中国临床研究 Chin J Clin Res, June 2024, Vol.37, No.6
Cite as: Pan HG, Xie RG, Long QH, Zhu XM. Genetic causes of isolated congenital heart disease [J]. Chin J Clin Res, 2024, 37(6):830-844.
DOI: 10.13429/j.cnki.cjcr.2024.06.003

Genetic causes of isolated congenital heart disease

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Abstract: The genetic mechanism of congenital heart disease (CHD) is complex and currently lacks a clear understanding. Literature studies on CHD often report the presence of concurrent extracardiac anomalies, but since the majority of CHD cases are isolated, presenting only a single cardiac malformation, the etiological mechanisms remain uncertain, especially regarding the genetic aspects. Furthermore, there is a scarcity of case studies focusing on isolated CHD, resulting in a lack of comprehensive research data. Therefore, elucidating the genetic causes of isolated CHD and providing guidance for its clinical treatment have become urgent issues for researchers. This article reviews the known genetic causes and potential genetic mechanisms of isolated CHD, as well as provides recommendations for genetic testing in patients with isolated CHD.

Keywords: Congenital heart disease, isolated; Genetics; Single gene; Copy number variation; Mosaicism; Chromosomal microarray analysis; Whole-exome sequencing
Fund program: Self-financing Scientific Research Project of Health Commission of Guangxi Zhuang Autonomous Region (Z-J20221800)

Congenital heart disease (CHD) refers to abnormal development of the heart and large blood vessels during the early fetal process, or anatomical abnormalities of the heart and blood vessels caused by channel closure disorders. CHD includes a variety of cardiac malformations, which are usually grouped according to the nature of cardiac structural malformations, blood flow patterns, recurrence risks, and related susceptibility genes. According to whether other organs are involved, CHD is divided into syndromic CHD and isolated CHD. The former usually merges with other system defects, such as developmental delay, intellectual disability, etc., while the latter is only a single cardiac defect without congenital defects in other systems. About 40% of CHD can identify known genetic factors, but still about 50% of CHD cannot determine the clear pathogenesis. For isolated CHD, because the lesions only involve a single cardiac defect without affecting other organ systems, coupled with the genetic heterogeneity, penetrance, and the influence of multiple genes and other non-genetic factors of CHD, the proportion of identifiable genetic factors is lower.

1 Epidemiological Characteristics of CHD

CHD is a common birth defect in the world and one of the main causes of neonatal death. The incidence rate in live-born infants is about 1%. Isolated septal defect is the most common type of CHD, and it is estimated that there are 3,570 cases of septal defect per million newborns. There are approximately 200,000 newborns with CHD born in China every year. With the advancement of surgical techniques, the probability of CHD patients surviving to adulthood after one year of age has greatly increased, but CHD remains the leading cause of death from birth defects. About 40% of CHD can be attributed to genetic causes, including 10% with severe aneuploidy, 25% with pathogenic copy number variations (CNV), and 5% with monogenic diseases. For isolated CHD, which is less frequently studied in the literature, existing studies have also revealed that pathogenic CNVs can account for 10% [1].

2 Evidence of Genetic Basis of CHD

The view that the occurrence of CHD is closely related to genetic factors, environmental factors, and the interaction between them is widely recognized by scholars. Bruneau *et al.* [2] found that the possibility of normal phenotype couples having CHD children again when they have already had CHD children is about 3%, and the incidence of CHD increases with the increase of birth times, reaching 10%. In addition, there are differences in the incidence of CHD among different genders and races. Overall, some types of CHD are more likely to be found in European races, and the severity of male lesions is higher. Studies have shown that CHD is prone to form family clustering. The possibility of twins with CHD is 6 times higher than that of ordinary infants. About one-third of the CHD phenotypes can be explained from the perspective of family inheritance, indicating that genetic factors play an important role in the occurrence and development of

CHD [3]. The pathogenesis of CHD is complex and lacks a deep understanding of genetic mechanisms. Among the known variations, chromosomal aneuploidy, CNV, and single-gene mutations usually disrupt genes that play important roles in normal heart development, leading to the occurrence of CHD. However, only 20% of cases can be attributed to a single genetic factor, and the proportion of identifiable single genetic factors causing isolated CHD is even lower [4-5]. With the advancement of next-generation sequencing technology and the emergence of innovative genetic assessment methods, more and more genetic variations have attracted the attention of researchers and been included in the list related to isolated CHD. Researchers are eager to find evidence related to isolated CHD by understanding the key CNVs or genes that play a crucial role in syndromic CHD, so as to identify the genetic etiology of isolated CHD.

3 Single Gene and Isolated CHD

Sufficient research evidence has shown that the T-box family series (*TBX1*, *TBX5*, *TBX20*), *SMAD2*, *SMAD6*, *NKX2.5*, *MYH6*, *MYH7*, etc., play a regulatory role in the temporal and spatial expression throughout the process of heart development, especially playing a key role in the initial differentiation of atrioventricular chambers, the formation of ventricles, and the septum of the heart [6-9]. For isolated CHD, due to the influence of multiple factors such as genetic heterogeneity and incomplete segregation, the frequency of gene mutations is much lower than that of syndromic CHD, indicating that the genetic mechanism of isolated CHD is different from syndromic CHD [10]. Earlier studies identified genes that cause CHD, including *NKX2.5*, *TBX5*, *GATA4*, etc. [11], which play an important role in the heart development process of CHD fetuses. These genes are mainly discovered through linkage analysis and positional cloning and may lead to defects in the cardiac conduction system [12]. In recent years, with the gradual promotion and use of genome sequencing methods, the types of genes related to patients with isolated CHD have rapidly increased. Unlike syndromic CHD, most of the identified genes are transcription factors, signaling molecules, and structural proteins [13]. Current evidence has proved that there is a correlation between single genes and isolated CHD, and as the list of mutations rapidly expands, the single-gene genetic mechanism of isolated CHD will gradually become clearer.

3.1 Structural Proteins

A recent study involving over 4,000 CHD patients in Europe has uncovered new evidence related to CHD. Among these, *MACROD2*, previously considered unrelated to CHD but closely associated with cancer, is now believed to be highly expressed in heart cells. Additionally, the study identified *GOSR2*, *WNT3*, *MSX1*, and *Ythdc2* as playing transcriptional regulatory roles in certain CHD conditions such as aortic stenosis, patent ductus arteriosus, and transposition of the great arteries [14]. Notably, a newly discovered coding gene, *CDkn1A*, has also been implicated in cardiovascular biology-related gene regions [15]. However, the specific targets and potential mechanisms of these candidate genes remain unexplained.

3.2 Transcription Factors

Currently, transcription factors are considered major contributors to syndromic CHD. Among the known transcription factors, *NKX2.5* was the first gene discovered to be associated with CHD. Mutations in *NKX2.5* primarily manifest as atrial septal defects, accounting for approximately 4% of isolated CHD cases. The mechanism underlying CHD caused by *NKX2.5* may involve reduced DNA binding activity due to base mutations, leading to transcriptional repression and affecting heart development [16]. Another example is *TBX-5* from the T-box family, which acts as a dose-sensitive factor regulating heart development. Mutations in *TBX-5* can lead to Holt-Oram Syndrome, an autosomal dominant genetic disease. *KLF13* can synergistically activate cardiac gene transcription with *TBX-5*. Furthermore, data confirm that *KLF13* modifies *TBX-5* in Holt-Oram Syndrome, suggesting similar roles for both genes in syndromic CHD [17]. Additionally, mutations in *KLF13* disrupt its synergistic interaction with the GATA family [18], which has been implicated in isolated CHD, such as *GATA4* mutations leading to various CHD formations, including atrial/ventricular septal defects, patent ductus arteriosus, tetralogy of Fallot (TOF), and endocardial cushion defects [19-20].

3.3 Cell Signaling Pathways

Evidence suggests that signaling pathways play crucial roles in the formation of cardiac progenitor cells and three-dimensional heart structures. In a study of over 2,000 patients with isolated CHD, a mutation in *PTPN11* was identified in a female patient. Although this indirectly suggests that *PTPN11* is not a major genetic cause of isolated CHD, it should not be overlooked that dysregulation of the RAS-MAPK signaling pathway is closely associated with the development of atrial septal defects and double inlet left ventricle [21-22]. Notably, isolated pulmonary valve stenosis is often present in patients with Noonan syndrome and is associated with *PTPN11* gene mutations [23]. Additionally, the Notch signaling pathway plays a vital role in the development of embryonic structures and organs, including the heart

[24]. Studies have shown that variations in Notch pathway genes can lead to cardiac abnormalities and isolated or syndromic CHD. Specifically, in patients with isolated tetralogy of Fallot, 4.5% carry a heterozygous mutation in *NOTCH1*, which is considered the most common genetic variant predisposing to isolated TOF [25]. Another variant, DLL4, was initially classified as a variant of unknown significance due to its early failure to meet the pathogenicity threshold. However, recent research on a family with isolated tetralogy of Fallot found that DLL4 variations may be harmful to protein function, leading to CHD [26]. Furthermore, the transforming growth factor (TGF)-β family of signaling pathways is crucial for heart development, with genes such as *BMP-2*, *BMP-4*, *TGF-β2*, *TGF-β3*, and *Nodal* implicated. Studies have identified various isolated CHD-related malformations, including dextroposition of the great arteries, double outlet right ventricle, TOF and isolated ventricular septal defects [27-28].

3.4 Ciliopathies

Cilia are cellular organelles that play crucial roles in cell signaling during embryonic development. Currently, abnormalities in ciliary structure or function have been associated with CHD. In certain CHD conditions, such as atrial septal defects and atrioventricular canal defects, primary cilia have been found to disrupt the Shh signaling pathway during heart development, leading to isolated CHD [29]. Additionally, genes expressed in ciliated cells of the respiratory and nervous systems, such as Foxj1, can cause hydrocephalus and airway diseases. Recent studies have reported that *Foxj11* variants, confirmed by whole-exome sequencing (WES) and functional testing, can cause abnormal cardiac circulation, leading to isolated CHD [30].

3.5 Modifier Genes

Zaidi *et al.* [31] found in their study on patients with severe CHD that approximately 10% of novel mutations were associated with methylation in *H3K4*. Among them, eight patients with *H3K4* methylation had different CHD phenotypes. The study also confirmed that *H3K4* methylation disrupted heart development, and its mechanism was related to the regulation of key heart development genes. Studying modifier genes like H3K4 methylation helps the further understanding in the pathogenesis of CHD, especially isolated CHD.

4 CNV and Isolated CHD

CNV is a common type of chromosomal structural abnormality, and pathogenic CNV is also an important cause of CHD. Besides causing common syndromic CHD such as Digeorge syndrome and Williams syndrome, it can also cause isolated CHD [32-33]. Some literature reports that CNV can affect heart development by altering gene dosage levels or functions, leading to the occurrence of syndromic CHD. The most common ones include 22q11.2 microdeletion and 15q11.2 microdeletion, which can be accompanied by varying degrees of neurological disorders and developmental delays [34-35]. A study on the pathogenic relationship between CNV and CHD found that the proportion of CHD fetuses with pathogenic abnormalities associated with extracardiac malformations could reach 30% [36]. Apart from being closely related to syndrome CHD, researchers have found in their studies on isolated CHD that the incidence of pathogenic CNV in isolated CHD is about 5.7%. Some CNV fragments smaller than 5 Mb can also lead to isolated CHD, such as Xp22.2 and 8p23.1, which can lead to atrioventricular septal defects, TOF, and other CHD. The most likely genetic cause of left/right ventricular outflow tract obstruction is related to microdeletion and microduplication [37-38]. According to literature reports, although the chromosome abnormality rate of isolated CHD is significantly lower than that of CHD combined with extracardiac abnormalities, the positive diagnostic rate of chromosome abnormalities still reaches 8.4%-9.6% [39-40]. In addition, researchers have detected several rare and newly-identified CNVs in isolated CHD children, which contain genes required for heart development, such as *TBX1*, *JAG1*, *NOTCH1*, etc. Researchers believed that about 10% of isolated CHD might be caused by genes in these regions [41]. Kim *et al.* [42] found in a cohort study of isolated CHD children that the incidence of newly-identified large CNVs in isolated CHD children was much higher than that in normal children, and children carrying such CNVs usually had poor prognosis, which may be related to the synergistic effect of genes in the region.

5 Mosaicism and Isolated CHD

Syndromic CHD often merges with neurological and motor system abnormalities, presenting with multi-organ symptoms such as developmental delay, intellectual disability, and craniofacial malformations. This type of CHD is the first to be widely studied due to its involvement in multiple system abnormalities and significant harm. Existing cytogenetic studies have shown that approximately 20% of CHD cases are diagnosed prenatally with chromosomal number and structural abnormalities. Foreign scholars' research has also shown that the proportion of syndromic CHD in patients with common chromosomal aneuploidy such as trisomy 21 and Turner syndrome is approximately 20% to 50% [43]. Compared with syndromic CHD, the genetic basis of isolated CHD is more complex. Existing cytogenetic studies have shown that mosaicism

individuals may have coronary heart disease without related syndrome characteristics. Any 45,X mosaic cell line may increase the risk of isolated CHD [44-45]. A study of heart tissue in CHD patients suggests that the mutation frequency of transcription factors such as *NKX2.5*, *GATA4*, *TBX5*, *MEF2C*, and *HEY2* detected by heart tissue sampling is significantly increased, indicating that mosaicism plays an important role in isolated CHD. In addition, some studies suggest that there is a dose-dependent relationship between the X chromosome and autosomal genes or aortic development loci. When the gene dose of the X chromosome is reduced, the inactivation of the X chromosome during development leads to the occurrence of CHD [46-47]. Furthermore, studies have found that mosaicism of chromosome 16 can also cause isolated CHD in fetuses [48]. Early ultrasound suggests an increase in nuchal translucency, which may be caused by cardiac dysfunction. Research suggests that the formation of chromosomal mosaicism may be related to "trisomic rescue," and the severity of clinical symptoms may be related to the mosaic ratio, which means that low-level mosaicism may occur in isolated CHD.

6 Detection Methods of Genetic Etiology of CHD

The genetic mechanism of CHD is complex, but existing research results have confirmed that genetic factors can lead to the occurrence of CHD, indicating that it plays an important role in the occurrence and development of CHD. However, due to the high heterogeneity of CHD and its wide phenotypic spectrum, as well as the difficulty in identifying malformed features during the heart development of newborns and infants, and the lag of cognition, it is more difficult to find the pathogenesis of CHD genetic factors. Recent research results show that next-generation sequencing technology is gradually expanding the screening range of CHD and providing new insights for further understanding the molecular mechanism of isolated CHD. Currently, when CHD is found by ultrasound and accompanied by varying degrees of extra-cardiac malformations, karyotype testing has become a consensus in the industry. In addition, when CHD is clinically considered to be associated with aneuploidy or 22q1.2 deletion, fluorescence in situ hybridization with obvious advantages can be selected for rapid prenatal diagnosis. Chromosomal microarray analysis (CMA) or CNV-seq is currently widely used for short-cycle, high-resolution detection of CNVs larger than ~100 kb, and has obvious cost-effectiveness in health economics. Current research results show that CMA or CNV-seq detection of various types of samples can increase the detection rate of pathogenic CNVs to 3% [50]. Although the selected CNVs are not always pathogenic, they may be familial, which is very important for finding the etiology of CHD. For negative CMA or CNV-seq tests, but CHD has obvious genetic etiology, WES can be performed to screen potential results and obtain more candidate protein-coding genes for isolated CHD [51]. It should be noted that clinically, genetic counseling capabilities should be provided for variants of uncertain significance (VOUS) and the possibility of secondary findings. In addition to WES, whole genome sequencing (WGS) has demonstrated technical advantages superior to WES, enabling the detection of mitochondrial sequences, mRNA, non-coding RNA, as well as promoters and regulatory sequences. WGS may be proven to have a higher diagnostic rate than WES and be widely used for diagnosing the genetic etiology of CHD. The use of rapid WGS in children with CHD can enable rapid treatment and reduce medical costs for this pediatric population. Furthermore, functional genomic studies using single-cell RNA sequencing, chromatin immunoprecipitation (ChIP)-seq, and assay for transposase-accessible chromatin (ATAC)-seq can also reveal the complex intrinsic genetic networks of CHD and elucidate the effects of specific cardiac lineages during early heart development.

7 Issues Faced by Isolated CHD Research

Due to the numerous genes that lead to CHD and the possible overlap between genes causing syndromic CHD and isolated CHD, it is more challenging to identify the genetic factors of isolated CHD. The phenotypic heterogeneity of CHD, that is, the same genetic sequence variation usually results in 50% of patients not always having the same cardiac phenotype. It is worth noting that due to the influence of epigenetics and mitochondrial inheritance, a comprehensive understanding of the genetic factors of isolated CHD still needs to consider the relationship between penetrance and pathogenic factors. In addition, since extracardiac malformations usually occur later than intracardiac malformations, it becomes particularly important to continue medical follow-up for patients with isolated congenital CHD caused by syndrome-related genes.

8 Prospects

Currently, research on isolated CHD is insufficient both domestically and internationally, and due to the involvement of environmental and genetic factors in the pathogenesis of CHD, it is difficult to explain the genesis of most CHD through a single study, and further research is needed. Although there is abundant evidence that isolated CHD is affected by genetic factors, research on isolated CHD still mostly focuses on genetic testing. Using mouse models with isolated CHD gene mutations will be an important means to study the

mechanism of mutant genes. Through the study of animal models, perhaps new methods for clinical treatment of isolated CHD can be found to improve the prognosis of isolated CHD.

Conflict of Interest None

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Submission received: 2023-07-25 / **Revised**:2023-09-14

· 研究进展 ·

孤立型先天性心脏病的遗传学病因

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摘要：先天性心脏病（CHD）的遗传机制复杂且目前对其缺乏确切的了解。文献中有关 CHD 的报道通常合并有心外畸形，但绝大多数 CHD 为孤立型，仅表现为单一的心脏畸形，其病因机制尚不十分确切，且遗传学机制更难以阐明，加之目前针对孤立型 CHD 的病例研究极少，缺乏相关的系统深入的研究数据，因此阐明孤立型 CHD 患者的遗传学病因并为其进一步的临床治疗提供指导是 CHD 领域具有重要研究价值的课题之一。本文回顾了孤立型先天性心脏病目前已知的遗传学病因及潜在的遗传学机制，以及对孤立型 CHD 患者进行遗传学检测的建议。

关键词：先天性心脏病，孤立型；遗传学；单基因；拷贝数变异；嵌合体；染色体微阵列分析；全外显子组测序
中图分类号：R725.4　R394　**文献标识码**：A　**文章编号**：1674-8182(2024)06-0830-05

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Abstract: The genetic mechanism of congenital heart disease (CHD) is complex and currently lacks a clear understanding. Literature studies on CHD often report the presence of concurrent extracardiac anomalies, but since the majority of CHD cases are isolated, presenting only a single cardiac malformation, the etiological mechanisms remain uncertain, especially regarding the genetic aspects. Furthermore, there is a scarcity of case studies focusing on isolated CHD, resulting in a lack of comprehensive research data. Therefore, elucidating the genetic causes of isolated CHD and providing guidance for its clinical treatment holds significant research value in the field of CHD. This article reviews the known genetic causes and potential genetic mechanisms of isolated CHD, as well as provides recommendations for genetic testing in patients with isolated CHD.

Keywords: Congenital heart disease, isolated; Genetics; Single gene; Copy number variation; Mosaicism; Chromosomal microarray analysis; Whole-exome sequencing
Fund program: Self-financing Scientific Research Project of Health Commission of Guangxi Zhuang Autonomous Region (Z-J20221800)

先天性心脏病（congenital heart disease, CHD）又称先心病，是指胎儿早期过程中心脏和大血管发育异常，或者因通道闭合障碍引起心脏及血管结构解剖异常。CHD 包括多种心脏畸形，通常根据心脏结构畸形的性质、血流模式、复发风险及相关易感基因进行分组。根据是否有其他器官受累而将先天性心脏病分为综合征型 CHD 和孤立型 CHD，前者通常合并其他系统缺陷，如发育迟缓、智力低下等，而后者仅为单一的心脏缺陷而无其他系统的先天缺陷。大约 40% 的 CHD 可以识别已知的遗传因素，尽管如此，仍有约 50% 的 CHD 无法确定明确的发病机制。对于孤立型 CHD 而言，由于病变仅涉及单一的心脏缺陷而未波及其他器官系统，加之 CHD 的遗传异质性、外显率及多基因和其他非遗传因素的影响，单一可识别的遗传因素比例更低。

1　CHD 的流行病学特征

CHD 是世界上常见的一种出生缺陷病，也是新生儿死亡的主要原因之一，在活产婴儿中的发病率约为 1% 左右，孤立型间隔缺损是最常见的 CHD 类型，估计每百万新生儿中间隔缺损数可达 3 570 例。据估算，我国每年约有 20 万例 CHD 患儿出生。随着外科手术技术的进步，CHD 患者一岁后存活至成年的概率大大增加，但 CHD 仍然是出生缺陷死亡的主要原因。大约 40% 的 CHD 可发现遗传学方面的病因，其中 10% 为严重的非整倍体异常，25% 为致病性拷贝数变异（copy number variation, CNV），5% 为单基因疾病，而对于文献较少涉及的孤立型 CHD，已有研究也揭示了致病性 CNV 占比可达到 10%[1]。

DOI：10.13429/j.cnki.cjcr.2024.06.003
基金项目：广西壮族自治区卫生健康委员会自筹经费科研课题（Z-J20221800）
出版日期：2024-06-20

QR code for English version

2　CHD 的遗传基础证据

CHD 的发生与遗传因素、环境因素以及两者共同作用密切相关这一观点为广大学者所认同。Bruneau 等[2]的研究发现,表型正常的夫妇曾育有 CHD 患儿时再次生育 CHD 患儿的可能性约为 3%,且 CHD 的发生率随着生育次数的增加而增加,可达到 10%。此外,不同性别及种族间的 CHD 发病率存在差异,总体而言,某些 CHD 类型在欧洲人种中更易发现,且男性病变严重程度更高。研究表明,CHD 较容易形成家族聚集,双胞胎 CHD 的患病的可能性是一般婴儿的 6 倍,约三分之一的 CHD 表型可以从家族遗传方面得到解释,这说明了遗传因素在 CHD 的发生发展中尤为重要[3]。CHD 的发病机制较为复杂且目前缺乏遗传机制的深入了解,目前所知的变异中,染色体非整倍体、CNV 及单基因变异通常会破坏正常心脏发育中起重要作用的基因,导致 CHD 的发生,但仅有 20%的病例可以确定为单一遗传因素引起,对于孤立型的 CHD 而言,可识别的由单一遗传因素引起的比例更低[4-5]。随着下一代测序技术的进步及创新性的遗传评估方法的出现,越来越多的基因变异得到了研究人员的关注,并被纳入到了与孤立型 CHD 相关的列表之中。研究者们亟待通过了解综合征型 CHD 中发挥关键作用的 CNVs 或基因寻找可能与孤立型 CHD 相关的证据,从而能够识别孤立型 CHD 的遗传学病因机制。

3　单基因与孤立型 CHD

目前已有足够的研究证据表明 T-box 家族系列(*TBX1*、*TBX5*、*TBX20*)、*SMAD2*、*SMAD6*、*NKX2.5*、*MYH6*、*MYH7* 等在心脏发育全过程中起到调控时间及空间上的表达,特别是在房室腔的初始分化、心室和心脏间隔的形成发挥了关键作用[6-9]。对于孤立型 CHD,由于受到遗传异质性、不完全分离等多因素影响,基因变异的发生频率远低于综合征型 CHD,这表明了孤立型 CHD 的遗传机制有别于综合征型 CHD[10]。早年的研究定位了引起 CHD 的基因,包括 *NKX2.5*、*TBX5*、*GATA4* 等[11],在 CHD 胎儿的心脏发育过程发挥了重要作用,这些基因主要通过是通过连锁分析以及定位克隆法发现,并可能导致心脏传导系统缺陷[12]。近年来,随着基因组测序方法的逐步推广使用,与孤立型 CHD 患者相关的基因种类迅速扩大,与综合征型 CHD 不同的是,已鉴定的基因多数是转录因子、信号分子及结构蛋白等[13]。目前现有的证据已证明单基因与孤立型 CHD 存在关联,且随着变异清单的迅速扩大,孤立型 CHD 的单基因遗传机制将逐渐变得清晰。

3.1　结构蛋白　欧洲一项超过 4 000 例 CHD 患者的研究新发现一组与 CHD 相关的新证据,其中 *MACROD2* 此前被认为是与 CHD 无关而与恶性肿瘤发生密切相关,研究者认为 *MACROD2* 在心脏细胞中高度表达,除此之外,研究还发现了 *GOSR2*、*WNT3*、*MSX1* 以及 *Ythdc2* 在某些 CHD 如主动脉缩窄、动脉导管未闭及大动脉转位等发挥转录调节的作用[14]。除此之外,新近发现的编码基因 *CDkn1A* 也被认为与心血管生物学相关性的基因区显著相关[15]。值得关注的是,这些候选基因的特定靶点及潜在的作用机制至今仍未得到解释。

3.2　转录因子　目前转录因子被认为是综合征型 CHD 的主要诱因在已知的转录因子中,*NKX2.5* 是第一个被发现与 CHD 相关的基因,*NKX2.5* 突变主要表现为房间隔缺损,解释了约 4%的孤立型 CHD 病例,其引起 CHD 的机制可能是碱基突变使 DNA 结合活性降低导致转录受到抑制而影响心脏发育[16]。如 T-box 家族中的 *TBX-5*,起着调节心脏发育的剂量敏感因子,当 *TBX-5* 突变时可导致常染色体显性遗传病——Holt-Oram 综合征,而 *KLF13* 可与 *TBX-5* 协同激活心脏的基因转录,此外数据证实,*KLF13* 是 Holt-Oram 综合征 *TBX-5* 的修饰基因,这说明 *KLF13* 和 *TBX-5* 在综合征型 CHD 中发挥了类似的作用[17]。除此之外,*KLF13* 突变还会破坏其在 GATA 家族中的协同作用[18],而后者已被证实与孤立型 CHD 有关,如 *GATA4* 基因突变可导致多种类型的 CHD 形成,如房/室间隔缺损、动脉导管未闭、法洛四联征、心内膜缺损等[19-20]。

3.3　细胞信号传导　已有的证据表明,信号通路在心脏祖细胞以及三维心脏结构的形成过程中发挥着重要作用。在一项 2 000 多例孤立型 CHD 患者的研究中,有一名女性患者发现了 *PTPN11* 基因的突变,尽管这间接提示了 *PTPN11* 并非孤立型 CHD 的主要遗传学病因,但不应忽视的是,RAS-MAPK 信号通路的失控与房间隔缺损及双尖瓣主动脉瓣的发生发展密切相关[21-22]。值得注意的是,孤立型肺动脉瓣狭窄常存在于 Noonan 综合征患者中,并且与 *PTPN11* 基因变异相关[23]。此外,Notch 信号通路通过在胚胎结构和器官(包括心脏)的发育中发挥着重要作用[24]。研究表明 Notch 通路基因的变异可导致心脏病变以及孤立型或综合征性冠心病,尤其在孤立型法洛四联征患者中,4.5%的患者携带了 *NOTCH1* 杂合变异,该位点被认为是易患孤立型法洛四联征的遗传变异最常见的位点[25]。另一个变异类型 *DLL4* 由于早期没有通过致病性阈值的评估而被归类为致病性未知的变异。然而,近期对一个孤立型法洛四联征家系的研究中发现,*DLL4* 变异可能对蛋白质的功能有害,从而导致 CHD 的发生[26]。此外,心脏发育至关重要的信号通路为转化生长因子(transforming growth factor, TGF)-β 家族,其中发现与心脏发育相关的基因有 *BMP-2*、*BMP-4*、*TGF-β2* 和 *TGF-β3* 以及 *NODAL*,目前研究发现的孤立型 CHD 相关的畸变类型包括大动脉 D 转位、右心室双出口、法洛四联征和孤立型室间隔缺损[27-28]。

3.4　纤毛(ciliopathies)　纤毛是胚胎发育过程中在细胞信号传导方面起重要作用的细胞器。目前发现的纤毛结构或功能异常均与 CHD 相关。对于某些 CHD 如房室间隔缺损、房室管畸形,初级纤毛逐渐被发现可在心脏发育过程中破坏 shh 信号,导致孤立型 CHD 发生[29]。某些表达在呼吸系统、神经系统的纤毛细胞上的基因如 *Foxj1* 可导致脑积水及起到疾病,最近的研究报道了经全外显子组测存(whole-exome sequencing, WES)确认的,经功能试验证实,*Foxj11* 变异体可引起异常的心脏循环,导致孤立型 CHD 发生[30]。

3.5　修饰基因　Zaidi 等[31]在对严重 CHD 患者的研究中发现,约 10%的新发突变与 *H3K4* 甲基化有关,其中有 8 例 *H3K4* 甲基化突变的患者的 CHD 表型并不相同,研究同时证实了 *H3K4* 甲基化突变时破坏了心脏的发育,其机制与调节关键的心脏发育基因有关。对类似 *H3K4* 甲基化等修饰基因的研究有助于对 CHD 特别是孤立型 CHD 的发病机制进一步了解。

4　CNV 与孤立型 CHD

CNV 是染色体结构异常的常见类型,致病性 CNV 也是导致 CHD 的重要原因,除了引起常见的综合征型 CHD 如 Digeorge 综合征和 Williams 综合征外,还可引起孤立型 CHD[32-33]。有文献报道,CNV 可通过改变基因的剂量水平或功能改变而影响心脏的发育,导致综合征型 CHD 的发生,最常见的如 22q11.2 微缺失、15q11.2 微缺失等可伴有不同程度上的神经系统障碍、发育迟缓等表型[34-35]。一项关于 CNV 与 CHD 病因关系的研究发现,合并心外畸形的 CHD 胎儿致病性异常的比例可达到 30%[36]。除了与综合征型 CHD 密切相关外,研究人员针对孤立型 CHD 的研究中发现,致病性 CNV 在孤立型 CHD 中的发生率约 5.7%,部分片段小于 5 Mb 的 CNV 也可导致孤立型 CHD 的发生,如 Xp22.2、8p23.1 等均可导致房室间隔缺损、法洛四联征等 CHD,而左/右心室流出道阻塞的遗传学病因最有可能为微缺失微重复有关[37-38]。文献报道,孤立型 CHD 的染色体异常率虽然显著低于 CHD 合并心外异常组,但其染色体异常阳性诊断率仍达 8.4%~9.6%[39-40]。此外,研究人员还从孤立型 CHD 患儿中检出了几种罕见新发的 CNVs,这些 CNVs 包含了心脏发育过程中所需要的基因,如 *TBX1*、*JAG1*、*NOTCH1* 等,研究者认为约 10%的孤立型 CHD 可能是由这些区域的基因引起[41]。Kim 等[42]在一项对孤立型 CHD 患儿的队列研究中发现,新发的大片段 CNVs 孤立型 CHD 患儿中的发生率远高于正常儿童,通常携带有此类 CNV 的患儿预后较差,这可能与区域内基因的协同作用有关。

5　嵌合体与孤立型 CHD

综合征型 CHD 常合并神经、运动等系统异常,可表现出发育迟缓、智力低下、颜面部畸形等多器官系统症状。现有的细胞遗传学研究表明,约 20%的 CHD 经产前诊断为胎儿染色体数目和结构异常。国外学者的研究也显示在常见染色体非整倍体如 21 三体综合征、特纳综合征患者中综合征型 CHD 的比例约为 20%~50%[43]。与综合征型 CHD 相比,孤立型 CHD 的遗传基础更为复杂,现有细胞遗传学研究表明,嵌合体个体中可能患有冠心病而没有相关的综合征特征,任何 45,X 的嵌合体细胞系中,可能都会增加孤立型 CHD 的风险[44-45]。一项针对 CHD 患者心脏组织的研究认为,心脏组织取样检测发现的 *NKX2.5*、*GATA4*、*TBX5*、*MEF2C* 和 *HEY2* 等转录因子的突变频率明显增加,表明嵌合体在孤立型 CHD 中发挥了重要作用。此外,有研究认为,X 染色体与常染色体基因或主动脉发育基因座之间具有剂量依赖性,当 X 染色体基因剂量减少,在发育过程中由于 X 染色体的失活,导致 CHD 发生[46-47]。此外,研究发现,16 号染色体的嵌合也会引起胎儿孤立型 CHD[48],早期的超声提示有颈项透明层厚度增加,原因可能是心功能障碍所致。研究认为,导致染色体嵌合的形成原因可能与“三体自救”有关,同时临床症状的严重程度可能与嵌合比率相关,这意味着低水平的嵌合可能会在孤立型 CHD 中出现。

6　CHD 的遗传学病因检测方法

CHD 遗传机制复杂,但现有的研究结果也已证实了遗传因素可导致 CHD 的发生,提示其在 CHD 的发生发展中扮演着重要角色。但由于 CHD 是一种具有高度异质性的疾病,其表型谱广泛,加之新生儿和婴幼儿在心脏发育过程中较难识别畸形特征,且因认知滞后,更增加了寻找 CHD 遗传学因素发病机制的难度。近期研究结果表明,下一代测序技术正逐渐扩大 CHD 的筛查范围,并为进一步了解孤立型 CHD 的分子机制提供新的见解。目前,超声发现的 CHD 多伴有不同程度的心外畸形时,进行核型检测已成为业内共识。此外,当临床考虑 CHD 与非整倍体或 22q1.2 缺失时,可选择具有明显优势的荧光原位杂交进行快速产前诊断。染色体微阵列分析(chromosomal microarray analysis, CMA)或 CNV-seq 目前广泛应用于~100 kb 以上大小的 CNV 进行短周期、高分辨的检测,在卫生经济学方面具有明显的成本效益[49]。目前的研究结果表明,对各种类型样本进行 CMA 或 CNV-seq 检测可将致病性 CNVs 的检出率提升至 3%[50]。尽管筛选出的 CNV 并不总是致病性 CNVs,却有可能是家族性的,这对于寻找 CHD 的病因非常重要。对于 CMA 或 CNV-seq 检测阴性,但 CHD 具有明显的遗传学病因时,可进行 WES,对检测的潜在结果作出筛选并获得更多关于孤立型 CHD 的候选蛋白编码基因[51]。需要注意的是,临床上应能提供针对临床意义未明的(variant of uncertain significance, VOUS)以及二次发现的可能性的遗传咨询能力。除 WES 外,全基因组测序(whole genome sequencing, WGS)展示了更优于 WES 的技术优势,能够对线粒体序列、mRNA、非编码 RNA 以及启动子和调控序列进行检测。WGS 可能会被证明较 WES 具有更高的诊断率而被广泛用于诊断 CHD 的遗传学病因,在对 CHD 患儿使用极速 WGS 可以使 CHD 这一儿童群体得到快速治疗并降低医疗成本[52]。此外,使用单细胞 RNA 测序、染色质免疫共沉淀(chromatin immunoprecipitation, ChIP)-seq 和染色质转座酶可及性测序(assay for transposase-accessible chromatin, ATAC)-seq 的功能基因组研究亦可揭示 CHD 复杂的内在遗传网络,并阐述早期心脏发育中的特定心脏谱系产生的影响。

7　孤立型 CHD 研究面临的问题

由于导致 CHD 的基因数量众多,且导致综合征型 CHD 与孤立型 CHD 的基因可能存在重叠,识别孤立型 CHD 的遗传学因素更具有挑战性。而 CHD 的表型异质性,即相同基因序列变异通常有 50%的患者心脏表型并不总是相同。值得注意的是,受表观遗传学以及线粒体遗传的影响,全面了解孤立型

CHD 的遗传学因素仍需要考虑外显率与发病因素间的关系。此外,由于心外畸形通常晚于心内,对综合征相关基因引起的孤立型 CHD 患者继续进行医学随访变得尤为重要。

8 展　望

国内外目前对于孤立型 CHD 的研究并不充分,且由于 CHD 的发病原因涉及环境因素、遗传因素,单一的研究难以解释大多数 CHD 的产生机制,需要进一步深入研究。尽管大量的证据证实孤立型 CHD 受遗传因素影响,然而,针对孤立型 CHD 的研究更多地仍停留在遗传学检测上。利用具有孤立型 CHD 基因突变的小鼠模型将是对突变基因机制研究的重要手段,通过动物模型的研究,或许可找到供临床治疗孤立型 CHD 的新方法,提高孤立型 CHD 的预后。

利益冲突　无

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收稿日期:2023-07-25　**修回日期**:2023-09-14　**编辑**:王娜娜