conduction system disease) and upper limb malformations, could be caused by the loss-of-function mutations in *TBX5*, which is notably expressed in the upper limbs and heart.³⁷⁻³⁸ Nonsense or frameshift mutations of *TBX5* may be responsible for this syndrome.¹² Functional deficiency of the conserved DNA-binding motif in the transcription factor encoded by *TBX5* may be the etiology of Holt-Oram syndrome.³⁹

GATA4, encoding one of the GATA zinc-finger transcription factors, is a deeply studied gene and is essential for cardiogenesis. Mutations in *GATA4* are reported to be implicated in cardiac septal defects.³¹ Over 100 mutations in *GATA4* coding region have been identified in patients with CHD, and more mutations will be identified with intensive research.⁴⁰ Among these mutations, 11 sites (two synonymous mutations, seven missense mutations and two frameshift mutations) have been studied in familial cases, which highlight the significance of these sites in the development of CHD (figure 1B). Multiple mutations identified from other transcription factors,

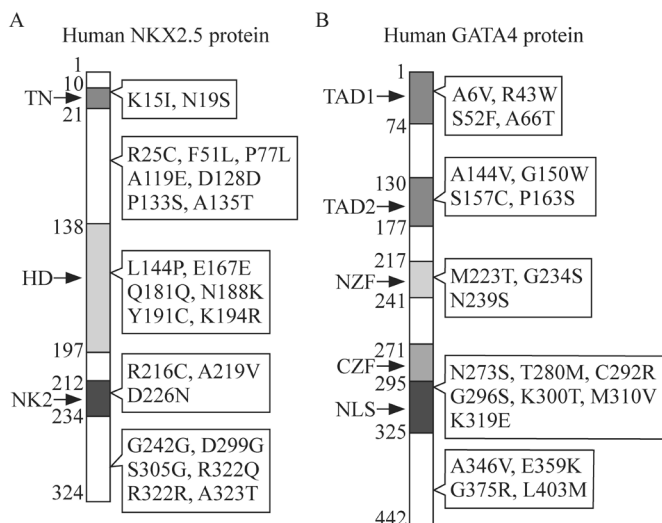


Figure 1 Spectrum of some significant mutant sites of (A) NKX2.5 and (B) GATA4 observed in the occurrence of congenital heart defects. CZF, C-terminal zinc finger domain; HD, homeodomain; NK2, NK-2-specific domain; NLS, nuclear localization signal; NZF, N-terminal zinc finger domain; TAD1, transcription activation domain 1; TAD2, transcription activation domain 2; TN, tin-man domain/transcriptional repression domain.

including *NKX2.6*,⁴¹ *GATA5*,^{42–44} *GATA6*,⁴⁵ *TFAP2 β* ,⁴⁶ *TBX1*,⁴⁷ *TBX20*^{48,49} and *ZIC3*,⁵⁰ have also been reported to be associated with the incidence of CHD.

Mutations of genes encoding signal proteins in CHD

Signaling pathways involved in the occurrence of CHD are widely studied. Genes involved in these different signaling pathways can converge into a large and sophisticated regulatory network that plays an important role in cardiac development and pathogenesis of CHD. Recent studies have also suggested the potential contributions of vascular endothelial growth factor-A (VEGFA), Notch signaling, Wnt signaling, transforming growth factor- β (TGF- β), bone morphogenic protein (BMP) signaling and cpathway to the occurrence of CHD.^{51–57} In these pathways, some are essential for the formation of cardiac septum, valves and the construction of cardiac outflow tracts, while others are associated with the asymmetric development of the heart. Gene mutations in renin-angiotensin system mitogen-activated protein kinase (RAS-MAPK) signal transduction pathway can lead to Noonan syndrome with the typical phenotype of pulmonary valve stenosis and hypertrophic cardiomyopathy.^{58–61}

Six missense variants (*COL6A1*, *COL6A2*, *CRELD1*, *FBLN2*, *FRZB* and *GATA5*), acting in the VEGF-A pathway, were found to be damaged in individuals with complete atrioventricular septal defect (AVSD),⁵¹ suggesting that rare variants in the VEGF-A pathway might play a role in the development of AVSD. In addition, mutations in VEGF-A have been reported to be associated with congenital left ventricular outflow tract obstruction.⁶² Notch signaling is a highly conserved pathway involved in developmental process of heart. *JAG1* encodes a ligand in

the Notch signaling pathway, which leads to localization of Notch to the nucleus and downstream activation of target genes. Mutations in *JAG1* have been found in over 90% cases of Alagille syndrome.^{63,64} Some cases (~2%) that have mutations in *NOTCH2*, a NOTCH receptor gene, are also correlated with Alagille syndrome.⁶⁵ However, variants of *NOTCH1* that belongs to the Notch signaling pathway have been identified to be associated with Adams-Oliver syndrome.¹⁵ Mind bomb 1 (Mib1) is a vital protein that promotes ubiquitination, endocytosis and subsequent activation of Notch ligands to activate the Notch signaling pathway. Mutations in *MIB1* have been identified to be associated with cardiac deformity such as ASD, AVSD and VSD, through a lower level of *JAG1* ubiquitination and Notch signaling induction.⁶⁶ In addition, mutations in other genes of Notch signaling pathway include *MAML1*,⁶⁷ *DLL4*⁶⁸ and *GALNT11*⁶⁹ are also involved in CHD.

AXIN2 is involved in the regulation network of cardiac valve formation and elongation, and its expression product is a negative regulator of Wnt/ β -catenin signaling pathway.^{70,71} It has been found that mutations in *AXIN2* can result in CHD with the phenotype of congenital valve defect.⁷² *HHEX*, a member of the Homeobox gene family, is an important cardiac determinant and controls the early differentiation, migration and development of cardiomyocytes.⁷³ Foley *et al*⁷⁴ reported that mutations in *HHEX* could lead to a phenotype of abnormal developmental endogenous cardiac or ectopic heart, which was similar to the antagonistic effect of Dickkopf-1 to Wnt signaling pathway. Aberrant Wnt signaling pathways implicated in CHD have been summarized in a previous excellent literature.⁷⁵ We describe the aberrant expression of genes associated with CHD within the Wnt signaling pathways in figure 2. TGF- β signaling pathway has important role in the development and remodeling of cardiovascular system. Aberrant TGF- β signaling pathway is involved in the pathogenesis of several human cardiovascular diseases through the epithelial-to-mesenchymal transition (EMT) of resident fibroblasts, circulating progenitors, pericytes, epithelial cells and/or the endothelial-mesenchymal transdifferentiation of endothelial cells.^{76,77} For example, mutations in *TGF- β 1*, one major subtype of TGF- β family, have been reported to be associated with CHD in pediatric patients.⁷⁸ In general, BMP synergizes with TGF- β signaling to activate the downstream genes, such as *Smad1*, *Smad5* or *Smad8*, with the changed transcription of target genes. Mutations in the genes that encode transducers of the TGF- β and BMP signaling pathway have been identified in the pathogenesis of cardiovascular diseases, such as Marfan syndrome and Loey-Dietz syndrome.^{55,79} Nodal signaling pathway is involved in the left-right patterning and development of the heart and in abnormal gene products throughout the pathway that are clearly associated with CHD. Roessler *et al*⁵⁶ previously demonstrated that reduced nodal signaling strength via mutation of *FOXH1* was linked to human heart defects. SHH, one morphogen

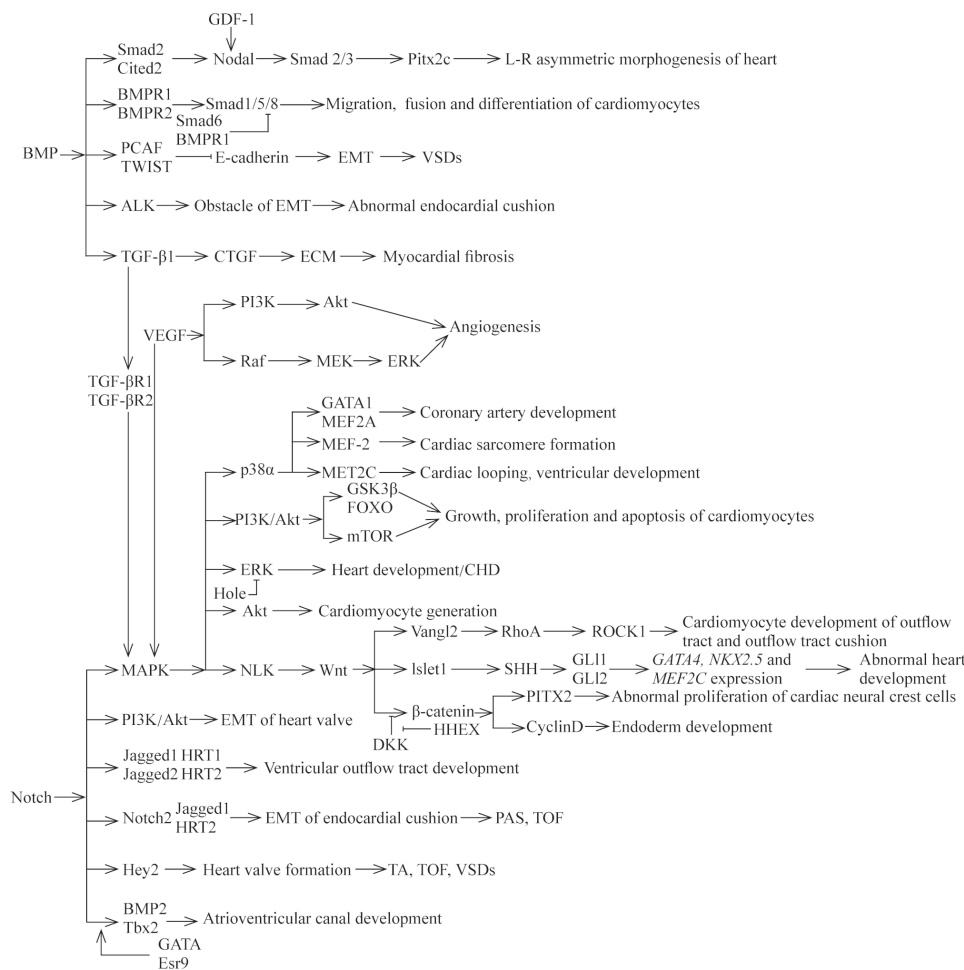


Figure 2 The relationships of different signaling pathways and some responsible mutated genes within these pathways in cardiac development and CHD. CHD, congenital heart disease; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; PAS, pulmonary artery stenosis; TA, tricuspid atresia; TOF, tetralogy of Fallot; VSD, ventricular septal defect.

of hedgehog (HH) family proteins, is involved in a remarkably wide variety of process, including cardiovascular development. Previous evidence demonstrated the mutations in genes of HH signaling were implicated in the occurrence of CHD with a phenotype of ASD, VSD or AVSD, and the responsible mutated genes include *SHH*, *Gli3* and *MKSI*.^{80–83} Noonan syndrome, one of the most common genetic syndromes of CHD, is caused by mutations in genes of the RAS-MAPK pathway.⁸⁴ At present, several genes have been verified to be responsible for the development of Noonan syndrome and other disorders. Mutations in genes, encoding molecules implicated in the RAS-MAPK signaling pathway, account for approximately 90% of affected CHD cases. These genes include: *PTPN11*, *SOS1*, *KRAS*, *RAF1*, *BRAF*, *SHOC2*, *NRAS*, *HRAS*, *CBL*, *MEK1*, *MEK2*, *PPP1CB*, *RIT1* and *SOS2*.^{15 58 61 84}

Currently, most studies have only stayed on the basic exploration of a single gene, which cannot fully explain the pathogenesis of CHD. The interactions of various signaling pathways involved in heart development are still incompletely understood. Some responsible mutated genes have been proposed to clarify the interactions of the signaling pathways implicated in CHD (figure 2).

More researches are needed to help elaborate the pathogenic mechanisms of CHD for the intricate network of signaling pathway.

Mutations of genes encoding cardiac structural proteins in CHD

Several studies use targeted whole-genome sequencing to investigate the genes that encode cardiac structural proteins to elucidate the monogenic cause of CHD. Some rare missense mutations or premature termination mutations in myosin heavy chain 6 (*MYH6*), a marker gene of myocardial cell, could result in ASD.^{85 86} However, mutations in *MYH7* have been confirmed in bilobal aortic valve.⁸⁷ CHD with apical hypertrophic cardiomyopathy, left ventricular non-compaction and ASD could be caused by the mutations in *ACTC*, which is a cardiac actin gene and is essential for cardiac contraction.^{88 89} *MYH11*, encoding smooth muscle myosin heavy chain, is another gene that has been reported to be involved in CHD with the phenotype of dominant thoracic aortic aneurysm.^{90 91} Moreover, loss-of-function mutations in the elastin gene (*ELN*) could result in CHD with the presence of Williams-Beuren syndrome and non-syndromic SVAS.¹⁷

EPIGENETIC MODIFICATIONS UNDERLYING CHD

Epigenetics, which refers to the mechanisms of changed gene expression that are independent of DNA sequence, provides a new way to understand the pathogenesis of CHD. To date, three canonical mechanisms of epigenetics include DNA methylation, histone modification and non-coding RNAs. The increasing evidence suggests that the aberrant regulation of gene expression by epigenetics is a key factor in the development of cardiovascular diseases, which have attracted attention to focus on the role of epigenetics in CHD.

DNA methylation modifications in CHD

DNA methylation, the most widely studied epigenetic mechanism, refers to the formation of a methyl group (–CH₃) in the 5' carbon of cytosine (CpG islands), which induces an alteration of the structure of DNA. The methylation process is catalyzed by DNA methyltransferases (DNMTs), comprising DNMT1, DNMT3A and DNMT3B. The dysregulation of DNA methylation during different stages of development could lead to the transcriptional repression and functional inactivation of tissue-specific genes, resulting in increased risk for several diseases, including cardiac malformation. Alterations of DNA methylation, especially in CpG islands, which are close to core transcription factors and genes in signaling pathway, have been reported in patients with cardiac malformations.⁹²

DNA methylation plays a critical role in the development of the heart. The expression of hyaluronan synthase 2 (*Has2*) is necessary for the formation of the heart valves, septa and epicardium. However, *Has* expression was found to be downregulated via DNA methylation in the heart at E14.5 embryos.⁹³ Furthermore, gene knockout model indicated that expression of *Has2* is downregulated via DNA methyltransferase 3B (DNMT3B), which was coexpressed with *Has2* in the region of cardiac valve, suggesting that changes in DNA methylation might be involved in the regulatory function of *Has2* enhancer. Aberrant methylation of *CITED2* may play an important role in the development of VSD, ASD and TOF.⁹⁴ Aberrant methylations of *CITED2* could decrease its mRNA expression and be regarded as the prime cause of CHD.⁹⁵ The presence of aberrant hypomethylation in the CpG region of *BRG1* was found to exist in patients with ASD and interventricular septal defect.⁹⁶ It has been shown that the promoter region of *CX43* plays an essential role in the development of the heart outflow tract, and the aberrant hypomethylation of this enhancer region of *CX43* could be considered as one of the etiologies of CHD.^{97 98}

In another study, two encoding transcription factors, zinc-finger in cerebellum 3 and nuclear receptor subfamily 2 group F member 2, were found to be hypermethylated in monozygotic twins with DORV, suggesting that the differential methylation of these transcription factors could be regarded as a potential pathogenesis of the diseased twin.⁹⁹ Hypermethylation of the promoter

region of *SCO2*, a cytochrome oxidase, has been found in patients with TOF and VSD. The methylation of CpG islands located in the promoter of *SCO2* result in reduced expression of *SCO2*, which may be the mechanism of the occurrence of these diseases.¹⁰⁰ Further studies discovered that hypermethylation of CpG islands of *NKX2.5*, *HAND1* and *RXRα* was also essential for CHD, including VSD and TOF.^{101 102} Multiple differential methylated genes have been identified from cardiomyocytes of newborn and adults, and adult failing hearts have revealed a highly dynamic of DNA methylation under specific developmental or pathological conditions. DNA methylation is tightly regulated during cardiac differentiation and maturation.¹⁰³ Taken together, these findings highlight the importance of DNA methylation in cardiac morphogenesis and CHD formation.

Histone modifications in CHD

Histones, including H1, H2A, H2B, H3 and H4, and DNA constitute the nucleosome, which is the basic structural unit of chromatin in eukaryotic cells.¹⁰⁴ Altering the histone-DNA contacts effectively via histone, post-translational modifications could loosen or tighten the chromatin architecture to control the availability of gene transcription or expression. Histone post-translational modifications could be modified by the catalysis of histone-modifying enzymes, such as histone methylases, demethylases, acetylases and deacetylases, ubiquitin enzymes and phosphorylases. Aberrant expression and mutation of the histone modifiers during the development of heart can influence the response of heart to pathological stresses.¹⁰⁵ Moreover, increasing researches show that the interplay between these different cardiac transcription factors and histone modifiers plays a significant role in heart development. Numerous studies have shown that methylation and acetylation of histone is an emerging epigenetic mechanism for the regulation of gene transcription. Therefore, we focused only the mechanism of histone methylation and acetylation in CHD.

Methylation mainly occurs at the core histones H3 and H4, which is performed by the catalysis of histone methyltransferases. H3K4, H3K36 and H3K79 methylation leads to the transcriptional activation, whereas transcriptional repression can be induced by H3K9, H3K27 and H4K20 methylation.¹⁰⁶ CHD associated with Wolf-Hirschhorn syndrome exists deletion of *Wolf-Hirschhorn candidate protein 1* (*Whsc1*), which encodes the H3K36me₃-specific methyltransferase. Nimura *et al.*¹⁰⁷ found that the pathogenic role of *Whsc1* was associated with the transcriptional activation of *Nkx2.5* in its target sites through the model of *Whsc1*-knockout murine. Another studies revealed that the interactions of JARID2 and SETDB1, which was an H3K9me₃-specific methyltransferase, could elucidate the role of *Jarid2* in the occurrence of VSD, DORV and impaired ventricular compaction induced by hypertrabeculation.^{108 109} *TBX1*, one member of T-box transcription factor family, was shown to interact with H3K4 and H3K27 via two domains of T-box to regulate

gene expression, and aberrant expression could result in CHD including TOF, VSD and aortic arch interruption.^{110 111} A later study further identified the interactions of *TBX1* and *BAF* chromatin remodeling complex regulate *Wnt5a* expression, and the insufficient expression of *TBX1* or *Wnt5a* could result in the phenotypes of hypoplastic right heart.¹¹² DPf3, an evolutionarily conserved protein, binds methylated and acetylated lysine residues of histone 3 and 4 to regulate gene expression. *Dpf3* is expressed in the heart during development and has been found as significantly upregulated in the right ventricular myocardium of patients with TOF.¹¹³

Histone acetylation and deacetylation are always in a dynamic state, which is associated with transcriptional activation or transcriptional repression, gene silencing and cell cycle. Histone acetylation and deacetylation have been studied extensively in recent years.¹¹⁴ It has been shown that some histone acetylases, such as EP300, KAT2A and hNAT1, are associated with the development of the heart. Aberrant expression of these acetylases could lead to CHD with ASD, VSD, AVSD and valve dysplasia.^{115 116} Previous studies have revealed that histone deacetylases (HDACs) are also implicated in CHD. For example, *HDAC3* regulates the cellular acetylation level affecting cardiogenesis, and the absence of *HDAC3* could cause PDA and TOF.¹¹⁷ In addition, down-regulation of *HDAC5* and *HDAC9* simultaneously could result in CHD with VSD.^{118 119} Abnormal regulation of *SIRT1* has been reported to be involved in ventricular hypoplasia.¹²⁰ Park *et al*¹²¹ revealed that histone methyltransferase *SMYD1* was associated with ventricular hypoplasia. Some other histone modifiers have been shown to be important for the development of the heart through numerous studies, such as *G9a*, *Ezh2/PRC2*, *Baf60c/Brg1*, *Jmjd3*, *UTX* and *MLL2*,¹²²⁻¹³⁰ and the aberrant modification of them could yield critical CHD phenotypes. The activity of cardiac transcription factor could be changed by histone modifiers, which means these modifiers could be strong candidates for CHD etiology and therapeutic targeting.

Non-coding RNA in CHD

Non-coding RNA is another type of epigenetic modification involved in the control of gene expression by post-transcriptional regulation. Among the various non-coding RNAs, microRNAs (miRNAs) and long non-coding RNAs (LncRNAs) are the two best studied groups. miRNAs are capable of regulating gene expression by interacting with mRNA transcript 3' untranslated regions (UTRs) to repress translation, whereas LncRNAs are able to regulate transcription by directly interacting with chromatin remodeling complexes.¹³¹ Emerging evidence that indicates the impact of non-coding RNAs to cardiac development was previously unappreciated but is becoming valuable.

A number of miRNAs have recently been shown to function in the heart.¹³² It has been shown miR-1 is important in cardiac development and is regarded as the most relevant miRNA leading to CHD.^{133 134} Li *et al* found that

expression of miRNA-1 decreased in patient with VSD.¹³⁵ Molecularly, miRNA-1 binds to its target gene *GJA1* and *SOX9*, regulating the formation of cardiac valves and septa, and therefore miRNA-1 dysregulation could result in CHD in human.¹³⁵ Further studies have demonstrated that myocyte enhancer factor 2 (*Mef2*) could upregulate the expression of miR-1, which suppress the cardiac transcription factor *Hand2* and HDAC translation.^{133 136} *Hand2* is known as a target of miRNA-1 and is involved in the growth of the embryonic heart; thus, *Hand2* could be associated with CHD.¹³³ Overexpression of miRNA-27b could repress the expression of *Mef2c* and affect the development of myocardial, leading to cardiac hypertrophy.¹³⁷ A recent study has confirmed that differentially expressed frataxin (*FXN*) regulates the development of CHD and that differential expression of *FXN* is under the control of miRNA-145.¹³⁸ Further investigation of this study demonstrated that overexpression of miRNA-145 could regulate apoptosis and mitochondrial function by repressing the expression of *FXN*, leading to the development of CHD.¹³⁸ In recent years, increasing evidence has confirmed that alterations of miRNAs expression are associated with human cardiovascular diseases, including CHD (reviewed by Nagy).¹³⁹ Furthermore, miRNAs are extensively studied as clinical biomarkers for their stability in blood, urine and other biological fluids and their ability to evade RNA degrading enzymes. The researchers demonstrated that miRNAs in maternal serum could be used as candidate biomarkers for prenatal detection of fetal CHD in early pregnancy.^{140 141} These authors identified miR-19b and miR-29c significantly upregulated in patients with VSD, while miR-19, miR-22, miR-29c and miR-375 upregulated in patients with TOF. In recent years, increasing new miRNAs have been found to be associated with CHD, suggesting a potential value of miRNAs as diagnostic markers in human cardiovascular diseases.

LncRNA regulates cell growth, differentiation, cell proliferation and apoptosis by controlling gene expression. To date, little information of the functional role of lncRNAs in CHD has been reported, and only scant evidence has demonstrated the involvement of lncRNAs in CHD, particularly on VSD and TOF. Jiang *et al*¹⁴² reported the increased expression of *SNHG6* in fetal cardiac tissues of VSD patients and suggested *SNHG6* might be involved in VSD by the mechanistic link between *SNHG6* upregulation, miR-100 downregulation, Wnt/ β -catenin activation and the formation of VSD. The author also identified that expression of *HOTAIR* was increased in right atrial biopsies of CHD patients with ASD and VSD and postulated *HOTAIR* as a biomarker for CHD. Another study identified a polymorphism of *MALAT1* associated with ASD and VSD.¹⁴³ LncRNA *TUC40* has been reported to reduce the expression of *PBX1* and to affect the differentiation of cardiomyocytes, which may be a potential pathological etiology of VSD.¹⁴⁴ In addition to the importance of LncRNAs in cardiac septal defects, the role of LncRNAs in the development of cyanotic heart disease

such as TOF has been reported. Wang *et al*¹⁴⁵ identified that high expression of *HAI17* was associated with adverse outcomes in TOF patients, although the mechanism of *HAI17* in TOF remained unclear. In a recent study by Gu *et al*,¹⁴⁶ the circulating plasma lncRNAs have been implicated in cardiovascular diseases for its potential as new biomarkers of diagnostic and prognostic in clinical treatments. The aberrant expression of the following lncRNAs: ENST00000436681, ENST00000422826, AA584040, AA709223 and BX478947 are associated with CHD. These specific lncRNAs identified from the plasma of pregnant women with typical fetal CHD may play an important role in the development and prenatal diagnosis of fetal CHD.

CONCLUSIONS

Normal development of the heart is a complex process involving many regulatory factors, including genetics and epigenetics. Cardiac malformations are congenital developmental disorders that can be induced by the dysregulations of genetic and epigenetic factors. Abundant seminal studies have found that conventional genetic factors could not elucidate the pathogenesis of CHD alone. Epigenetic regulation is also an important aspect of normal cardiac development as well as of defective function in disease situations such as CHD. The interactions at multiple levels provide insights of the combinatorial regulation into the morphogenesis of heart and suggest they can partially compensate each other's function. Phenotypic heterogeneity and incomplete penetrance of CHD complicate our understanding of the interactions between genetics and epigenetics in CHD. Future studies to focus on elucidating the epigenetic signals of genes associated with cardiac development pathways could throw light on the genetic and epigenetic mechanisms in the development of CHD.

With the great progress in basic researches carried out in model systems (eg, *in vivo*: animal models; *in vitro*: cell and tissue engineered models) and with the improving limits of detection, the study of CHD has entered a new era for clearer understanding of its etiology. The wide application of these new detection technologies would provide an effective method for the prevention, diagnosis and treatment of CHD, opening up new avenues of individualized care for patients with CHD.

Contributors YW and XJ contributed to conceptualization and data curation. YW, XJ, YZ and JZ performed writing and revising the original draft. RY was responsible for conceptualization and conducted all procedures, including data curation, writing the original draft and revised manuscript. All authors read and approved the final manuscript.

Funding This work was supported by the National Key R&D Program of China (grant number 2018YFC1002700) and the National Key R&D Program of China (grant number 2018YFC1002703).

Disclaimer The funder of the work had no role in: study design, or collection, analysis, and interpretation of data, or writing the manuscript or decision to submit.

Competing interests The authors declare that they have no conflicts of interest to report regarding the present study.

Patient consent for publication Not required.

Ethics approval Not required for this review article.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available in a public, open access repository.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

REFERENCES

- Zaidi S, Brueckner M. Genetics and genomics of congenital heart disease. *Circ Res* 2017;120:923–40.
- Fahed AC, Gelb BD, Seidman JG, *et al*. Genetics of congenital heart disease: the glass half empty. *Circ Res* 2013;112:707–20.
- Marelli AJ, Ionescu-Ittu R, Mackie AS, *et al*. Lifetime prevalence of congenital heart disease in the general population from 2000 to 2010. *Circulation* 2014;130:749–56.
- Baumgartner H, De Backer J, Babu-Narayan SV, *et al*. 2020 ESC guidelines for the management of adult congenital heart disease. *Eur Heart J* 2021;42:563–645.
- Kalisch-Smith JI, Ved N, Sparrow DB. Environmental risk factors for congenital heart disease. *Cold Spring Harb Perspect Biol* 2020;12. doi:10.1101/cshperspect.a037234. [Epub ahead of print: 02 Apr 2020].
- Li YJ, Yang YQ. An update on the molecular diagnosis of congenital heart disease: focus on loss-of-function mutations. *Expert Rev Mol Diagn* 2017;17:393–401.
- Pierpont ME, Basson CT, Benson DW, *et al*. 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* 2007;115:3015–38.
- Pont SJ, Robbins JM, Bird TM, *et al*. Congenital malformations among liveborn infants with trisomies 18 and 13. *Am J Med Genet A* 2006;140:1749–56.
- Petry P, Polli JB, Mattos VF, *et al*. Clinical features and prognosis of a sample of patients with trisomy 13 (Patau syndrome) from Brazil. *Am J Med Genet A* 2013;161A:1278–83.
- Cho YG, Kim DS, Lee HS, *et al*. A case of 49,XXXXX in which the extra X chromosomes were maternal in origin. *J Clin Pathol* 2004;57:1004–6.
- Costain G, Silversides CK, Bassett AS. The importance of copy number variation in congenital heart disease. *NPJ Genom Med* 2016;1:16031.
- Geng J, Picker J, Zheng Z, *et al*. Chromosome microarray testing for patients with congenital heart defects reveals novel disease causing loci and high diagnostic yield. *BMC Genomics* 2014;15:1127.
- Momma K. Cardiovascular anomalies associated with chromosome 22q11.2 deletion syndrome. *Am J Cardiol* 2010;105:1617–24.
- Merscher S, Funke B, Epstein JA, *et al*. Tbx1 is responsible for cardiovascular defects in velo-cardio-facial/digeorge syndrome. *Cell* 2001;104:619–29.
- Digilio MC, Marino B. What is new in genetics of congenital heart defects? *Front Pediatr* 2016;4:120.
- Pober BR. Williams-Beuren syndrome. *N Engl J Med* 2010;362:239–52.
- Metcalfe K, Rucka AK, Smoot L, *et al*. Elastin: mutational spectrum in supravalvular aortic stenosis. *Eur J Hum Genet* 2000;8:955–63.
- Pehlivan T, Pober BR, Brueckner M, *et al*. Gata4 haploinsufficiency in patients with interstitial deletion of chromosome region 8p23.1 and congenital heart disease. *Am J Med Genet* 1999;83:201–6.
- Kumar V, Roy S, Kumar G. An interesting and unique case of 8p23.3p23.1 deletion and 8p23.1p11.1 interstitial duplication syndrome. *J Pediatr Genet* 2018;7:125–9.
- Carey AS, Liang L, Edwards J, *et al*. Effect of copy number variants on outcomes for infants with single ventricle heart defects. *Circ Cardiovasc Genet* 2013;6:444–51.
- Osogawa K, Iovannisci DM, Lin B, *et al*. Identification of novel candidate gene loci and increased sex chromosome aneuploidy among infants with conotruncal heart defects. *Am J Med Genet A* 2014;164A:397–406.

- 22 Grossfeld PD, Mattina T, Lai Z, *et al.* The 11q terminal deletion disorder: a prospective study of 110 cases. *Am J Med Genet A* 2004;129A:51–61.
- 23 Ye M, Coldren C, Liang X, *et al.* Deletion of Ets-1, a gene in the Jacobsen syndrome critical region, causes ventricular septal defects and abnormal ventricular morphology in mice. *Hum Mol Genet* 2010;19:648–56.
- 24 Christiansen J, Dyck JD, Elyas BG, *et al.* Chromosome 1q21.1 contiguous gene deletion is associated with congenital heart disease. *Circ Res* 2004;94:1429–35.
- 25 Silversides CK, Lionel AC, Costain G, *et al.* Rare copy number variations in adults with tetralogy of Fallot implicate novel risk gene pathways. *PLoS Genet* 2012;8:e1002843.
- 26 Greenway SC, Pereira AC, Lin JC, *et al.* De novo copy number variants identify new genes and loci in isolated sporadic tetralogy of Fallot. *Nat Genet* 2009;41:931–5.
- 27 Battaglia A, Filippi T, Carey JC. Update on the clinical features and natural history of Wolf-Hirschhorn (4p-) syndrome: experience with 87 patients and recommendations for routine health supervision. *Am J Med Genet C Semin Med Genet* 2008;148C:246–51.
- 28 Soemedi R, Wilson IJ, Bentham J, *et al.* Contribution of global rare copy-number variants to the risk of sporadic congenital heart disease. *Am J Hum Genet* 2012;91:489–501.
- 29 Kleefstra T, Brunner HG, Amiel J, *et al.* Loss-of-function mutations in euchromatin histone methyl transferase 1 (EHMT1) cause the 9q34 subtelomeric deletion syndrome. *Am J Hum Genet* 2006;79:370–7.
- 30 Bruneau BG. Signaling and transcriptional networks in heart development and regeneration. *Cold Spring Harb Perspect Biol* 2013;5:a008292.
- 31 Garg V, Kathiriyia IS, Barnes R, *et al.* Gata4 mutations cause human congenital heart defects and reveal an interaction with Tbx5. *Nature* 2003;424:443–7.
- 32 Tong YF. Mutations of Nkx2.5 and GATA4 genes in the development of congenital heart disease. *Gene* 2016;588:86–94.
- 33 Chung IM, Rajakumar G. Genetics of congenital heart defects: the Nkx2-5 gene, a key player. *Genes* 2016;7. doi:10.3390/genes7020006. [Epub ahead of print: 23 Jan 2016].
- 34 McElhinney DB, Geiger E, Blinder J, *et al.* Nkx2.5 mutations in patients with congenital heart disease. *J Am Coll Cardiol* 2003;42:1650–5.
- 35 Schott JJ, Benson DW, Basson CT, *et al.* Congenital heart disease caused by mutations in the transcription factor Nkx2-5. *Science* 1998;281:108–11.
- 36 Benson DW, Silberbach GM, Kavanaugh-McHugh A, *et al.* Mutations in the cardiac transcription factor Nkx2.5 affect diverse cardiac developmental pathways. *J Clin Invest* 1999;104:1567–73.
- 37 Basson CT, Bachinsky DR, Lin RC, *et al.* Mutations in human TBX5 [corrected] cause limb and cardiac malformation in Holt-Oram syndrome. *Nat Genet* 1997;15:30–5.
- 38 Li QY, Newbury-Ecob RA, Terrett JA, *et al.* Holt-Oram syndrome is caused by mutations in Tbx5, a member of the Brachyury (T) gene family. *Nat Genet* 1997;15:21–9.
- 39 Basson CT, Huang T, Lin RC, *et al.* Different Tbx5 interactions in heart and limb defined by Holt-Oram syndrome mutations. *Proc Natl Acad Sci U S A* 1999;96:2919–24.
- 40 Yu Y, Lei W, Yang J, *et al.* Functional mutant GATA4 identification and potential application in preimplantation diagnosis of congenital heart diseases. *Gene* 2018;641:349–54.
- 41 Heathcote K, Braybrook C, Abushaban L, *et al.* Common arterial trunk associated with a homeodomain mutation of NKX2.6. *Hum Mol Genet* 2005;14:585–93.
- 42 Wei D, Bao H, Zhou N, *et al.* Gata5 loss-of-function mutation responsible for the congenital ventriculoseptal defect. *Pediatr Cardiol* 2013;34:504–11.
- 43 Padang R, Bagnall RD, Richmond DR, *et al.* Rare non-synonymous variations in the transcriptional activation domains of GATA5 in bicuspid aortic valve disease. *J Mol Cell Cardiol* 2012;53:277–81.
- 44 Lentjes MHFM, Niessen HEC, Akiyama Y, *et al.* The emerging role of GATA transcription factors in development and disease. *Expert Rev Mol Med* 2016;18:e3.
- 45 Zheng GF, Wei D, Zhao H, *et al.* A novel GATA6 mutation associated with congenital ventricular septal defect. *Int J Mol Med* 2012;29:1065–71.
- 46 Satoda M, Zhao F, Diaz GA, *et al.* Mutations in TFAP2B cause char syndrome, a familial form of patent ductus arteriosus. *Nat Genet* 2000;25:42–6.
- 47 Gong W, Gottlieb S, Collins J, *et al.* Mutation analysis of Tbx1 in non-deleted patients with features of DGS/VCFS or isolated cardiovascular defects. *J Med Genet* 2001;38:45e–45.
- 48 Kirk EP, Sunde M, Costa MW, *et al.* Mutations in cardiac T-box factor gene TBX20 are associated with diverse cardiac pathologies, including defects of septation and valvulogenesis and cardiomyopathy. *Am J Hum Genet* 2007;81:280–91.
- 49 Posch MG, Gramlich M, Sunde M, *et al.* A gain-of-function TBX20 mutation causes congenital atrial septal defects, patent foramen ovale and cardiac valve defects. *J Med Genet* 2010;47:230–5.
- 50 Ware SM, Peng J, Zhu L, *et al.* Identification and functional analysis of ZIC3 mutations in heterotaxy and related congenital heart defects. *Am J Hum Genet* 2004;74:93–105.
- 51 Ackerman C, Locke AE, Feingold E, *et al.* An excess of deleterious variants in VEGF-A pathway genes in Down-syndrome-associated atrioventricular septal defects. *Am J Hum Genet* 2012;91:646–59.
- 52 Luxán G, D'Amato G, MacGrogan D, *et al.* Endocardial Notch signaling in cardiac development and disease. *Circ Res* 2016;118:e1–18.
- 53 Gillers BS, Chiplunkar A, Aly H, *et al.* Canonical Wnt signaling regulates atrioventricular junction programming and electrophysiological properties. *Circ Res* 2015;116:398–406.
- 54 Kodo K, Ong SG, Jahanbani F, *et al.* iPSC-derived cardiomyocytes reveal abnormal TGF- β signalling in left ventricular non-compaction cardiomyopathy. *Nat Cell Biol* 2016;18:1031–42.
- 55 Quarto N, Li S, Renda A, *et al.* Exogenous activation of BMP-2 signaling overcomes TGF β -mediated inhibition of osteogenesis in Marfan embryonic stem cells and Marfan patient-specific induced pluripotent stem cells. *Stem Cells* 2012;30:2709–19.
- 56 Roessler E, Ouspenskaia MV, Karkera JD, *et al.* Reduced nodal signaling strength via mutation of several pathway members including FoxH1 is linked to human heart defects and holoprosencephaly. *Am J Hum Genet* 2008;83:18–29.
- 57 Thomas NA, Koudijs M, van Eeden FJM, *et al.* Hedgehog signaling plays a cell-autonomous role in maximizing cardiac developmental potential. *Development* 2008;135:3789–99.
- 58 Tartaglia M, Mehler EL, Goldberg R, *et al.* Mutations in PTPN11, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. *Nat Genet* 2001;29:465–8.
- 59 Tartaglia M, Pennacchio LA, Zhao C, *et al.* Gain-of-function Sos1 mutations cause a distinctive form of Noonan syndrome. *Nat Genet* 2007;39:75–9.
- 60 Pandit B, Sarkozy A, Pennacchio LA, *et al.* Gain-of-function Raf1 mutations cause Noonan and LEOPARD syndromes with hypertrophic cardiomyopathy. *Nat Genet* 2007;39:1007–12.
- 61 Romano AA, Allanson JE, Dahlgren J, *et al.* Noonan syndrome: clinical features, diagnosis, and management guidelines. *Pediatrics* 2010;126:746–59.
- 62 Zhao W, Wang J, Shen J, *et al.* Mutations in VEGFA are associated with congenital left ventricular outflow tract obstruction. *Biochem Biophys Res Commun* 2010;396:483–8.
- 63 Li L, Krantz ID, Deng Y, *et al.* Alagille syndrome is caused by mutations in human Jagged1, which encodes a ligand for Notch1. *Nat Genet* 1997;16:243–51.
- 64 Oda T, Elkahloun AG, Pike BL, *et al.* Mutations in the human Jagged1 gene are responsible for Alagille syndrome. *Nat Genet* 1997;16:235–42.
- 65 McDaniel R, Warthen DM, Sanchez-Lara PA, *et al.* Notch2 mutations cause Alagille syndrome, a heterogeneous disorder of the Notch signaling pathway. *Am J Hum Genet* 2006;79:169–73.
- 66 Li B, Yu L, Liu D, *et al.* Mib1 mutations reduce Notch signaling activation and contribute to congenital heart disease. *Clin Sci* 2018;132:2483–91.
- 67 Preuss C, Capredon M, Wünnemann F, *et al.* Family based whole exome sequencing reveals the multifaceted role of Notch signaling in congenital heart disease. *PLoS Genet* 2016;12:e1006335.
- 68 Meester JAN, Southgate L, Stittrich AB, *et al.* Heterozygous loss-of-function mutations in Dll4 cause Adams-Oliver syndrome. *Am J Hum Genet* 2015;97:475–82.
- 69 Boskovski MT, Yuan S, Pedersen NB, *et al.* The heterotaxy gene GALNT11 glycosylates Notch to orchestrate cilia type and laterality. *Nature* 2013;504:456–9.
- 70 Jho EH, Zhang T, Domon C, *et al.* Wnt/beta-catenin/Tcf signaling induces the transcription of Axin2, a negative regulator of the signaling pathway. *Mol Cell Biol* 2002;22:1172–83.
- 71 Bosada FM, Devasthali V, Jones KA, *et al.* Wnt/ β -catenin signaling enables developmental transitions during valvulogenesis. *Development* 2016;143:1041–54.
- 72 Hulin A, Moore V, James JM, *et al.* Loss of AXIN2 results in impaired heart valve maturation and subsequent myxomatous valve disease. *Cardiovasc Res* 2017;113:40–51.
- 73 Hallaq H, Pinter E, Enciso J, *et al.* A null mutation of Hhex results in abnormal cardiac development, defective vasculogenesis and elevated VEGFA levels. *Development* 2004;131:5197–209.

- 74 Foley AC, Mercola M. Heart induction by Wnt antagonists depends on the homeodomain transcription factor Hex. *Genes Dev* 2005;19:387–96.
- 75 Mohamed IA, El-Badri N, Zaher A. Wnt signaling: the double-edged sword diminishing the potential of stem cell therapy in congenital heart disease. *Life Sci* 2019;239:116937.
- 76 Pardali E, Goumans MJ, ten Dijke P. Signaling by members of the TGF-beta family in vascular morphogenesis and disease. *Trends Cell Biol* 2010;20:556–67.
- 77 ten Dijke P, Arthur HM. Extracellular control of TGFbeta signalling in vascular development and disease. *Nat Rev Mol Cell Biol* 2007;8:857–69.
- 78 Guerri-Guttenberg RA, Castilla R, Francos GC, et al. Transforming growth factor β 1 and coronary intimal hyperplasia in pediatric patients with congenital heart disease. *Can J Cardiol* 2013;29:849–57.
- 79 Loeys BL, Mortier G, Dietz HC. Bone lessons from Marfan syndrome and related disorders: fibrillin, TGF-B and BMP at the balance of too long and too short. *Pediatr Endocrinol Rev* 2013;10:417–23.
- 80 Currier DG, Polk RC, Reeves RH. A sonic hedgehog (Shh) response deficit in trisomic cells may be a common denominator for multiple features of Down syndrome. *Prog Brain Res* 2012;197:223–36.
- 81 Petrova R, Garcia ADR, Joyner AL. Titration of Gli3 repressor activity by sonic hedgehog signaling is critical for maintaining multiple adult neural stem cell and astrocyte functions. *J Neurosci* 2013;33:17490–505.
- 82 Sund KL, Roelker S, Ramachandran V, et al. Analysis of Ellis van Creveld syndrome gene products: implications for cardiovascular development and disease. *Hum Mol Genet* 2009;18:1813–24.
- 83 Abdelhamed ZA, Whewy G, Szymanska K, et al. Variable expressivity of ciliopathy neurological phenotypes that encompass Meckel-Gruber syndrome and Joubert syndrome is caused by complex de-regulated ciliogenesis, Shh and Wnt signalling defects. *Hum Mol Genet* 2013;22:1358–72.
- 84 Tidyman WE, Rauhen KA. The RASopathies: developmental syndromes of Ras/MAPK pathway dysregulation. *Curr Opin Genet Dev* 2009;19:230–6.
- 85 Ching YH, Ghosh TK, Cross SJ, et al. Mutation in myosin heavy chain 6 causes atrial septal defect. *Nat Genet* 2005;37:423–8.
- 86 Granados-Riveron JT, Ghosh TK, Pope M, et al. Alpha-cardiac myosin heavy chain (MYH6) mutations affecting myofibril formation are associated with congenital heart defects. *Hum Mol Genet* 2010;19:4007–16.
- 87 Postma AV, van Engelen K, van de Meerakker J, et al. Mutations in the sarcomere gene MYH7 in Ebstein anomaly. *Circ Cardiovasc Genet* 2011;4:43–50.
- 88 Monserrat L, Hermida-Prieto M, Fernandez X, et al. Mutation in the alpha-cardiac actin gene associated with apical hypertrophic cardiomyopathy, left ventricular non-compaction, and septal defects. *Eur Heart J* 2007;28:1953–61.
- 89 Matsson H, Eason J, Bookwalter CS, et al. Alpha-cardiac actin mutations produce atrial septal defects. *Hum Mol Genet* 2008;17:256–65.
- 90 Zhu L, Vranckx R, Khau Van Kien P, et al. Mutations in myosin heavy chain 11 cause a syndrome associating thoracic aortic aneurysm/aortic dissection and patent ductus arteriosus. *Nat Genet* 2006;38:343–9.
- 91 Pucci L, Pointet A, Good JM, et al. A new variant in the MYH11 gene in a familial case of thoracic aortic aneurysm. *Ann Thorac Surg* 2020;109:e279–e281.
- 92 Moore-Morris T, van Vliet PP, Andelfinger G, et al. Role of epigenetics in cardiac development and congenital diseases. *Physiol Rev* 2018;98:2453–75.
- 93 Chamberlain AA, Lin M, Lister RL, et al. Dna methylation is developmentally regulated for genes essential for cardiogenesis. *J Am Heart Assoc* 2014;3:e000976.
- 94 Sperling S, Grimm CH, Dunkel I, et al. Identification and functional analysis of Cited2 mutations in patients with congenital heart defects. *Hum Mutat* 2005;26:575–82.
- 95 Xu M, Wu X, Li Y, et al. Cited2 mutation and methylation in children with congenital heart disease. *J Biomed Sci* 2014;21:7.
- 96 Hang CT, Yang J, Han P, et al. Chromatin regulation by BRG1 underlies heart muscle development and disease. *Nature* 2010;466:62–7.
- 97 Thibodeau IL, Xu J, Li Q, et al. Paradigm of genetic mosaicism and lone atrial fibrillation: physiological characterization of a connexin 43-deletion mutant identified from atrial tissue. *Circulation* 2010;122:236–44.
- 98 Gu R, Xu J, Lin Y, et al. The role of histone modification and a regulatory single-nucleotide polymorphism (rs2071166) in the Cx43 promoter in patients with TOF. *Sci Rep* 2017;7:10435.
- 99 Lyu G, Zhang C, Ling T, et al. Genome and epigenome analysis of monozygotic twins discordant for congenital heart disease. *BMC Genomics* 2018;19:428.
- 100 Grunert M, Dorn C, Cui H, et al. Comparative DNA methylation and gene expression analysis identifies novel genes for structural congenital heart diseases. *Cardiovasc Res* 2016;112:464–77.
- 101 Sheng W, Qian Y, Wang H, et al. Dna methylation status of Nkx2-5, GATA4 and HAND1 in patients with tetralogy of Fallot. *BMC Med Genomics* 2013;6:46.
- 102 Zhang J, Ma X, Wang H, et al. Elevated methylation of the RXRA promoter region may be responsible for its downregulated expression in the myocardium of patients with TOF. *Pediatr Res* 2014;75:588–94.
- 103 Gilsbach R, Preissl S, Grüning BA, et al. Dynamic DNA methylation orchestrates cardiomyocyte development, maturation and disease. *Nat Commun* 2014;5:5288.
- 104 Richmond TJ, Davey CA. The structure of DNA in the nucleosome core. *Nature* 2003;423:145–50.
- 105 Zaidi S, Choi M, Wakimoto H, et al. De novo mutations in histone-modifying genes in congenital heart disease. *Nature* 2013;498:220–3.
- 106 Barski A, Cuddapah S, Cui K, et al. High-resolution profiling of histone methylations in the human genome. *Cell* 2007;129:823–37.
- 107 Shimura K, Ura K, Shiratori H, et al. A histone H3 lysine 36 trimethyltransferase links Nkx2-5 to Wolf-Hirschhorn syndrome. *Nature* 2009;460:287–91.
- 108 Mysliwiec MR, Bresnick EH, Lee Y. Endothelial Jarid2/Jumonji is required for normal cardiac development and proper Notch1 expression. *J Biol Chem* 2011;286:17193–204.
- 109 Mysliwiec MR, Carlson CD, Tietjen J, et al. Jarid2 (Jumonji, at rich interactive domain 2) regulates Notch1 expression via histone modification in the developing heart. *J Biol Chem* 2012;287:1235–41.
- 110 Miller SA, Huang AC, Miazgowiec MM, et al. Coordinated but physically separable interaction with H3K27-demethylase and H3K4-methyltransferase activities are required for T-box protein-mediated activation of developmental gene expression. *Genes Dev* 2008;22:2980–93.
- 111 Zhang M, Li FX, Liu XY, et al. Tbx1 loss-of-function mutation contributes to congenital conotruncal defects. *Exp Ther Med* 2018;15:447–53.
- 112 Chen L, Fulcoli FG, Ferrentino R, et al. Transcriptional control in cardiac progenitors: Tbx1 interacts with the BAF chromatin remodeling complex and regulates Wnt5a. *PLoS Genet* 2012;8:e1002571.
- 113 Lange M, Kaynak B, Forster UB, et al. Regulation of muscle development by DPFF3, a novel histone acetylation and methylation reader of the BAF chromatin remodeling complex. *Genes Dev* 2008;22:2370–84.
- 114 Jiang YZ, Manduchi E, Jiménez JM, et al. Endothelial epigenetics in biomechanical stress: disturbed flow-mediated epigenomic plasticity in vivo and in vitro. *Arterioscler Thromb Vasc Biol* 2015;35:1317–26.
- 115 Han Y, Tanios F, Reeps C, et al. Histone acetylation and histone acetyltransferases show significant alterations in human abdominal aortic aneurysm. *Clin Epigenetics* 2016;8:3.
- 116 Matthis AL, Zhang B, Denson LA, et al. Importance of the evaluation of N-acetyltransferase enzyme activity prior to 5-aminosalicylic acid medication for ulcerative colitis. *Inflamm Bowel Dis* 2016;22:1793–802.
- 117 Lewandowski SL, Janardhan HP, Trivedi CM. Histone deacetylase 3 coordinates deacetylase-independent epigenetic silencing of transforming growth factor- β 1 (TGF- β 1) to orchestrate second heart field development. *J Biol Chem* 2015;290:27067–89.
- 118 Chang S, McKinsey TA, Zhang CL, et al. Histone deacetylases 5 and 9 govern responsiveness of the heart to a subset of stress signals and play redundant roles in heart development. *Mol Cell Biol* 2004;24:8467–76.
- 119 Guise AJ, Cristea IM. Approaches for studying the subcellular localization, interactions, and regulation of histone deacetylase 5 (HDAC5). *Methods Mol Biol* 2016;1436:47–84.
- 120 Cheng HL, Mostoslavsky R, Saito S, Saito S, et al. Developmental defects and p53 hyperacetylation in Sir2 homolog (SIRT1)-deficient mice. *Proc Natl Acad Sci U S A* 2003;100:10794–9.
- 121 Park CY, Pierce SA, von Drehle M, et al. skNAC, a Smyd1-interacting transcription factor, is involved in cardiac development and skeletal muscle growth and regeneration. *Proc Natl Acad Sci U S A* 2010;107:20750–5.



- 122 Ow JR, Palanichamy Kala M, Rao VK, *et al.* G9A inhibits MEF2C activity to control sarcomere assembly. *Sci Rep* 2016;6:34163.
- 123 Chen L, Ma Y, Kim EY, *et al.* Conditional ablation of EZH2 in murine hearts reveals its essential roles in endocardial cushion formation, cardiomyocyte proliferation and survival. *PLoS One* 2012;7:e31005.
- 124 He HH, Meyer CA, Shin H, *et al.* Nucleosome dynamics define transcriptional enhancers. *Nat Genet* 2010;42:343–7.
- 125 Lickert H, Takeuchi JK, Von Both I, Both V I, *et al.* Baf60C is essential for function of BAF chromatin remodelling complexes in heart development. *Nature* 2004;432:107–12.
- 126 Takeuchi JK, Lou X, Alexander JM, *et al.* Chromatin remodelling complex dosage modulates transcription factor function in heart development. *Nat Commun* 2011;2:187.
- 127 Ohtani K, Zhao C, Dobreva G, *et al.* Jmjd3 controls mesodermal and cardiovascular differentiation of embryonic stem cells. *Circ Res* 2013;113:856–62.
- 128 Lee S, Lee JW, Lee SK. Utx, a histone H3-lysine 27 demethylase, acts as a critical switch to activate the cardiac developmental program. *Dev Cell* 2012;22:25–37.
- 129 Wan X, Liu L, Ding X, *et al.* Mll2 controls cardiac lineage differentiation of mouse embryonic stem cells by promoting H3K4me3 deposition at cardiac-specific genes. *Stem Cell Rev Rep* 2014;10:643–52.
- 130 Ng SB, Bigham AW, Buckingham KJ, *et al.* Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. *Nat Genet* 2010;42:790–3.
- 131 Tsai MC, Manor O, Wan Y, *et al.* Long noncoding RNA as modular scaffold of histone modification complexes. *Science* 2010;329:689–93.
- 132 Dueñas A, Expósito A, Aranega A, *et al.* The role of non-coding RNA in congenital heart diseases. *J Cardiovasc Dev Dis* 2019;6. doi:10.3390/jcdd6020015. [Epub ahead of print: 01 Apr 2019].
- 133 Zhao Y, Samal E, Srivastava D. Serum response factor regulates a muscle-specific microRNA that targets HAND2 during cardiogenesis. *Nature* 2005;436:214–20.
- 134 Zhao Y, Ransom JF, Li A, *et al.* Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1-2. *Cell* 2007;129:303–17.
- 135 Li J, Cao Y, Ma XJ, *et al.* Roles of miR-1-1 and miR-181c in ventricular septal defects. *Int J Cardiol* 2013;168:1441–6.
- 136 Chen JF, Mandel EM, Thomson JM, *et al.* The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nat Genet* 2006;38:228–33.
- 137 Chinchilla A, Lozano E, Daimi H, *et al.* MicroRNA profiling during mouse ventricular maturation: a role for miR-27 modulating MEF2C expression. *Cardiovasc Res* 2011;89:98–108.
- 138 Wang L, Tian D, Hu J, *et al.* MiRNA-145 regulates the development of congenital heart disease through targeting FXN. *Pediatr Cardiol* 2016;37:629–36.
- 139 Nagy O, Baráth S, Ujfalusi A. The role of microRNAs in congenital heart disease. *EJIFCC* 2019;30:165–78.
- 140 Yu Z, Han S, Hu P, *et al.* Potential role of maternal serum microRNAs as a biomarker for fetal congenital heart defects. *Med Hypotheses* 2011;76:424–6.
- 141 Zhu S, Cao L, Zhu J, *et al.* Identification of maternal serum microRNAs as novel non-invasive biomarkers for prenatal detection of fetal congenital heart defects. *Clin Chim Acta* 2013;424:66–72.
- 142 Jiang Y, Zhuang J, Lin Y, *et al.* Long noncoding RNA SNHG6 contributes to ventricular septal defect formation via negative regulation of miR-101 and activation of Wnt/ β -catenin pathway. *Pharmazie* 2019;74:23–8.
- 143 Li Q, Zhu W, Zhang B, *et al.* The MALAT1 gene polymorphism and its relationship with the onset of congenital heart disease in Chinese. *Biosci Rep* 2018;38. doi:10.1042/BSR20171381. [Epub ahead of print: 29 May 2018].
- 144 Li H, Jiang L, Yu Z, *et al.* The role of a novel long noncoding RNA TUC40- in cardiomyocyte induction and maturation in P19 cells. *Am J Med Sci* 2017;354:608–16.
- 145 Wang B, Shi G, Zhu Z, *et al.* Sexual difference of small RNA expression in tetralogy of Fallot. *Sci Rep* 2018;8:12847.
- 146 Gu M, Zheng A, Tu W, *et al.* Circulating lncRNAs as novel, non-invasive biomarkers for prenatal detection of fetal congenital heart defects. *Cell Physiol Biochem* 2016;38:1459–71.

3/17/2021

RightsLink Printable License

**OXFORD UNIVERSITY PRESS LICENSE
TERMS AND CONDITIONS**

Mar 17, 2021

This Agreement between Department of Genetic and Metabolic Diseases, The Children's Hospital, Zhejiang University School of Medicine -- Yue Wu ("You") and Oxford University Press ("Oxford University Press") consists of your license details and the terms and conditions provided by Oxford University Press and Copyright Clearance Center.

The publisher has provided special terms related to this request that can be found at the end of the Publisher's Terms and Conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

License Number 5025060940256

License date Mar 09, 2021

Licensed Content Publisher Oxford University Press

Licensed Content Publication European Heart Journal

Licensed Content Title 2020 ESC Guidelines for the management of adult congenital heart disease: The Task Force for the management of adult congenital heart disease of the European Society of Cardiology (ESC)

Licensed Content Author Baumgartner, Helmut; De Backer, Julie

Licensed Content Date Aug 29, 2020

Type of Use Journal

3/17/2021

RightsLink Printable License

Requestor type Educational Institution/Non-commercial/ Not for-profit

Pharmaceutical support or sponsorship for this project No

Format Print and electronic

Portion Figure/table

Number of figures/tables 1

Will you be translating? No

Circulation/distribution 1

Title of new article Genetic and Epigenetic Mechanisms in the Development of Congenital Heart Diseases

Lead author Yue Wu

Title of targeted journal World Journal of Pediatric Surgery

Publisher BMJ

Expected publication date Mar 2021

Portions Table 4. Classification of congenital heart disease complexity

Requestor Location Department of Genetic and Metabolic Diseases, The Children's Hospital, Zhejiang University School of Medicine
No 3333, Binsheng Road

Hangzhou, 310052
China

3/17/2021

RightsLink Printable License

Attn: Department of Genetic and Metabolic Diseases, The
Children's Hospital, Zhejiang University School of Medicine

Publisher Tax ID GB125506730

Billing Type Credit Card

Credit card info Master Card ending in 1493

Credit card expiration 05/2024

Total 253.50 USD

Terms and Conditions

**STANDARD TERMS AND CONDITIONS FOR REPRODUCTION OF MATERIAL
FROM AN OXFORD UNIVERSITY PRESS JOURNAL**

1. Use of the material is restricted to the type of use specified in your order details.
2. This permission covers the use of the material in the English language in the following territory: world. If you have requested additional permission to translate this material, the terms and conditions of this reuse will be set out in clause 12.
3. This permission is limited to the particular use authorized in (1) above and does not allow you to sanction its use elsewhere in any other format other than specified above, nor does it apply to quotations, images, artistic works etc that have been reproduced from other sources which may be part of the material to be used.
4. No alteration, omission or addition is made to the material without our written consent. Permission must be re-cleared with Oxford University Press if/when you decide to reprint.
5. The following credit line appears wherever the material is used: author, title, journal, year, volume, issue number, pagination, by permission of Oxford University Press or the sponsoring society if the journal is a society journal. Where a journal is being published on behalf of a learned society, the details of that society must be included in the credit line.
6. For the reproduction of a full article from an Oxford University Press journal for whatever purpose, the corresponding author of the material concerned should be informed of the proposed use. Contact details for the corresponding authors of all Oxford University Press journal contact can be found alongside either the abstract or full text of the article concerned, accessible from www.oxfordjournals.org Should there be a problem clearing these rights, please contact journals.permissions@oup.com
7. If the credit line or acknowledgement in our publication indicates that any of the figures, images or photos was reproduced, drawn or modified from an earlier source it will be

3/17/2021

RightsLink Printable License

necessary for you to clear this permission with the original publisher as well. If this permission has not been obtained, please note that this material cannot be included in your publication/photocopies.

8. While you may exercise the rights licensed immediately upon issuance of the license at the end of the licensing process for the transaction, provided that you have disclosed complete and accurate details of your proposed use, no license is finally effective unless and until full payment is received from you (either by Oxford University Press or by Copyright Clearance Center (CCC)) as provided in CCC's Billing and Payment terms and conditions. If full payment is not received on a timely basis, then any license preliminarily granted shall be deemed automatically revoked and shall be void as if never granted. Further, in the event that you breach any of these terms and conditions or any of CCC's Billing and Payment terms and conditions, the license is automatically revoked and shall be void as if never granted. Use of materials as described in a revoked license, as well as any use of the materials beyond the scope of an unrevoked license, may constitute copyright infringement and Oxford University Press reserves the right to take any and all action to protect its copyright in the materials.

9. This license is personal to you and may not be sublicensed, assigned or transferred by you to any other person without Oxford University Press's written permission.

10. Oxford University Press reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

11. You hereby indemnify and agree to hold harmless Oxford University Press and CCC, and their respective officers, directors, employs and agents, from and against any and all claims arising out of your use of the licensed material other than as specifically authorized pursuant to this license.

12. Other Terms and Conditions: -Permission is granted on the basis that no pharmaceutical or medical product companies are directly involved or sponsoring this reuse. -Inclusion under a Creative Commons license or any other Open Access license allowing onward reuse is prohibited. -The credit line and any copyright/ disclaimer notices provided by OUP shall be included on the same page as the Material: Baumgartner H, De Backer J, Babu-Narayan SV, et al. 2020 ESC Guidelines for the management of adult congenital heart disease: The Task Force for the management of adult congenital heart disease of the European Society of Cardiology (ESC), European Heart Journal 2020; 1-83 doi:10.1093/eurheartj/ehaa554. Reprinted by permission of Oxford University Press on behalf of the European Society of Cardiology.

v1.4

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

3/17/2021

RightsLink Printable License

**OXFORD UNIVERSITY PRESS LICENSE
TERMS AND CONDITIONS**

Mar 17, 2021

This Agreement between Department of Genetic and Metabolic Diseases, The Children's Hospital, Zhejiang University School of Medicine -- Yue Wu ("You") and Oxford University Press ("Oxford University Press") consists of your license details and the terms and conditions provided by Oxford University Press and Copyright Clearance Center.

The publisher has provided special terms related to this request that can be found at the end of the Publisher's Terms and Conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

License Number	5025060940256
License date	Mar 09, 2021
Licensed Content Publisher	Oxford University Press
Licensed Content Publication	European Heart Journal
Licensed Content Title	2020 ESC Guidelines for the management of adult congenital heart disease: The Task Force for the management of adult congenital heart disease of the European Society of Cardiology (ESC)
Licensed Content Author	Baumgartner, Helmut; De Backer, Julie
Licensed Content Date	Aug 29, 2020
Type of Use	Journal

3/17/2021

RightsLink Printable License

Requestor type Educational Institution/Non-commercial/ Not for-profit

Pharmaceutical support or sponsorship for this project No

Format Print and electronic

Portion Figure/table

Number of figures/tables 1

Will you be translating? No

Circulation/distribution 1

Title of new article Genetic and Epigenetic Mechanisms in the Development of Congenital Heart Diseases

Lead author Yue Wu

Title of targeted journal World Journal of Pediatric Surgery

Publisher BMJ

Expected publication date Mar 2021

Portions Table 4. Classification of congenital heart disease complexity

Requestor Location Department of Genetic and Metabolic Diseases, The Children's Hospital, Zhejiang University School of Medicine
No 3333, Binsheng Road

Hangzhou, 310052
China

3/17/2021

RightsLink Printable License

Attn: Department of Genetic and Metabolic Diseases, The
Children's Hospital, Zhejiang University School of Medicine

Publisher Tax ID GB125506730

Billing Type Credit Card

Credit card info Master Card ending in 1493

Credit card expiration 05/2024

Total 253.50 USD

Terms and Conditions

**STANDARD TERMS AND CONDITIONS FOR REPRODUCTION OF MATERIAL
FROM AN OXFORD UNIVERSITY PRESS JOURNAL**

1. Use of the material is restricted to the type of use specified in your order details.
2. This permission covers the use of the material in the English language in the following territory: world. If you have requested additional permission to translate this material, the terms and conditions of this reuse will be set out in clause 12.
3. This permission is limited to the particular use authorized in (1) above and does not allow you to sanction its use elsewhere in any other format other than specified above, nor does it apply to quotations, images, artistic works etc that have been reproduced from other sources which may be part of the material to be used.
4. No alteration, omission or addition is made to the material without our written consent. Permission must be re-cleared with Oxford University Press if/when you decide to reprint.
5. The following credit line appears wherever the material is used: author, title, journal, year, volume, issue number, pagination, by permission of Oxford University Press or the sponsoring society if the journal is a society journal. Where a journal is being published on behalf of a learned society, the details of that society must be included in the credit line.
6. For the reproduction of a full article from an Oxford University Press journal for whatever purpose, the corresponding author of the material concerned should be informed of the proposed use. Contact details for the corresponding authors of all Oxford University Press journal contact can be found alongside either the abstract or full text of the article concerned, accessible from www.oxfordjournals.org Should there be a problem clearing these rights, please contact journals.permissions@oup.com
7. If the credit line or acknowledgement in our publication indicates that any of the figures, images or photos was reproduced, drawn or modified from an earlier source it will be

3/17/2021

RightsLink Printable License

necessary for you to clear this permission with the original publisher as well. If this permission has not been obtained, please note that this material cannot be included in your publication/photocopies.

8. While you may exercise the rights licensed immediately upon issuance of the license at the end of the licensing process for the transaction, provided that you have disclosed complete and accurate details of your proposed use, no license is finally effective unless and until full payment is received from you (either by Oxford University Press or by Copyright Clearance Center (CCC)) as provided in CCC's Billing and Payment terms and conditions. If full payment is not received on a timely basis, then any license preliminarily granted shall be deemed automatically revoked and shall be void as if never granted. Further, in the event that you breach any of these terms and conditions or any of CCC's Billing and Payment terms and conditions, the license is automatically revoked and shall be void as if never granted. Use of materials as described in a revoked license, as well as any use of the materials beyond the scope of an unrevoked license, may constitute copyright infringement and Oxford University Press reserves the right to take any and all action to protect its copyright in the materials.

9. This license is personal to you and may not be sublicensed, assigned or transferred by you to any other person without Oxford University Press's written permission.

10. Oxford University Press reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

11. You hereby indemnify and agree to hold harmless Oxford University Press and CCC, and their respective officers, directors, employs and agents, from and against any and all claims arising out of your use of the licensed material other than as specifically authorized pursuant to this license.

12. Other Terms and Conditions: -Permission is granted on the basis that no pharmaceutical or medical product companies are directly involved or sponsoring this reuse. -Inclusion under a Creative Commons license or any other Open Access license allowing onward reuse is prohibited. -The credit line and any copyright/ disclaimer notices provided by OUP shall be included on the same page as the Material: Baumgartner H, De Backer J, Babu-Narayan SV, et al. 2020 ESC Guidelines for the management of adult congenital heart disease: The Task Force for the management of adult congenital heart disease of the European Society of Cardiology (ESC), *European Heart Journal* 2020; 1-83 doi:10.1093/eurheartj/ehaa554. Reprinted by permission of Oxford University Press on behalf of the European Society of Cardiology.

v1.4

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.