

Congenital Heart Disease

Subjects: Cardiac & Cardiovascular Systems

Contributor: Francesco Nappi

The use of next-generation sequencing has provided new insights into the causes and mechanisms of congenital heart disease (CHD). Examinations of the whole exome sequence have detected detrimental gene variations modifying single or contiguous nucleotides, which are characterised as pathogenic based on statistical assessments of families and correlations with congenital heart disease, elevated expression during heart development, and reductions in harmful protein-coding mutations in the general population.

Keywords: congenital heart disease ; first heart field ; second heart field ; whole-exome sequencing ; whole-genome sequencing

1. Current Knowledge

Congenital heart disease is widely acknowledged as the most frequent and frequently serious abnormality present at birth, with a prevalence of 6–13 per 1000 newborn infants ^{[1][2][3][4][5][6]}. CHD encompasses a broad spectrum of heart abnormalities, ranging from a single defect, such as an atrial septal defect (ASD) or a ventricular septal defect (VSD), or an isolated dysplastic valve, to more complex disorders. Cardiac conditions such as tetralogy of Fallot (TOF) or hypoplastic left heart syndrome (HLHS) are characterised by multiple defects. **Figure 1** and **Figure 2** show the improved operative and management techniques that have led to a significant reduction in the mortality rate of CHD. These techniques are used to treat critical malformations that require early intervention for survival. Over 90% of CHD patients survive into adulthood ^[7], resulting in a higher prevalence of CHD in the general population. In addition, the extended lifespan of patients with CHD has prompted greater acknowledgement of the related comorbidities. Childhood extracardiac structural or functional abnormalities and neurodevelopmental delays occur in approximately 13% of newborns with CHD ^{[3][8]}. CHD is considered syndromic if other diagnoses are likely to have resulted from the same cause. Syndromic CHD often results in complex heart malformations that can cause lifelong health problems affecting various bodily systems.

Therefore, an understanding of the causes and underlying pathways of CHD can provide valuable information about both the healthy and abnormal development of multiple organs.

Genetics

Due to significant technical advances in human genome research, genetics has now been found to play a crucial role in the development of CHD. Evidence initially emerged from examining patients with syndromic CHD through karyotyping, which identified aneuploidies such as trisomy 13, 18, or 21 (Down syndrome) and monosomy X (Turner syndrome) in approximately 12% of CHD patients ^[9]. Cytogenetic analyses and genomic arrays have aided in the understanding of subchromosomal structural rearrangements. These analyses have highlighted frequent 3 Mb deletions, which are also associated with DiGeorge (velocardiofacial) syndrome ^{[10][11][12][13]}. Deletions of 22q11.2, as determined by fluorescence in situ hybridisation (FISH) ^{[14][15]} and targeted amplification ^[16], are present in approximately 2% of all cases of congenital heart disease and in 13% of individuals with selected heart malformations. About 25% of sporadic CHD cases are caused by the coexistence of karyotype or microarray-detected abnormalities ^{[17][18]}. The preliminary detection of the monogenic causes of familial CHD has been facilitated by genome-wide linkage causes of familial CHD, encompassing variations in genes encoding transcription factors and transcriptional regulators of genes involved in heart growth ^{[19][20]}. Enhanced comprehension of the human genome at the base pair level, along with advancements in sequencing technologies, has facilitated the identification of genes linked to CHD. Harmful variants, such as missense mutations, loss-of-function mutations (LOFs), small insertions or deletions, copy number variants (CNVs), and structural defects, can be identified using advanced whole-exome sequencing (WES) and whole-genome sequencing (WGS) platforms, along with bioinformatic tools and large sequencing datasets from population-based studies. These techniques have been used to identify harmful variants present in individuals with a family history of congenital heart disease, new mutations in CHD

subjects with healthy parents, and a significantly higher frequency of mutations in approximately 20% of CHD patients in large-cohort studies [18][19][20][21][22][23][24][25].

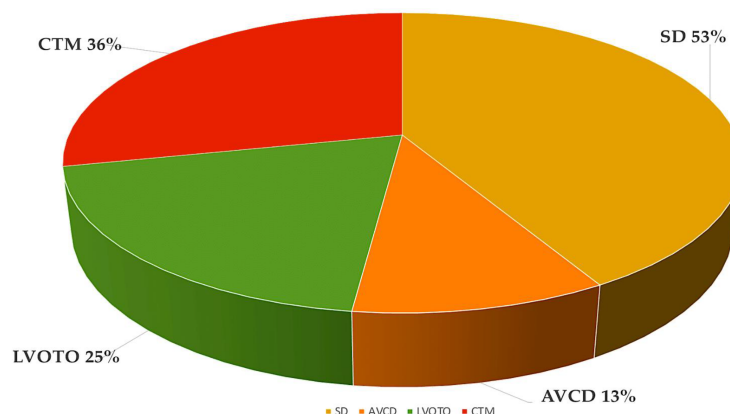


Figure 1. The most frequent forms of CHD from the Paediatric Cardiac Genomics Consortium, with percentages indicating the prevalence of the malformation in the patient population. It is important to note that the percentages exceed 100% as a result of concomitant structural cardiac abnormalities in the individuals. Abbreviations: AVCD, atrioventricular canal defect; CHD, congenital heart disease; CTM, conotruncal malformation; LVOTO, left ventricular outflow tract obstruction; SD, septal defect.

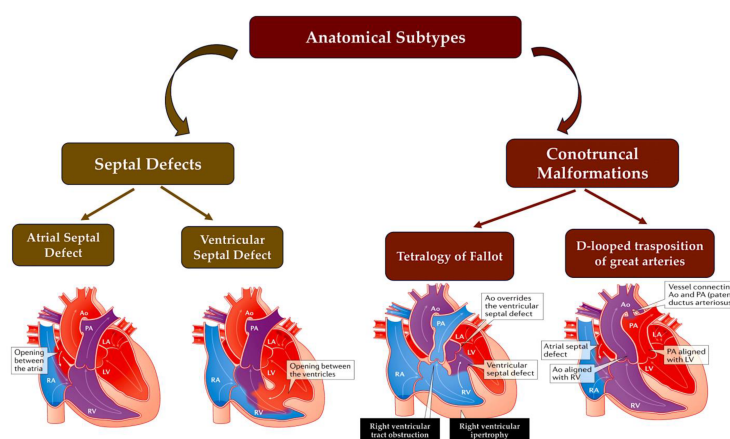


Figure 2. A simplified scheme of CHD defects divided according to anatomical subtype and their corresponding oxygen saturation levels. Anomalies in the communication between the left and right ventricles, such as ASD or VSD or atrioventricular canal defects, usually result in acyanotic states due to a left-to-right shunting of oxygen-rich blood into the pulmonary circulation. LVOTOs can range from isolated aortic stenosis to a combination of anomalies, such as hypoplastic left heart syndrome with mitral and aortic stenosis, which can cause cyanosis. Conotruncal malformations, such as TOF and TGV, are often associated with cyanosis due to the shunting of deoxygenated blood into the systemic circulation. This information is from the Paediatric Cardiac Genomics Consortium. Abbreviations: Ao, aorta; LA, left atrium; LV, left ventricle; PA, pulmonary artery; RA, right atrium; RV, right ventricle; TGV, transposition of great vessel; TOF, tetralogy of Fallot.

In 45% of patients with CHD, these methods can detect harmful coding mutations in genes that are definitively or potentially linked to CHD [18][19][20][21][22][23][24][25]. Genes that are strongly associated with CHD exhibit mutations that significantly co-segregate within families affected by CHD, are significantly more prevalent in unrelated patients with CHD compared to control groups, or cause heart defects in individuals with CHD linked to syndromes. CHD candidate genes share common features, but their properties lack statistical significance for definitive categorisation. Different types of variants, such as aneuploidies, copy number variants, structural genetic variations, and LOF variations, affect the dosage of genes related to CHD. However, deleterious missense variants can preserve the physiologic gene dosage whilst impairing the function of the encoded protein [21][24]. Please refer to **Figure 3**.

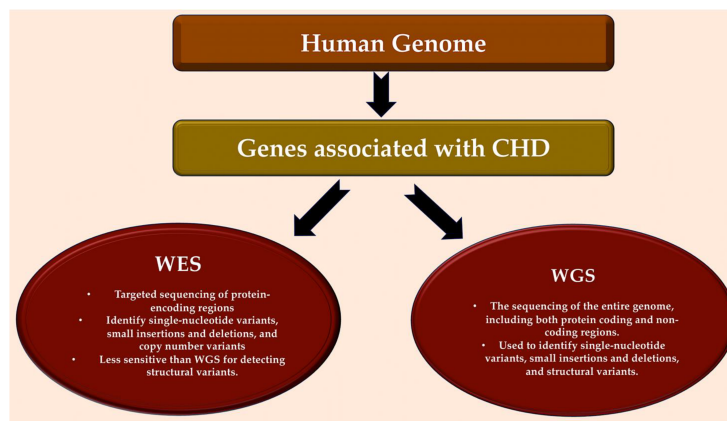


Figure 3. Detection of genes associated with CHD in human genome. Bioinformatic analytical tools such as WES and WGS platforms can be used to identify deleterious missense and LOF variants, small insertions or deletions, CNVs, and structural variants in large, aggregated, population-based sequencing datasets. Abbreviations: CHD, congenital heart disease; CNV, copy number variant; LOF, loss-of-function; WES, whole-exome sequencing; WGS, whole-genome sequencing.

Genes linked to CHD provide new knowledge about the essential regulatory molecules and pathways involved in cardiogenesis, complementing and extending the wealth of information gained from studying heart development in experimental models. Studying spontaneous gene variants in sporadic CHD can reveal novel genes implicated in cardiogenesis while providing detailed information [17][21][24]. Clinical evaluations enable the identification of a wider range of cardiac and extracardiac phenotypes than studies conducted on experimental models. Most human variants have a heterozygous dosage, and while experimental models usually examine homozygous variants, data on congenital heart disease obtained from human studies can reveal information about less obvious impacts on heart formation. Furthermore, patients with critical and complex malformations display the greatest frequency of de novo damaging variants in genes linked to CHD, which provides convincing evidence of severe adverse effects on reproductive health and the evolutionary restriction on numerous genes connected with CHD [8][9][10][11][12][13][14][15][16][17][18][19][20][21][22][23][24][25].

The question of why CHD is the most prevalent congenital abnormality is intriguing. One hypothesis suggests that the intricate developmental processes involved in heart formation are highly responsive to alterations in gene dosage for many essential genes and pathways. Such changes can cause developmental errors, many of which can be readily detected by the 18-week gestational age screening ultrasonography [26][27]. To showcase the intricate molecular orchestration of heart formation, researchers provide a brief summary of cardiac development. Following this, the latest genomic findings and approaches that have been at the forefront of current CHD science over the last decade are discussed. Key signals that govern cardiac morphogenesis have been extensively examined in several informative reviews, which are recommended for further details, including publications [28][29][30][31][32][33][34][35]. Genes with harmful variants found in people with CHD are highlighted in bold during the report (Figure 4, [22][23][36][37][38][39][40]).

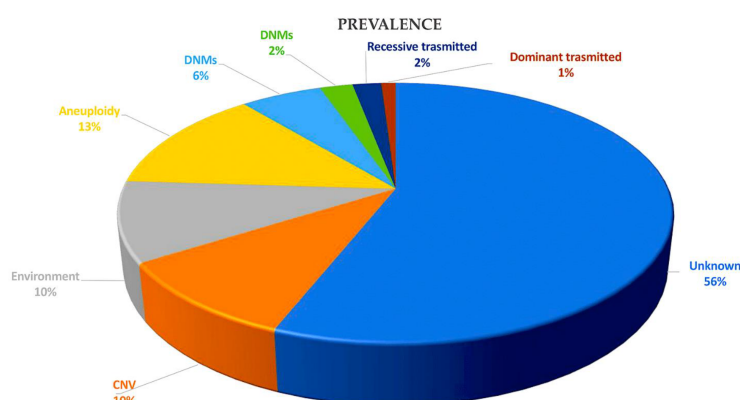


Figure 4. This pie chart illustrates the various causes of CHD and their relative proportions in contributing to the disease. Recessive transmitted causes involve variants in cilia and known CHD genes. Dominant causes of TOF include LOFs associated with *FLT4*, *KDR*, *TBX1*, and *NOTCH1*. DNMs may involve chromatin modifiers (2%) and dominant CHD genes (6%). Aneuploidy aetiology is related to trisomy 13, 18, or 21 and monosomy X. Non-genetic causes of CHD include teratogenic exposures such as alcohol; antiseizure and antiretroviral medication; and illnesses and infectious agents, such as diabetes, hypercholesterolemia, and rubella. The causes of CHD are investigated through gene–gene interactions, gene–environment interactions, polygenic inheritance, and epigenetics. CNVs, including Del22q11.2, Del20p12,

Del17q11, Del11q24-25, Del8p23.1, Del1q21.1, Dup12q24, and Dup7q11.23, are also considered. Abbreviations: CHD, congenital heart disease; CNVs, copy number variations; Del, deletion, DMSs, de novo mutations; Dup, duplication; LOF, loss of function.

2. Development of the Heart

The heart develops during the early stages of embryogenesis. The cardiac precursor fields differentiate and coalesce to create the cardiac crescent. During human gestation, the heart tube grows, undergoes regional transformation, and emerges as a nearly fully formed organ by 8 weeks. This process involves a linear and then looped structure. During heart development, differentiating precursor cell clusters interact to generate specialised heart cells with a well-defined three-dimensional architecture within restricted regions.

2.1. A Look at the Lines of Progenitor Cells and Their Morphogenesis

The heart is formed by two fields: the first heart field (FHF) from the lateral plate mesoderm and the second heart field (SHF) from the lateral plate splanchnic mesoderm and the migrating cardiac neural crest. These three cell lineages are major contributors to the formation of heart structures in the III, IV, and VI pharyngeal arches [35][41][42][43] (**Figure 5**). The FHF and SHF, which are symmetrical, merge anteriorly to form the heart crescent, which then coalesces into a linear cardiac tube with ventricular and atrial precursors aligned along the anterior–posterior axis. The heart cells originating from the FHF are involved in the development of the atria and the left ventricle, while the cells from the SHF are involved in the development of the right ventricle and the outflow tract, as well as contributing to both atria. Asymmetric growth results in the ballooning of the external curvature of the cardiac tube. This increases the size of the ventricular chamber [44][45]. The subsequent outflow tract separation and smooth muscle differentiation occur with the contribution of the cardiac neural crest.

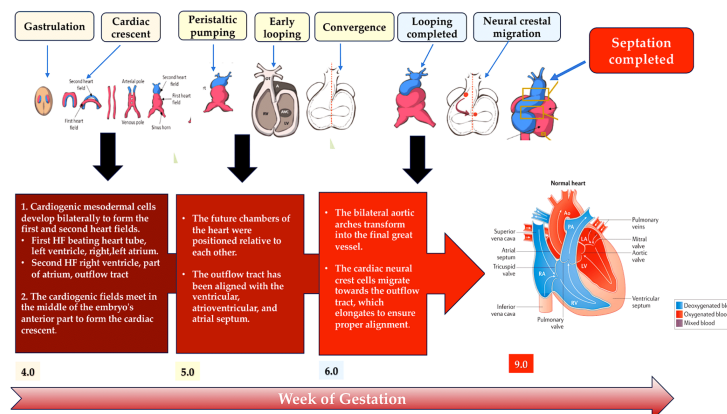


Figure 5. This schematic illustrates the embryonic development of the human heart, including first and second heart field formation, heart tube formation and pumping, looping, neural crest migration, and septation. The process results in a fully developed heart at the end of gestation. Abbreviation: HF, heart field.

2.2. Heart Morphogenesis under Genetic Control

Several important biomolecules play a role in differentiating progenitor cells into cardiac cell lineages and directing cardiac morphogenesis. Genes associated with CHD have identified some of these molecules. The FHF arises from the cardiac mesoderm through signalling, including the bone morphogenetic protein (BMP) and fibroblast growth factor (FGF), which are induced by the adjacent endoderm [46][47][48][49]. The expression of key cardiac transcripts required for cardiomyocyte lineage commitment is reduced in experimental models by the deletion of genes encoding BMPs [50]. The expressions of genes such as *GATA4*, *GATA6*, *NKX2-5*, and *TBX5* control sarcomere formation and contraction [28][29][30][31][32][33][51]. Ventricular cardiomyocyte specification is promoted by the expression of *IRX4* in both the FHF and SHF of the cardiac crescent [52]. Factors such as retinoic acid within the mesodermal progenitor fields initiate commitment to an atrial lineage [53][54]. Further specification leads to cardiomyocytes, which populate the left ventricle (*IRX4*, *HAND1*, and *MSX1*) [55][56][57], the right ventricle (*IRX4* and *GATA6*) [55][58], and the outflow tract (*GATA6*, *HAND2*, *ARID3B*, and *TEA*) [56][58][59][60]. FGF signalling is required for SHF contributions to the outflow tract, allowing for neural crest migration and the induction of *tbx1* expression in the SHF in zebrafish [61]. While there are established differentiation pathways, the progenitor fields in cardiac development still maintain plasticity. For instance, removing FHF cells in zebrafish leads to SHF compensation, which regenerates ventricular cardiomyocytes [62]. Additional pathways and gene networks involved in cardiac neural crest differentiation have been discovered. In mice, the normal differentiation of cardiac neural crest cells is prevented in the

absence of retinoic acid signalling [63], which can be ameliorated by maternal vitamin A supplementation [64]. This highlights the genotype–environment interactions in CHD. In contrast, a missense variant of *GATA6*, which is frequently found in patients with CHD and impairs outflow track formation, significantly enhances retinoic acid signalling [58]. *FOXC2* expression is essential for cardiac neural crest migration, as it mediates the activation of *Sema3c* expression in the SHF. *TBX1* expression, however, results in the counterbalancing of inhibitory signals [65][66].

In the atrioventricular canal and outflow tract, the heart contains non-chambered cardiomyocytes, as well as valvular endothelial and smooth muscle cells. Regulatory proteins, such as *TBX2*, *TBX3* [67], *BMP2*, and *BMP4* [68], repress chamber-specific genes to specify the non-chamber myocardium. In order to populate the endocardial cushions, valvular endothelial cells undergo endothelial-to-mesenchymal transition. This process is controlled by signalling cascades. These include vascular endothelial growth factor (VEGF) [69] and transforming growth factor- β (TGF β) [70]. Investigations into the differentiation of the outflow tract in mouse models have also shown that histone deacetylase 3 (encoded by *Hdac3*) is necessary for the differentiation of smooth muscle, whereas FGF and sonic hedgehog signalling are needed for the pulmonary arteries and veins [71][72][73]. Through transdifferentiation, cardiomyocytes can also change into cells that are not cardiac chambers. During the development of the mouse heart, a population of cardiomyocytes was found to transdifferentiate into vascular smooth muscle cells of the great arteries [74].

The left–right signalling axis is established in the embryo within the embryonic node. This occurs through signals mediated by motile and sensory cilia, leading to the asymmetric expression of genes that encode downstream regulatory signals.

Among the genes that encode downstream regulatory signals are those that encode the TGF β family members *Nodal*, *Lefty1*, *Lefty2*, and *Zic3* [75][76][77]. These signals are critical for the correct rightward looping of the linear heart tube, which ensures the correct positioning of the atria, ventricles, and outflow tract and gives rise to the sinoatrial node in the right atrium. Knockout mutations in *Lefty2*, which encodes left–right axis signalling proteins and transcriptional regulators, or the disruption of Sonic hedgehog or activin signalling in the chick embryo [78] can cause defects in cardiac looping, resulting in congenital heart defects in mice [79].

3. New CHD Genes Discovered in Cohort Studies

Analyses of CHD cohorts recruited from single centres or through national and international collaborations are increasingly identifying genes associated with congenital heart disease [22][25][80]. The majority of studies include patients with severe or critical CHD. There is an under-representation of patients with prevalent, simple CHD findings and an absence of patients with confirmed genetic syndromes [81]. WES and case–control analyses are commonly used to detect variants. These studies suggest that people with congenital heart disease have similar proportions of total de novo variants to the general population; however, they have significantly higher numbers of rare harmful mutations (allele frequency $\leq 1 \times 10^{-5}$ in the general population), including a 9% increase in de novo harmful variants, a 7% higher frequency of dominant inherited variants, and a 1% increase in recessive variants [22][23][24][25].

Deleterious mutations are found in an estimated 8–10% of patients with unspecified sporadic CHD [22][25]. The highest rate of de novo harmful variants is found in patients with syndromic congenital heart disease, while the highest rate of heritable LOF variants is found among patients with isolated CHD [25]. The importance of predefined biological pathways in the pathogenesis of CHD has been supported by cohort studies. The newly identified CHD-associated genes include those involved in the Notch [82] and VEGF [82] signalling cascades. However, the most significant functional class of these genes is chromatin remodelling [23][36]. Patients with CHD who have extracardiac anomalies and a neurodevelopmental delay most often have deleterious variants in these genes encoding chromatin remodelling proteins. The broad expression pattern of the genes suggests that organ development uses common epigenetic and transcriptional regulatory pathways.

The importance of ciliary function in cardiogenesis is supported by WES data from patients with CHD [83]. Deleterious mutations in cilia-related genes are often inherited from a healthy parent, as has been shown in other recessive diseases. In contrast, harmful variations in genes associated with chromatin alterations are more likely to occur de novo, which indicates that the damage caused by even a single mutant allele in this class of genes is significant.

- CHD definitive genes.

Studies were conducted on WES data from two cohorts of patients with congenital heart defects: 2056 patients with isolated congenital heart defects, 2994 with syndromic congenital heart defects, and 808 with congenital heart defects and an unknown extracardiac status. The statistical power of the analysis was sufficient to detect a substantial high frequency of harmful mutations in individuals with congenital heart disease compared to their matched counterparts, with correction

for analyses of all protein-coding genes ($p \leq 2 \times 10^{-6}$) [22][25][84]. In these cohorts, the genes that were identified had a definitive direct link to CHD, as with the genes related to the rare familial forms of CHD. However, a greater contribution to the overall population prevalence of CHD was made by genes identified in large-scale study populations. A total of 18 genes showed significant enrichment in these two WES cohorts, namely, *ADNP*, *ANKRD11*, *CDK13*, *CHD4*, *CHD7*, *DDX3X*, *DYRK1A*, *FLT4*, *KMT2A*, *KMT2D*, *NOTCH1*, *NSD1*, *PACS1*, *PRKD1*, *PTPN11*, *RBFOX2*, *SMAD6*, and *TAB2* [22][25][85][86]. Patients with syndromic congenital heart disease were screened out at enrolment, but mutations in *CHD7* associated with CHARGE syndrome, *KMT2D* associated with Kabuki syndrome, *NSD1* associated with Sotos syndrome, and *PTPN11* associated with Noonan syndrome were identified in the study. This suggests that formes frustes of syndromic phenotypes, which escape clinical recognition, may arise from some of the variants associated with syndromic CHD. *CDK13*, *CHD4*, and *PRKD1* are mutated most frequently in syndromic CHDs, whereas *SMAD6* is mutated most frequently in isolated CHDs [87]. Previous studies have emphasised the biological significance of certain genes associated with CHD. In mice, heart malformations have been observed as a result of null mutations in the murine homologues of *SMAD6* and *NOTCH1* [88][89]. *RBFOX2* transcript levels are reduced in some CHD patient tissues compared to in controls [90]. Additionally, the *DYRK1A* protein is a potential therapeutic candidate for treating neurodevelopmental disorders in trisomy 21 patients [91][92]. Recurrent variants in other genes have led to the discovery of new phenotype correlations. In one study, it was found that *NOTCH1* and *FLT4* mutations are causative factors in TOF, affecting 7% of patients [22][85][86][93]. Additionally, genes previously linked to intellectual disability, such as *ADNP* [94], *ANKRD11* (KBG syndrome) [95], *CDK13* [96][97], and *DDX3X* [98], are now accepted as being implicated in CHD. However, many definitive roles of CHD in heart development remain unknown.

- Genes linked to CHD that have deleterious variants in humans.

WES data suggest that CHD may be influenced by approximately 400 genes, a significantly higher number than currently known [36]. While some of these genes may contain harmful variants in CHD patients, they cannot be classified as definitive CHD-associated genes due to a lack of statistical significance [22][25][84][85]. Morton et al. performed a meta-analysis of whole-exome sequencing data from two large cohorts of patients with congenital heart disease to increase statistical power. The study aimed to improve the understanding of the genetic basis of congenital heart disease. Patients with aneuploidies or deletions at the 22q11 locus were excluded. The study analysed the WES data along with data from a cohort of 128 subjects without congenital heart disease, as well as results from the Genome Aggregation Database. It identified 132 genes associated with CHD, of which 66 had a high frequency of loss-of-function variants and 78 had a high frequency of deleterious missense variants. Patients with aneuploidies or 22q11 deletions were excluded from the analysis [84].

This meta-analysis provides evidence for the involvement of genes associated with coronary heart disease (CHD) in the regulation of transcription. Some of the genes linked to CHD due to loss-of-function variants have functional associations with chromatin remodelling, transcription factors, or RNA processing, and they have been investigated in detail elsewhere. These genes have been implicated in CHD or organogenesis, which explains the associated extracardiac phenotypes. Patients with loss-of-function variants in the group of definitive and candidate genes for the regulation of gene expression in congenital heart disease exhibited a higher incidence of extracardiac phenotypes than those with damaging missense variants [22][25]. The analysis confirmed that new loss-of-function mutations were more common in genes associated with chromatin modification in patients with congenital heart disease and extracardiac abnormalities. Patients with CHD and extracardiac abnormalities showed an enrichment for inherited missense variants. In contrast, patients with isolated CHD showed an enrichment for transmitted LOF variants. These observations suggest that there may be a genetic basis for the presence or absence of extracardiac abnormalities in CHD. It is possible that certain chromatin-modifying proteins have a specific function in the heart.

New technologies have enabled the comparison of results from studies on people of different ethnic and racial backgrounds [22][25][99]. Evaluating harmful missense genetic variations may reclassify some candidate variant genes for CHD as definitive. This will enhance genotype–phenotype correlations and the understanding of their role in heart development.

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