

REVIEW

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Congenital heart diseases: genetics, non-inherited risk factors, and signaling pathways

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Abstract

Background: Congenital heart diseases (CHDs) are the most common congenital anomalies with an estimated prevalence of 8 in 1000 live births. CHDs occur as a result of abnormal embryogenesis of the heart. Congenital heart diseases are associated with significant mortality and morbidity. The damage of the heart is irreversible due to a lack of regeneration potential, and usually, the patients may require surgical intervention. Studying the developmental biology of the heart is essential not only in understanding the mechanisms and pathogenesis of congenital heart diseases but also in providing us with insight towards developing new preventive and treatment methods.

Main body: The etiology of congenital heart diseases is still elusive. Both genetic and environmental factors have been implicated to play a role in the pathogenesis of the diseases. Recently, cardiac transcription factors, cardiac-specific genes, and signaling pathways, which are responsible for early cardiac morphogenesis have been extensively studied in both human and animal experiments but leave much to be desired. The discovery of novel genetic methods such as next generation sequencing and chromosomal microarrays have led to further study the genes, non-coding RNAs and subtle chromosomal changes, elucidating their implications to the etiology of congenital heart diseases. Studies have also implicated non-hereditary risk factors such as rubella infection, teratogens, maternal age, diabetes mellitus, and abnormal hemodynamics in causing CHDs. These etiological factors raise questions on multifactorial etiology of CHDs. It is therefore important to endeavor in research based on finding the causes of CHDs. Finding causative factors will enable us to plan intervention strategies and mitigate the consequences associated with CHDs. This review, therefore, puts forward the genetic and non-genetic causes of congenital heart diseases. Besides, it discusses crucial signaling pathways which are involved in early cardiac morphogenesis. Consequently, we aim to consolidate our knowledge on multifactorial causes of CHDs so as to pave a way for further research regarding CHDs.

Conclusion: The multifactorial etiology of congenital heart diseases gives us a challenge to explicitly establishing specific causative factors and therefore plan intervention strategies. More well-designed studies and the use of novel genetic technologies could be the way through the discovery of etiological factors implicated in the pathogenesis of congenital heart diseases.

Keywords: Congenital heart diseases, Long non-coding RNAs, MicroRNAs, Cardiac transcription factor genes, Copy number variants, Signaling pathways

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Background

Congenital heart diseases (CHDs) are the most frequent congenital anomalies among infants and account for approximately 4–10 in 1000 live births [1]. The most common congenital heart defects are ventricular septal defect (VSD), atrial septal defects (ASD), transposition of greater vessels (TGV), patent ductus arteriosus (PDA), and tetralogy of Fallot (TOF) [2]. The prevalence of CHDs varies globally [3–15]. Congenital heart defects are classified clinically, as cyanotic and acyanotic [16]. Bluish discoloration of mucous membrane clinically characterizes cyanotic heart defects due to an increased level of deoxygenated hemoglobin. Therefore, cyanotic congenital heart defects are regarded as the most severe forms of CHDs [17].

Clinical presentations and severity of CHDs depend on their types or sub-types. However, most children with CHDs present with failure to thrive, cough, repeated chest infections, difficulty in breathing, exercise intolerance, and bluish discoloration of mucous membranes (cyanosis) [18]. Congenital heart defects are associated with serious complications such as cognitive impairment [19–27] and often affect families and individuals both emotionally and financially [23, 28–31].

Recent improvements of surgical and diagnostic procedures [32–34] have improved the survival of patients with congenital heart diseases; however, still significant mortalities among infants associated with CHDs are observed [13, 14, 35–37]. The mortality is higher for low birth weight infants even after surgical intervention [38].

Several studies have been conducted to investigate the etiology of CHDs, but the molecular etiology and mechanisms leading to CHD are still the subject of debate. Nonetheless, advances in molecular techniques give the possibility to study the developmental defects of the heart, thus closing the gap of knowledge between the morphology and genetics [39]. Genetic factors are postulated to play a significant role in the pathogenesis of CHDs [40].

Point mutations of cardiac transcription factor genes, single nucleotide polymorphism (SNPs), aneuploidy, and chromosomal copy number variants (CNV) are directly associated with CHDs [41–43]. Similarly, mutations in genes encoding for receptors and ligands, which are responsible for cardiac morphogenesis signaling pathways such as Notch and Jagged respectively are implicated in the etiology of CHDs [44–46].

Several well-established cardiac transcription factors that are highly expressed in cardiogenic plates such as NKX2.5, GATA4, and TBX5 have been extensively studied in both human [47] and animal experiments [48, 49]. Mutations in these cardiac transcription factors are associated with most non-syndromic CHDs [50]. Functional studies on NKX2.5, GATA4, and TBX5 in animal experiments have indicated high reproducibility of the results

[51], suggesting the monogenic inheritance model of CHD pathogenesis. However, this model (monogenic inheritance) of CHDs pathogenesis raises two important questions; firstly, why do we observe different CHDs phenotypes associated with the same type of single-gene mutations? Secondly, why do we observe the same kind of CHDs phenotypes in different single-gene mutations? These two questions suggest there might be many molecules (multifactorial inheritance) involved in the etiology of CHDs.

Perhaps the most intriguing feature is that the prevalence of the CHDs remains the same notwithstanding the decrease in reproductive potential of the patients with CHDs [52, 53], suggesting that the mutations might not be inherited but rather *de novo* [54, 55]. Evolutionarily, we would expect the prevalence of CHDs to be decreasing as the negative selection could have removed the inherited mutation. The reasons for the expected decrease in prevalence being the high mortality rate associated with CHDs and the decrease in reproductive potential among CHD patients.

The questions also remain if the CHDs mode of inheritance is a familial and whether its mode of inheritance is autosomal dominant or autosomal recessive. This enigma is highlighted by the fact that the autosomal dominant mutation is usually expressed in high penetrance, and we would expect the high percentage of first degree relatives to acquire the CHDs phenotypes, but the study indicates the opposite [56]. The autosomal recessive fashion might be the most appropriate model to define the hereditary bases of CHDs. Studies show that the consanguineous marriages resulted in an increased risk of CHDs [57, 58].

Genomic wide association studies (GWAS) involve the comparison of genetic variants of different affected individuals within the society and whether the variants are associated with a certain trait. GWAS, therefore, can be used to detect multiple genetic risk factors which contribute to congenital heart diseases [59]. CHDs being heterogeneous group of diseases, the use GWAS may give us an insight to the etiology of CHDs [60].

Understanding of the hereditary causes will provide us with an insight to the biological basis of the congenital heart defects; therefore, allow the definition of disease risk, which is the base of disease prevention. It will also illuminate not only the developmental biology of the heart, but also plans for the possibility of future novel treatment of congenital heart diseases. Studying the etiology of CHDs is essential because it may predict clinical outcomes, get to know genetic reproductive risks, and screen for genetic risk factors within the families. Also, it is important to investigate genotype-phenotype correlation as it can provide us with an opportunity to predict the prognosis. Previous studies show that patients with

CHDs may develop pulmonary hypertension (PHT), and certain types of mutations are associated with its rapid development [61, 62]. In this case, early intervention is required.

Main text

Embryogenesis of the heart

The heart develops from the mesoderm [63]. It is the first organ to develop and starts beating at approximately 22 days of gestation [64]. Its development (embryogenesis) in early days of fetal life involves a series of events which include the following: precardiac cell migration from primitive streak, the formation of 2 primitive heart tubes, fusion of primitive tubes, cardiac looping, cardiac septation, cardiac chamber formation, formation of cardiac conducting system, and the coronary arteries [65–67].

The developmental process of the heart is a tightly regulated process that requires intricate interplay between transcription factors, several cardiac-specific genes, and signaling pathways [68]. Both genetic [69, 70] and environmental factors [71] play essential roles during cardiac development. Gene mutations [72] and some teratogens [73, 74] can interfere with normal development (embryogenesis) of the heart leading to congenital heart defects.

Endodermal delivered signals such as BMP2, FGF8, crescent, and Shh act as inducers of cardiac mesoderm while inhibitory signals such as chordin, noggin, wnt1, and serrate (ser) are mesodermal delivered [75, 76].

The heart looping, chamber formation, and left to right asymmetry are under the control of cardiogenic plate expressing genes NKX2.5, GATA, HANDS, and TBX5 [77]. During early cardiac development, NKX2.5 [78] and MHC2A [79] genes are essential, and in later stages, MEF2 [80], HAND1, and HAND2 genes [81, 82] are actively transcribed. The regulation process during cardiac development is under the control of transcription factor genes GATA and TBX5, growth factors such as VEGF, FGF, and PDGF, and morphogenic proteins (BMP2, BMP4, BMP5) [79]. Besides, the Foxf genes which control the second heart field in the hedgehog pathway through interaction with TBX are actively involved [83].

Transcription factors genes, cardiac-specific genes, and CHDs

NKX2.5, GATA4, GATA6, and TBX5 are the most studied transcription factors genes. These genes are the key transcription factors which are involved in early cardiac development [84, 85]. Studies have revealed mutations and single nucleotide polymorphism (SNPs) in these essential transcription factor genes result in CHDs occurrence [47, 86, 87]. In one large cohort study, they identified the novel mutations in NKX2.5 and GATA4, both mutations were associated with mutations of

MYH6 gene (encoding for cardiac (alpha) myosin), suggesting additive effect to the pathogenesis of CHDs [88]. Mutation in MYH6 is associated with familial atrial septal defect (ASD) [89]. A study among Chinese identified the missense mutations of GATA4 in patients with tetralogy of Fallot and membranous ventral septal defect. They also identified NKX2.5 mutations in patients with isthmus stenosis, ventral septal defect, and patent ductus arteriosus [90].

NKX2.5, a homeodomain transcription factor, is vital in early cardiogenesis in mammals [91]. Mutations in NKX2.5 have been reported in patients with ventral septal defect [92], tetralogy of Fallot, transposition of greater vessels, and patent ductus arteriosus [93], atrial septal defect and hypoplastic left heart syndrome (HLHS) [94]. The association of mutations with clinical manifestations is of paramount importance; NKX2.5 mutation associated ASD was found to present with cardiac conduction defects; thus, patients with mutation in NKX2.5 have an increased risk of cardiac arrest [95]. Overall, more than 40 NKX2.5 transcription factor different mutations have been identified, causing impaired protein function [96] and have a negative impact on transcriptional activity [97].

MEF2C, NKX2.5, GATA4, and GATA6 mutations have been identified in patients with cardiac outflow tract defect (OFT). Studies on the functional analysis have documented significant effect on their protein functions affecting early cardiac development [50]. MEF2C is an essential factor for cardiac development and its loss of function due to mutations results in double outlet ventricle, ventricular septal defects, and patent ductus arteriosus [98, 99].

GATA4, a zinc finger transcription factor, is expressed during cardiac development as well as in adult cardiomyocytes [100]. GATA4 mutations produce GATA4 protein which reduces cardiomyocytes proliferation in vivo, causing functional deficit [101]. GATA4 mutation is associated with cardioseptal defect [102], some of which are familial [103]. GATA4 mutations were identified in patients with atrial and ventral septal defects but these mutations did not alter the transcriptional activity when combined with other transcription factors such as NKX2.5 and TBX20, suggesting that GATA4, as a single factor may not have a direct effect on the heart by itself [104].

TBX5 transcription factor is an essential regulator in cardiac development [105] as it plays a role in gastrulation process and organogenesis in vertebrates [106]. TBX5 mutation is common in patients with Holt-Oram syndrome, a syndrome which is characterized by skeletal abnormalities and heart diseases [54, 107, 108]. TBX5 mutations were found in patients with tetralogy of Fallot associated with large ostium secundum defect and features of upper limb deformities [109], and also in

patients with the ventricular septal defects [110]. Some studies indicate that it is not TBX5 mutations that cause CHDs but the abnormality in the TBX5 expression levels is responsible for congenital heart diseases [111].

Mutations in other several genes have been reported to be associated with congenital heart diseases. These genes include the following: ankyrin repeat domain 1 (ANKRD1) associated with septal defects, NR2F2 gene associated with double outlet ventricle, HAND2 gene associated with familial ventral septal defect, pulmonary stenosis, double outlet right ventricle, tetralogy of Fallot, and CASZ1 gene associated with the ventral septal defects [112–116].

Cardiac-specific genes are those genes which encode for cardiac-specific proteins. MYH7 encodes for beta myosin heavy chain and MYH11 encodes for the smooth muscle heavy chain. Mutation in MYH7 is associated with Ebstein anomaly, the condition which is characterized by the fusion of posterior and anterior leaflets of tricuspid valves and left ventricular non-compaction [117]. ACTC is another important gene which encodes for cardiac actin, changes in ACTC due to mutation results into disruption of actin composition of fetal heart leading to severe deficit in both function and structure of the heart [118, 119]. See summary in Table 1.

MicroRNAs and CHDs

MicroRNAs (miRNAs) are small non-coding RNA molecules (18~26 nucleotides) that regulate eukaryotic gene expression at the post-transcription level [120]. The microRNAs blocks gene expression by interacting with the 3UTR region of the messenger RNA [121]. They act as gene suppressors by inhibiting messenger RNA translation or cause the degradation of messenger RNA. MicroRNAs cause the RNA strand cleavage, shortening of polyA tail of messenger RNA, and making the

messenger RNA less efficient to be translated by ribosomes [122].

A single microRNA has an ability to interfere with multiple target genes; thus, perturb the entire network, which can eventually result in a serious pathological state [123]. miRNA exerts its canonical repressive effect by binding to Argonaute (AGO) proteins [124]. Recent studies have shown that miRNA can bind to other non-AGO RNA-binding proteins. miR-328 has been found to bind to heterogeneous nuclear ribonucleoproteins (rhRNPE2) within the CCAAT Enhancer Binding Protein Alpha (CEBPA) of mRNA mediating transcriptional inhibition [125].

Apart from interfering with the messenger RNA by its repressive activity, the miRNAs have other unconventional roles. These roles include up regulation of protein expression upon cell cycle arrest [126] and target mitochondrial transcript by inhibiting cytochrome c-oxidase subunit (MT-COX 1). In this case, miRNA-181c translocates into the mitochondria and exerts its action by enhancing (MT-COX 2) mRNA expression through miRNA-181c binding [127]. miRNAs can also activate Toll receptor (TLR). TLR plays a crucial role in innate immunity. Let-7 has been found to activate TLR7 causing neurodegeneration [128].

miRNAs play a crucial role in cardiac morphogenesis with regards to patterning, proliferation, and differentiation of myocardium [129]. Therefore, microRNAs are important therapeutic target and potential in regenerative medicine [130]. We can use miRNAs as biomarkers for the diagnosis of congenital heart diseases [131], for instance, miR-499 is regarded as a useful biomarker for congenital heart disease diagnosis [132]. miRNAs are significantly upregulated, and target genes that are important for cardiac development such as TBX5 gene [133] and NOTCH 1, HAND1, and GATA3 genes which

Table 1 Summary of genes involved in cardiac morphogenesis. Mutations of these important genes lead to various phenotypes of CHDs

Candidate gene	CHD phenotype	References
NKX2.5	Tetralogy of Fallot, patent ductus arteriosus, transposition of greater vessels, atrial septal defects with conduction defects, and hypoplastic left heart syndrome	[93–95]
MEF2C	Double outlet ventricle, ventricular septal defects, and patent ductus arteriosus	[98, 99]
GATA4	Atrial and ventral septal defects	[102, 104]
TBX5	Holt-Oram syndrome, ostium secundum defects, tetralogy of Fallot, and ventricular septal defects	[109, 110]
ANKRD1	Septal defects	[113]
NR2F2	Double outlet ventricle	[112]
HAND2	Familial ventral septal defect, pulmonary stenosis, double outlet ventricle, and tetralogy of Fallot	[115]
CASZ1	Ventral septal defects	[114]
MYH6	Familial atrial septal defects	[89]
MYH7	Ebstein anomaly and left ventricular non-compaction	[117]

are vital for the right ventricle development [134]. miRNA dysregulation is known to cause severe congenital anomaly of the heart such as hypoplastic left heart [135], a condition which is largely characterized by maldevelopment of the right side of the heart.

miRNA normally interact with the 3UTR site of the gene; thus, a single nucleotide polymorphism to the 3UTR site of cardiac transcription factor gene GATA4 is associated with congenital heart disease [136]. miRNA-145 targets the FXN gene (gene making a protein fra-taxin, which is abundant in the heart) by negatively regulating its function through apoptosis and mitochondrial function [137]. The downregulation of miRNA-199a-5p favors the unfolded protein response, thus protecting the myocardium against hypoxia-induced endoplasmic reticulum stress in patients with congenital heart disease and mechanistically by binding to GTP78 and ATP 6 changing their gene expression [138].

miRNA-84 has been found to be significantly down-regulated and associated with cyanotic congenital heart diseases; miRNA-84 decreases proliferation and increases the apoptosis of cardiomyocytes [139]. In a different study, it has been demonstrated that miRNA-1 promotes cardiomyocyte proliferation through activation of HANDS2 (a key gene which is involved in cardiac development) and suppression of apoptosis by decreasing the caspase 3-cleavage [140]. The roles of these small non-coding RNAs are stressed in a study which found miRNA-499 single nuclear polymorphism to be associated with congenital heart diseases. miRNA-499 inhibits the expression of methionine synthase (MTR), a key enzyme in folate metabolism, thus interfering with early development of the heart [141]. Which specific miRNAs are upregulated or downregulated in different types of CHDs and mechanisms on how miRNAs cause CHDs is still elusive. High throughput miRNA sequencing in malformed heart will provide insight to the novel miRNAs, which are essential in cardiac morphogenesis.

Long non-coding RNAs and CHDs

Long non-coding RNAs (LncRNAs) are made up of more than 200 nucleotides with no coding function and they are actively transcribed in metazoans [142]. LncRNAs have been reported to play various biological processes by controlling gene expression through histone modifications or epigenetics [143]. LncRNAs are linked to the development of congenital heart diseases, and therefore can be used as the potential biomarkers for prediction of occurrence of fetal CHD in pregnant mothers [144].

Post-transcriptional regulation during cardiac embryogenesis is an essential part of normal cardiac development [145]. Long non-coding RNA and miRNAs have been implicated to play an indispensable role during

cardiac development by influencing gene expression and post-transcription regulation [146]. Indeed long coding RNAs are essential in the regulation of mammalian cardiac morphogenesis and play a role in pathogenesis of cardiac tissue diseases [147].

Dysregulation of LncRNA in cardiac tissue was found among patients with VSDs which indicates that there is an association between LncRNA and congenital heart diseases [148]. Aberrant expression of LncRNAs was found in pregnant mothers with fetal congenital heart diseases which designate that LncRNAs play a significant role in the development of congenital heart disease [144]. LncRNA uc 4 over expression inhibits the TGF- β signaling pathway resulting in the development of congenital heart diseases [149]. Long non-coding RNAs play a role in controlling the transcription of HANDS 2 which plays an important role in guiding cardiac morphogenesis [115], therefore blocking the transcription of long non-coding RNA (Uph) and results in right ventricular hypoplasia and early embryonic lethality in mice [150].

Chromosomal abnormalities, copy number variants, and CHDs

Congenital heart defects are the most common congenital malformations found among patients with chromosomal aberrations. Studies have shown that congenital heart defects are associated with some forms of chromosomal abnormalities. The commonest form of aneuploidy, the Down syndrome (trisomy 21), is usually associated with atrial septal defect (ASDs) [151, 152]. The abnormalities in chromosomal number (aneuploidy) are often associated with an increased risk of congenital heart diseases. Many different cardiac phenotypes are associated with aneuploidy. About 50% of patients with trisomy 21 and Turner's syndrome (45, X) develop congenital heart diseases, and in Turner's syndrome premature mortality is caused by CHDs complications [153, 154]. In the case of other trisomies such as trisomy 18 and trisomy 13, the prevalence of CHDs is more than 50% of the cases [155].

Children with congenital heart defects have a high incidence of pathological copy number variants [156], implying that subtle chromosomal changes (copy number variants) play an essential role in the etiology of congenital heart defects. It is, therefore, necessary to find out the copy number variants as it can give us an insight to the etiology of CHDs [157].

Karyotyping and chromosomal microarray (CMA) methods have been used to identify the number of abnormal chromosomes and copy number variants (CMV), respectively [158–160]. These methods are used widely for the diagnosis of fetal aneuploidy. Prenatal diagnosis using these technologies can help to predict the fetal outcomes. Recently, the chromosomal microarrays and

next generation sequencing are reliable tools for investigating the genetic abnormalities in patients with congenital heart diseases [161, 162]. We can also identify the copy number variants in a specific genomic region by multiplex ligation-independent probe amplification [156, 163] and its modified form which is known for its high sensitivity and specificity [164].

CMA and karyotyping are the two screening methods which can supplement each other, and thus, negative karyotyping results should be re-examined by using chromosomal microarrays [165]. Studies have associated the occurrence of copy number variants and congenital heart diseases [166, 167] and other malformations such as that of limbs [168]. Copy number variants (microdeletion) were found in VSD patients with normal karyotyping results [169]. Therefore, it is vital to have CMA even in a fetus with normal karyotype.

Chromosomal microarrays (CMA) can be used to detect the pathological CNVs in patients with CHDs. The detection of pathogenic CNVs in syndromic CHD patients using CMA has been found to have the diagnostic yield of up to 20% [170]. CMA can detect both pathological CNV and those CNV of unknown significance (VOUS) [171]. Copy number variant (gene deletion and duplications) was found in a patient with heterotaxy, functional analysis on the variant genes revealed that the genes are involved in left to right patterning of the heart [172]. Microdeletion and microduplication are highly implicated in pathogenesis of CHDs, 240 CNVs were found in genes which are involved in cardiac development including, NRP1, NTRK3, MESP1, ADAM19, and HAND1 [173].

A cohort study found that the gain of more than 200 kb or losses of 100 kb in different cardiac-specific genes was associated with CHDs; significant enrichment were found in patients with tetralogy of Fallot, atrial ventricular septal defect, truncus arteriosus, subaortic stenosis, and atrial ventricular canal [174]. In patients with Down syndrome, not only the chromosomal number abnormalities are implicated in causing CHDs, but also the combination of copy number variants and single nucleotide polymorphism in certain specific genes may be found in the same chromosome. Another cohort study found that rs2832616 and rs1943950 SNPs and CNV in RIPK4 and ZBTB21 genes within chromosome 21 in a Down syndrome patient, suggesting multiple gene aberrations [175]. 22q11 deletion, 17p13.3, 4q35, and TBX1 deletions are highly associated with conotruncal defect abnormalities [176] and 3.76 Mb de novo gain of 9q34.2-q34.3 is associated with tetralogy of Fallot with the absence of pulmonary valve [177]. In addition, serious cardiac malformations have been documented due to duplication and deletion in GATA4 and SOX7 genes respectively [178] as well as in NODAL gene [179].

Signaling pathways and CHDs

Cardiogenesis is a complex process which requires early cardiac stem cell fate, proliferation differentiation, and organ formation. It is therefore important for the developing embryo to tightly regulate these processes through signaling pathways. Early normal cardiac morphogenesis depends on perfect regulation of signaling pathways, and thus, during cardiac development intracellular crosstalk is important for spatial-temporal precision [68]. During the development of the heart, Notch signaling, BMP, and TGF- β are critical pathways; they work together to promote epithelial-mesenchymal transition (EMT) as well as mesenchyme cell invasiveness [180].

NOTCH signaling pathway is a highly conserved pathway which plays an important role in early cardiac development; it is therefore important for ultimate cellular development, differentiation, proliferation, apoptosis, adhesion, and epithelial-mesenchymal transition [181]. Thus, cardiac progenitor cell ability to differentiate from the mesoderm to mature cardiomyocytes is under the control of the NOTCH signaling pathway. NOTCH is also crucial for the repair of mature cardiomyocytes after myocardial injury [125] and protects the heart from hypertrophic responses as well as the survival of cardiomyocytes [182]. Notch receptor interacts with the endocardium and neurocrest cells forming signals to regulate the morphogenesis of the heart, in particular, the formation of the endocardium during chamber and valve development as well as during valve formation. NOTCH signaling pathway promotes epithelial-mesenchymal transition and controls endocardium-myocardium signals leading to the formation of trabecular myocytes [183]. NOTCH signaling pathway also participates in outflow tract formation and trabecular compaction [184]. It is, therefore, essential to understand that any perturbation in this important signaling pathway may cause congenital heart disease.

Animal experiments have revealed that mutations of NOTCH receptor and ligands can lead to several different types of congenital malformations including the congenital heart defects. NOTCH 1 receptor and its ligands jagged 1 mutations are associated with congenital heart defects [185]. Alterations of the NOTCH signaling pathway lead to abnormal ventricular chamber development, non-compaction, and cardiomyopathy [186]. Novel mutations of NOTCH 1 have been identified in patients with severe forms of congenital heart disease, the hypoplastic left heart syndrome (HLHS) [187].

A study has shown that the induced pluripotent stem cells generated from the patient with hypoplastic left heart syndrome when allowed to differentiate into mature cardiomyocytes in vitro, show reduced NOTCH receptor expression, disorganization of sarcomere, and low beating rate [188]. Also, in the same study, they found

that the activation of NOTCH receptor through jagged ligand interaction restores the cardiomyocytes' ability to differentiate and beating rate as well as reduces smooth muscle formation [188]. iPcs generated from the HLHS show reduced expression of NKX2-5, TBX2, and NOTCH genes with a consequent decrease in their differentiation potential. Co-transfection with these promoters restores the potential of cells to differentiate [189].

TGF- β signaling pathway has been found to play an essential part in the development of embryonic myocardium. Mutation in TBX20 transcription factor gene causes abnormal signaling of TGF- β pathway in induced pluripotent stem cells delivered from left ventricular non-compaction cardiomyopathy [190]. β 2-spectrin is an adaptor of Smads and it plays an important role in TGF- β signaling pathway. Animal experiments have found that β 2-spectrin loss leads to inactivation of TGF- β signaling and developmental defects of the heart, particularly affecting the ventricular wall [191].

Non-inherited risk factors and CHDs

Most studies have been done on genetic risk factors and their association with congenital heart defects, therefore giving us the way to define disease risk. However, little information is available for the modifiable risk factors associated with congenital heart defects. Studies have shown non-inherited risk factors are associated with congenital heart defects. During the embryonic period, which normally ends at 8 weeks of gestation, mothers are advised to avoid substances that are deemed as teratogens [192, 193]. Advanced maternal age has been implicated to the etiology of congenital heart diseases possibly because age increases the chances of chromosomal aberrations; however, little evidence is available with regard to advanced maternal age in the absence of chromosomal abnormality [194, 195].

There is a strong correlation between drug exposure, viral infection, and conotruncal defects [196]. Congenital rubella infection is a risk factor for many malformations including the CHDs [197, 198]. Smoking during pregnancy has been found to increase the risk of congenital heart defects [199, 200] and women who smoke cigarettes have a substantial possibility of having children with atrial septal defects [201]. Maternal obesity is associated with increased risk of birth defects [202] and pregestational high body mass index has been found to be a risk factor for CHD development, particularly the left and right ventricular outflow tracts, septal defects, and hypoplastic left heart syndrome [203, 204]. Maternal hyperglycemia is also an important risk factor implicated to CHDs [205]. Besides, folic acid intake has been associated with many congenital defects but many studies have indicated that there is no association of folic acid intake

and CHDs [206–208]. With all these environmental factors implicated, in causing CHDs, it is therefore reasonable to say both genetic and environmental factors are being associated with congenital heart defects [209].

Conclusions

The etiology congenital heart disease is a diverse and interesting area of the study. CHD multifactorial etiology imposes a challenge in defining its pathogenesis. Large genomic wide association studies (GWAS), well-designed studies on non-inherited risk factors, and use of contemporary technologies such as high throughput sequencing may eventually pave away for understanding the genetic and non-genetic causes of congenital heart diseases.

Abbreviations

ASD: Atrial septal defects; CHDs: Congenital heart diseases; CMA: Chromosomal microarrays; CNV: Copy number variants; GWAS: Genomic wide association studies; HLHS: Hypoplastic left heart syndrome; LncRNAs: Long non-coding RNAs; miRNAs: MicroRNAs; PDA: Patent ductus arteriosus; PHT: Pulmonary hypertension; SNPs: Single nucleotide polymorphism; TGV: Transposition of greater vessels; TOF: Tetralogy of Fallot; VOUS: Variations of unknown significance; VSD: Ventricular septal defect

Acknowledgements

We thank the University of Shandong for providing facilities required for effective writing and submitting the article for publication.

Authors' contributions

All authors did participate in the whole process of writing a review. ES contributed the concept, manuscript drafting, manuscript writing, and literature review. LS provided the guidelines, manuscript corrections, and supervision. QX did the literature review and manuscript writing. AM did the reference sorting and writing the manuscript. All the authors have read and approved the manuscript.

Funding

Chinese Government Scholarship (CSC), funded by the Government of China.

Availability of data and materials

All the data and publications used as references in this review were cited from PubMed. We used the Endnote software version 8 for data management and referencing.

Ethics approval and consent to participate

Not applicable

Consent for publication

Consent to publish the review was provided by the Department of Anatomy, School of Basic Sciences, Shandong University.

Competing interests

The authors declare that they have no competing interests.

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Received: 21 October 2019 Accepted: 5 February 2020

Published online: 28 February 2020

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