ORIGINAL RESEARCH

Exploring the genetic basis of pediatrics congenital heart disease

Thammana Soma Raju[1], Diggireddy Shilpa Reddy[2]

[1]Associate Professor, [2]Assistant Professor, Department of Pediatrics, Ayaan Institute of Medical Sciences, Teaching Hospital & Research Centre, Kanakamamidi (V), Moinabad (M), R,R, Dist, Telanaga, India

Corresponding Author
Diggireddy Shilpa Reddy
Assistant Professor, Department of Pediatrics, Ayaan Institute of Medical Sciences, Teaching Hospital & Research Centre, Kanakamamidi (V), Moinabad (M), R,R, Dist, Telanaga, India

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ABSTRACT

Objective: This study aimed to elucidate the genetic, epigenetic, and environmental determinants of pediatric congenital heart disease, a prevalent congenital anomaly affecting approximately 1% of live births globally. **Methodology:** The study was conducted as a case-control observational investigation, wherein two hundred pediatric patients clinically diagnosed with CHD were compared against three hundred age-matched healthy controls with no familial history of CHD. Participants were selected based on well-defined inclusion and exclusion criteria to ensure the reliability of findings. Comprehensive demographic, clinical, and genetic histories were meticulously collected through medical records and direct patient interviews. Peripheral blood samples were obtained from all participants, followed by whole-exome sequencing and targeted genetic screening to identify deleterious genetic variants associated with CHD. Bioinformatics tools and computational modeling were employed to annotate, map, and analyze the identified mutations, integrating comparative statistical analyses to infer their pathogenic implications. Additionally, epigenetic profiling, including DNA methylation and histone modification analyses, was performed to examine non-genetic regulatory mechanisms. The role of maternal environmental factors, such as teratogenic exposure, maternal diabetes, and folate deficiency, was assessed through gene-environment interaction modeling to evaluate their contribution to CHD susceptibility. **Results:** The study identified several pathogenic genetic variants implicated in CHD, with NKX2-5, GATA4, TBX5, NOTCH1, CHD7, and SMAD3 mutations emerging as the most frequently observed. NKX2-5 missense mutations were detected in 20% of CHD patients, linked to atrial septal defects and conduction abnormalities. GATA4 frameshift mutations were identified in 15% of cases, with a strong correlation to ventricular septal defects and Tetralogy of Fallot. TBX5 nonsense mutations, occurring in 12% of affected individuals, were significantly associated with atrioventricular septal defects and Holt-Oram syndrome. The study further revealed a substantial burden of de novo mutations in CHD7, NOTCH1, and SMAD3, reinforcing their critical role in cardiac valve formation and outflow tract development. Beyond genetic variations, epigenetic modifications were identified as significant contributors to CHD pathogenesis. Aberrant DNA methylation patterns, observed in 40% of CHD patients, led to the silencing of key cardiac transcription factors, particularly NKX2-5 and GATA4, thereby disrupting normal cardiac morphogenesis. Histone acetylation modifications affected TBX5 and SMAD3, altering their transcriptional activity and developmental regulation. Additionally, non-coding RNA dysregulation, affecting NOTCH1 and CHD7, was observed in 25% of CHD cases, implicating miRNA-mediated post-transcriptional regulation in disease progression. The study also confirmed significant gene-environment interactions, wherein maternal diabetes, teratogenic exposures, folate deficiency, and prenatal infections markedly influenced CHD risk. Maternal diabetes (OR = 3.5, $p < 0.001$) was strongly associated with mutations in GATA4 and TBX5, suggesting that hyperglycemia-induced oxidative stress altered fetal cardiac gene expression. Teratogenic exposures (OR = 4.2, $p < 0.005$) increased the prevalence of CHD7 and NOTCH1 mutations, reinforcing the role of prenatal toxins in disrupting cardiac development. Folate deficiency (OR = 2.8, $p < 0.01$) was linked to NKX2-5 and SMAD3 mutations, highlighting the necessity of maternal nutritional optimization during pregnancy. **Conclusion:** This study reaffirmed the multifactorial and polygenic nature of CHD, consolidating existing research while introducing novel genetic variants and epigenetic mechanisms contributing to disease susceptibility. The findings underscored the pivotal role of monogenic mutations, de novo variants, and gene-environment interactions in shaping CHD pathogenesis. By integrating genomic sequencing, epigenetic profiling, and maternal risk factor analyses, the study provided a comprehensive framework for understanding CHD etiology. The identification of pathogenic genetic variants highlighted the potential for early genetic screening, enabling personalized risk assessments and tailored interventions. Furthermore, the epigenetic findings suggested that targeted therapies aimed at reversing DNA methylation abnormalities or modulating histone modifications could hold promise for future treatment strategies. Considering these findings, future research should aim to validate these genetic and epigenetic associations across diverse populations while exploring the therapeutic potential of gene-editing technologies, such as CRISPR-Cas9, for CHD correction. These insights paved the way for the integration of genomic data into precision medicine frameworks, enhancing early diagnostics, individualized management, and therapeutic innovations for congenital heart disease.

Keywords: Congenital Heart Disease (CHD), Genetic Variants, Whole-Exome Sequencing, Epigenetic Modifications, DNA Methylation, Gene-Environment Interactions, Maternal Risk Factors, Precision Medicine, CRISPR-Cas9, Pediatric Cardiology

BACKGROUND

Congenital heart disease is the most prevalent congenital anomaly, affecting 1% of live births globally. It encompasses a heterogeneous group of structural heart defects that arise due to aberrant cardiac development during fatal life. The clinical spectrum of CHD is remarkably diverse, ranging from minor, asymptomatic malformations to severe, life-threatening anomalies necessitating immediate medical intervention(1). Despite significant strides in cardiovascular medicine and pediatric cardiology, the precise etiological underpinnings of CHD remain an enigma. However, a growing body of evidence underscores the pivotal role of genetic determinants in concert with environmental influences in shaping CHD pathogenesis.The genetic architecture of CHD is highly intricate and multifaceted. While certain congenital heart defects are linked to well-defined chromosomal aberrations and single-gene mutations, others arise from polygenic and multifactorial inheritance patterns(2). Innovative genomic methodologies, including genome-wide association studies, whole-exome sequencing, and whole-genome sequencing, have illuminated the genetic landscape of CHD. These investigations have identified critical genes orchestrating cardiac morphogenesis, encompassing transcription factors, signaling molecules, and structural proteins. Unraveling the genetic basis of CHD is paramount for enhancing diagnostic precision, risk stratification, and the development of targeted therapeutic strategies(3).

Monogenic etiologies of CHD have been delineated in both syndromic and non-syndromic manifestations. Syndromic CHD frequently coexists with extracardiac anomalies and developmental disorders, as exemplified by Down syndrome (trisomy 21), DiGeorge syndrome (22q11.2 deletion), and Noonan syndrome(4). In these conditions, chromosomal deletions, duplications, and pathogenic mutations in key cardiac regulatory genes perturb normal cardiac morphogenesis. Conversely, non-syndromic CHD is often attributable to single-gene mutations in fundamental cardiac transcriptional regulators, such as NKX2-5, GATA4, and TBX5, which govern critical processes like septation, valvulogenesis, and chamber formation(5).Beyond monogenic contributions, recent investigations have underscored the significance of de novo mutations in CHD pathogenesis. These spontaneous genetic alterations, arising in the germline, have been implicated in a substantial proportion of severe CHD cases. High-throughput sequencing technologies have identified recurrent de novo mutations in genes such as CHD7, NOTCH1, and SMAD3, which participate in pivotal signaling pathways modulating cardiac development. These findings have reinforced the notion that novel genetic variants exert profound influences on CHD etiology(6,7).

The burgeoning field of epigenetics has further enriched our understanding of CHD pathogenesis. Epigenetic mechanisms, including DNA methylation, histone modifications, and non-coding RNAs, regulate gene expression without altering the underlying DNA sequence. Dysregulation of these epigenetic processes can disrupt normal cardiac gene expression, thereby contributing to congenital heart anomalies(8). For instance, aberrant methylation patterns in cardiac transcription factors have been implicated in congenital malformations. Moreover, maternal environmental factors, such as teratogenic exposures, maternal diabetes, and folate deficiency, may modulate epigenetic landscapes and influence fetal cardiac development.Polygenic risk factors and gene-environment interactions further compound the complexity of CHD genetics(9,10). Unlike monogenic disorders, wherein a single genetic aberration precipitates disease, polygenic CHD arises from the cumulative effects of multiple genetic variants, each conferring modest individual risk. Advances in polygenic risk assessment have facilitated the identification of variant combinations that elevate CHD susceptibility(11). Additionally, environmental modulators, including maternal health conditions, prenatal infections, and lifestyle factors, interact with genetic predispositions to modulate CHD risk, underscoring the intricate interplay between genetic and non-genetic determinants(12).

Despite significant advancements, substantial gaps persist in our comprehension of CHD genetics. A considerable proportion of CHD cases remain genetically unexplained, suggesting the existence of undiscovered genes, regulatory elements, or intricate gene-gene interactions. Future research leveraging multi-omics approaches, such as transcriptomics, proteomics, and metabolomics, will be instrumental in bridging these knowledge gaps(13). Furthermore, the advent of gene-editing technologies, notably CRISPR-Cas9, holds immense promise for functional validation of candidate genes and the development of potential therapeutic interventions.Elucidating the genetic underpinnings of CHD carries profound clinical implications. Genetic screening and counselling empower early detection, risk assessment, and personalized management of affected individuals. Moreover, deciphering the molecular mechanisms underpinning CHD lays the groundwork for innovative therapeutic paradigms, including gene therapy and targeted pharmacological interventions.

As our comprehension of CHD genetics continues to evolve, the integration of genetic insights into precision medicine frameworks will augment patient outcomes and alleviate the burden of congenital heart disease.

Aim of the study

To elucidate the genetic underpinnings of pediatric congenital heart disease and its implications for diagnosis, prognosis, and targeted therapeutic strategies.

Objective of the study

To identify and characterise genetic variants associated with CHD using advanced genomic sequencing technologies.

Methodology

The study was conducted as an observational, case-control investigation to elucidate the genetic determinants of congenital heart disease. The participant selection process was meticulously structured based on well-defined inclusion and exclusion criteria to ensure the reliability and accuracy of the findings.

Inclusion Criteria

The inclusion criteria encompassed pediatric patients clinically diagnosed with congenital heart disease, confirmed through detailed medical assessments and echocardiographic evaluations. Additionally, age-matched healthy individuals with no personal or familial history of CHD were recruited as controls to facilitate comparative genetic analyses.

Exclusion Criteria

The exclusion criteria included individuals:

- Diagnosed with acquired heart conditions,
- Chromosomal abnormalities unrelated to CHD,
- Cases with insufficient or incomplete medical records.

Data Collection

Data collection was executed through a comprehensive approach involving retrieving detailed demographic, clinical, and genetic histories from medical records and direct patient interviews. Peripheral blood samples were obtained from all participants for genomic analysis, incorporating whole-exome sequencing and targeted genetic screening to identify pathogenic variants linked to CHD.

Data Analysis

The data analysis phase employed sophisticated bioinformatics tools and statistical methodologies to discern significant genetic associations. Identified genetic variants were systematically annotated and mapped to CHD phenotypes using computational modelling. Comparative statistical analyses between CHD cases and healthy controls were performed to validate the significance of observed genetic mutations and infer their pathogenic implications in congenital heart anomalies.

RESULTS

This table provided a comprehensive overview of the demographic attributes of the study cohort, encompassing a total of 600 individuals, equally divided between CHD cases and healthy controls. The CHD group, comprising 200 pediatric patients, had been clinically diagnosed with congenital heart disease, whereas the control group consisted of 300 age-matched, healthy individuals devoid of any personal or familial history of CHD. The mean age of the CHD group was 4.5 years, marginally lower than the 4.8 years recorded in the control group. Gender distribution indicated that males constituted 60% of the CHD cohort and 58% of the control group, whereas females accounted for 40% and 42%, respectively. These demographic parameters ensured a balanced and statistically robust dataset, facilitating reliable genetic comparisons and reinforcing the validity of subsequent analytical outcomes.

Table 1: Demographic Characteristics of Study Participants

Variable	CHD Group	Control Group
Total Participants	300	300
CHD Cases	200	-
Healthy Controls	-	300
Mean Age (years)	4.5	4.8
Male (%)	60	58
Female (%)	40	42

This table delineated the diverse phenotypic manifestations of congenital heart disease, offering critical insights into its heterogeneous clinical spectrum. Septal defects, characterized by anomalies in the atrial or ventricular septum, emerged as the most frequently observed defect, affecting 35% of CHD patients. Conotruncal defects, which disrupt the proper formation of the cardiac outflow tracts, accounted for 25% of cases. Left-sided and right-sided obstructive lesions were equally prevalent, each constituting 15% of the total CHD cases, underscoring the intricate variability in structural heart malformations. Notably, 10% of CHD patients exhibited single ventricle defects, which represent some of the most severe and complex congenital anomalies necessitating early surgical intervention

and lifelong cardiac management. These findings underscored the necessity for precise diagnostic methodologies and tailored therapeutic approaches, given the considerable heterogeneity in CHD presentations.

Table 2: Clinical Features of CHD Patients

Feature	Percentage (%)
Septal Defects	35
Cono truncal Defects	25
Left-sided Obstructions	15
Right-sided Obstructions	15
Single Ventricle Defects	10

This table provided a detailed exposition of the genetic underpinnings of CHD, highlighting the key pathogenic variants identified in affected individuals. Among the most frequently implicated genes, NKX2-5 mutations emerged as the most prevalent, detected in 20% of cases, primarily manifesting as missense mutations that alter the protein's functionality. GATA4 mutations, predominantly of the frameshift variety, were present in 15% of CHD patients, leading to significant disruptions in cardiac transcriptional regulation. TBX5, an essential regulator of cardiac and limb development, exhibited nonsense mutations in 12% of cases, reinforcing its role in congenital cardiac malformations. NOTCH1, crucial for valvular and endocardial cushion formation, displayed splice-site mutations in 10% of patients. Additionally, CHD7 deletions and SMAD3 insertions, observed in 8% and 5% of cases, respectively, further underscored the profound impact of genetic dysregulation on cardiac morphogenesis. These findings underscored the critical role of genetic mutations in CHD pathogenesis, highlighting potential targets for future diagnostic screening and therapeutic interventions.

Table 3: Identified Genetic Variants in CHD Patients

Gene	Mutation Type	Frequency (%)
NKX2-5	Missense	20
GATA4	Frameshift	15
TBX5	Nonsense	12
NOTCH1	Splice-site	10
CHD7	Deletion	8
SMAD3	Insertion	5

This table elucidated the significance of de novo mutations, which arise spontaneously and are not inherited, in the pathogenesis of CHD. A strikingly higher prevalence of CHD7 mutations was observed in 18% of CHD cases compared to only 2% in the control group, with an exceptionally strong statistical significance (p-value <0.001). Similarly, NOTCH1 mutations, essential for proper cardiac morphogenesis, were detected in 12% of CHD patients, whereas only 1% of controls exhibited these alterations (p-value <0.005). SMAD3 mutations, which play a pivotal role in TGF-β signaling and extracellular matrix remodeling, were identified in 10% of CHD cases, compared to an exceedingly low frequency (0.5%) in controls (p-value <0.01). Moreover, ZEB2 and FOXC1 mutations, crucial for cardiac transcriptional regulation, were significantly more frequent among CHD patients than in the healthy cohort. These findings reinforced the hypothesis that de novo mutations contribute profoundly to CHD pathogenesis, thereby emphasizing the importance of advanced genomic sequencing in unraveling novel pathogenic variants.

Table 4: Comparison of De Novo Mutations Between CHD and Controls

Gene	CHD Cases (%)	Controls (%)	P-Value
CHD7	18	2	<0.001
NOTCH1	12	1	<0.005
SMAD3	10	0.5	<0.01
ZEB2	7	0.3	<0.05
FOXC1	5	0.2	<0.05

This table explored the pivotal role of epigenetic modifications—including DNA methylation, histone modifications, and non-coding RNA dysregulation—in the molecular etiology of CHD. Aberrant DNA methylation patterns, observed in 40% of CHD patients, were primarily associated with NKX2-5 and GATA4, leading to gene silencing and disrupted transcriptional activity. Histone acetylation

modifications, detected in 30% of cases, prominently affected TBX5 and SMAD3, resulting in aberrant gene activation, which likely contributed to maladaptive cardiac development. Additionally, non-coding RNA dysregulation, implicated in post-transcriptional gene regulation, was noted in 25% of CHD patients, significantly altering the expression of NOTCH1 and CHD7, two genes essential for embryonic heart formation. These findings underscored the intricate role of epigenetic dysregulation in CHD pathogenesis, highlighting the necessity of integrating epigenetic screening in precision medicine frameworks to enhance early detection and therapeutic targeting.

Table 5: Epigenetic Modifications in CHD Patients

Modification	Affected Genes	Effect	Percentage of CHD Patients (%)
DNA Methylation	NKX2-5, GATA4	Silencing	40
Histone Acetylation	TBX5, SMAD3	Activation	30
Non-coding RNA Dysregulation	NOTCH1, CHD7	Post-transcriptional Regulation	25

This table illuminated the complex interplay between genetic predisposition and environmental exposures in shaping CHD susceptibility. Maternal diabetes emerged as a significant risk factor, exhibiting an odds ratio (OR) of 3.5, with a strong association with GATA4 and TBX5 mutations (p-value <0.001). Teratogenic exposures, encompassing maternal contact with harmful chemicals, medications, and environmental toxins, were linked to an OR of 4.2, affecting CHD7 and NOTCH1, with a high degree of statistical significance (p-value <0.005). Folate deficiency, a well-established prenatal risk factor, demonstrated a robust correlation with NKX2-5 and SMAD3 mutations, yielding an OR of 2.8 (p-value <0.01). Furthermore, prenatal infections, particularly viral and bacterial pathogens affecting embryonic heart formation, were associated with an OR of 3.1, significantly influencing ZEB2 and FOXC1 expression (p-value <0.05). These findings underscored the imperative need for targeted prenatal interventions, emphasizing the significance of maternal health optimization in mitigating CHD risk.

Table 6: Gene-Environment Interactions and CHD Risk

Factor	Associated Genes	Odds Ratio	P-Value
Maternal Diabetes	GATA4, TBX5	3.5	<0.001
Teratogenic Exposure	CHD7, NOTCH1	4.2	<0.005
Folate Deficiency	NKX2-5, SMAD3	2.8	<0.01
Prenatal Infections	ZEB2, FOXC1	3.1	<0.05

DISCUSSION

This study provided an intricate and multidimensional exploration of the genetic, epigenetic, and environmental determinants of pediatric congenital heart disease, reaffirming previous findings while uncovering novel genetic associations and mechanistic insights. By employing whole-exome sequencing and targeted genetic screening, the research delineated pathogenic variants in key regulatory genes, including NKX2-5, GATA4, TBX5, NOTCH1, CHD7, and SMAD3, all of which serve fundamental roles in cardiac morphogenesis, septation, and valvulogenesis. These findings substantiated the conclusions drawn by Pierpont et al., who emphasized the polygenic and multifactorial nature of CHD, illustrating that both inherited and de novo mutations significantly contribute to the disease's pathogenesis(14).A particularly compelling finding of this study was the high prevalence of NKX2-5 mutations (20%), which exhibited a strong correlation with atrial septal defects and conduction abnormalities. These results resonated with the landmark study by Pierpont et al., who first demonstrated that missense mutations in NKX2-5 disrupted cardiac septation and conduction system function(15). Similarly, Chun et al., validated these findings, establishing a recurring pattern of NKX2-5-related CHD phenotypes(16). The study also observed that GATA4 mutations (15%) were significantly associated with ventricular septal defectsand Tetralogy of Fallot, reinforcing the findings of Goldmuntzet al., who established that frameshift mutations in GATA4 compromised cardiac chamber formation and valvulogenesis(17).

Further strengthening the study's findings, TBX5 nonsense mutations (12%) were identified primarily in atrioventricular septal defects and Holt-Oram syndrome, corroborating the seminal work of Mazzeuet al., (18). Their research characterized TBX5 mutations as a definitive cause of Holt-Oram syndrome, highlighting the gene's dual role in limb development and cardiac septation, a conclusion that was reinforced by this study's findings. Furthermore, this study contributed additional evidence affirming the role of NOTCH1 mutations (10%) in CHD, particularly in left-sided obstructive lesions and outflow tract malformations, aligning with the work of Lin et al., who demonstrated that NOTCH1 dysregulation leads to defective cardiac valve morphogenesis(19).Beyond inherited mutations, this study revealed a considerable burden of de novo mutations in CHD7, NOTCH1, SMAD3, and ZEB2,

reinforcing the findings of Li et al., who established that spontaneous genetic alterations play a pivotal role in severe CHD cases. The de novo CHD7 mutations (18%) identified in this study, occurring at a significantly higher frequency in CHD patients than in healthy controls (2%), reaffirmed its role in CHARGE syndrome, a disorder characterized by congenital heart anomalies, coloboma, and craniofacial abnormalities(20). These findings paralleled those of Pandit et al., who provided conclusive evidence linking CHD7 haploinsufficiency to CHD(21).

Epigenetic modifications also emerged as significant contributors to CHD pathogenesis, particularly DNA methylation, histone acetylation, and non-coding RNA dysregulation. The presence of DNA methylation abnormalities in 40% of CHD patients, affecting NKX2-5 and GATA4, substantiated the findings of Kratz et al., who demonstrated that methylation-induced gene silencing in cardiac transcription factors disrupted normal heart development(22). Moreover, the study identified histone acetylation modifications in TBX5 and SMAD3, observed in 30% of cases, reinforcing the work of Digilio et al., who underscored the crucial role of chromatin remodelling in cardiac differentiation and morphogenesis(23). Additionally, non-coding RNA dysregulation was detected in 25% of CHD patients, particularly in NOTCH1 and CHD7, suggesting that miRNA-mediated post-transcriptional regulation significantly influences CHD susceptibility. This finding aligned with the work of Aoki et al., who demonstrated that microRNAs such as miR-1 and miR-133 played a critical role in modulating cardiac gene expression, cardiomyocyte proliferation, and septation, further reinforcing their pathogenic relevance in congenital heart disease(24).

The study further delved into gene-environment interactions, uncovering a strong correlation between maternal risk factors and CHD susceptibility, a finding that echoed numerous previous epidemiological studies. Maternal diabetes (OR = 3.5, $p < 0.001$) exhibited a significant association with GATA4 and TBX5 mutations, supporting the conclusions of Schubbert et al., who proposed that hyperglycemia-induced oxidative stress alters fetal cardiac gene expression, exacerbating congenital anomalies(25). Similarly, teratogenic exposures (OR = 4.2, $p < 0.005$), including prenatal exposure to anticonvulsants, retinoic acid, and alcohol, were linked to an increased prevalence of CHD7 and NOTCH1 mutations, reinforcing the findings of Satoda et al., who demonstrated that teratogens disrupt cardiac signaling pathways, leading to congenital malformations(26).The observed correlation between folate deficiency and NKX2-5/SMAD3 mutations (OR = 2.8, $p < 0.01$) further substantiated the research of Zhu et al., who demonstrated that maternal folate insufficiency impairs DNA methylation patterns essential for fetal cardiac development(27). Likewise, prenatal infections (OR = 3.1, $p < 0.05$) were found to significantly increase CHD susceptibility, particularly through their influence on ZEB2 and FOXC1 mutations, reinforcing the findings of Robbinson et al., who provided compelling evidence linking maternal infections to congenital heart defects via inflammatory and immune-mediated pathways(28).

While this study's findings aligned with existing research, several novel contributions set it apart. Notably, the identification of previously unreported de novo mutations in ZEB2 and FOXC1 suggested their potential involvement in transcriptional regulation and cardiac neural crest cell migration, two fundamental processes in heart development. Furthermore, the integration of multi-omics approaches, incorporating genomic sequencing, epigenetic profiling, and maternal risk factor assessments, provided a holistic and comprehensive perspective on CHD pathogenesis, surpassing previous studies that often examined these factors in isolation.

CONCLUSION

This study reaffirmed the polygenic, epigenetic, and environmental underpinnings of CHD, consolidating prior knowledge while introducing new genetic variants and mechanistic insights that significantly contributed to the evolving landscape of CHD research. By elucidating the intricate interplay of monogenic mutations, de novo variants, and maternal environmental influences, the study emphasized the importance of early genetic screening, targeted prenatal interventions, and the potential for precision medicine approaches in CHD management. These findings underscored the transformative potential of gene-editing technologies such as CRISPR-Cas9, which could pave the way for therapeutic gene corrections aimed at mitigating CHD severity. Future research should focus on validating these genetic and epigenetic associations across diverse populations while exploring the therapeutic potential of targeted molecular interventions in reducing the global burden of congenital heart disease.

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