Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 03 Sep 2026
Revised 05 Oct 2026
Accepted 25 Nov 2026

Natural Resources for Human Health 2026, Vol. 6 (23s): 1249-1262

KEYWORDS:
COUP-TFII; Non-coding variants; congenital heart disease; Exon; CTCF; Pathogenic variation

Molecular Characterization of NR2F2 First Exon Identifies a Rare 5′-UTR Variant Affecting Predicted CTCF Binding in Indonesian CHD Patients.

Wahidullah Mansoor[1], Dewi Imelda Roesma[2*], Djong Hon Tjong[3], Hirowati Ali[4], Kino Kino[5], Didik Hariyanto[6]

[1]Department of Biology, Universitas Andalas, Padang West Sumatra, Indonesia, mansoorwahid0300@gmail.com, (0009-0005-7026-3935).
[2] *Professor of Molecular Genetics, Department of Biology, Universitas Andalas, Padang West Sumatra, Indonesia, Tel/ Fax: +62-751-71671, dewiroesma@sci.unand.ac.id, (0000-0002-5901-1711).
[3] Professor of Molecular Genetics, Department of Biology, Universitas Andalas, Padang West Sumatra, Indonesia, djonghontjong@sci.unand.ac.id, (0000-0002-4743-9935).
[4] Department of Biochemistry, Faculty of medicine, Universitas Andalas, Padang West Sumatra, Indonesia, hirowatiali@med.unand.ac.id (0000-0002-1720-5147).
[5] Department of Cardiology and Vascular, Faculty of medicine, Universitas Andalas, Padang West Sumatra, Indonesia, kinokino@med.unand.ac.id
[6] Department of Pediatric, Faculty of medicine, Universitas Andalas, Padang West Sumatra, Indonesia, dhf1965@yahoo.co.id

ABSTRACT:

Background: NR2F2 (COUP-TFII) is an important transcription factor involved in cardiovascular development, rare genetic variants in NR2F2 have been associated with congenital heart disease (CHD). However, this study investigated genetic variation in the first exon and adjacent regulatory region of NR2F2 in Indonesian patients with CHD.

Methods: Genomic DNA from 35 CHD patients and 27 controls was analysed by Sanger sequencing. Identified variants were annotated using Ensembl Variant Effect Predictor and evaluated using gnomAD. Potential effects on transcription-factor binding were assessed using JASPAR and FIMO with the human CTCF motif MA0139.1. Fisher's exact test were used to evaluate the association between the identified variant and CHD.

Results: This study identified a rare heterozygous NR2F2 5'-UTR variant, (c.-97A>G), in one of 35 CHD patients and was absent in the 27 controls. The variant showed an overall gnomAD allele was extremely rare in East Asian populations (0.003612%). JASPAR identified the variant within a predicted CTCF-binding motif. FIMO analysis showed a substantial reduction in motif score from 3.4021 to −0.95082, with increases in p-value and q-value. Ensembl VEP classified the variant as a 5'-UTR variant affecting the protein-coding transcript ENST00000394166.8. However, no pathogenic variants were identified within the coding region of the first exon of NR2F2 gene.

Conclusion: These findings discover that pathogenic coding variants within this exon are unlikely frequent genetic cause in Indonesian CHD patients. The study highlights the variant is a rare non-coding that may weaken a predicted CTCF-binding site. Finding

suggests a potential regulatory role that warrants investigation in larger cohorts and functional studies.

1. INTRODUCTION

Congenital heart disease (CHD) is one of the most common and serious birth defects, affecting approximately 1–3% of all live births worldwide. These conditions range from mild to severe abnormalities in cardiac structure and function(Chatwal et al., 2023). CHD is the most frequent congenital anomaly globally and remains one of the leading causes of neonatal mortality.

Most neonatal deaths occur during the first week of life and are commonly associated with prematurity, birth asphyxia, infections, or congenital anomalies. During the first five years of life, the major causes of childhood mortality include pneumonia, malaria, and congenital disorders (Chowdhury, 2007).

Congenital heart disease develops when the fatal heart fails to form normally during pregnancy. Cardiac development occurs primarily between the second and tenth weeks of gestation and is regulated by complex genetic and environmental factors, including transcription factors, non-coding RNAs, and structural proteins that precisely control the timing and pattern of heart formation,(Y. Wu et al., 2021)(Pierpont et al., 2018) . Because cardio genesis is highly complex, CHDs are relatively common. Despite major advances in medical and surgical management, CHD remains a significant contributor to infant mortality among congenital disorders(Bahado-Singh et al., 2023).

The Asia–Pacific region accounts for approximately 68.5 million of the 136.7 million births worldwide each year, with five countries contributing nearly 85% of these births, including Indonesia (Octavius et al., 2024). In Asia, CHD is the most prevalent congenital disorder, with an estimated incidence of 9.3 per 1,000 live births(Zych-Krekora et al., 2023). In Indonesia, disparities in healthcare infrastructure, geographic accessibility, and availability of specialized medical personnel pose significant challenges to early diagnosis and management of CHD, particularly in rural areas. According to population data from 2021, Indonesia has approximately 281.6 million inhabitants and a crude birth rate of 16 births per 1,000 individuals. Although precise epidemiological data are limited, CHD is estimated to affect approximately 8 per 1,000 live births, resulting in nearly 50,000 infants born with CHD annually (Octavius et al., 2024).

The Indonesian Ministry of Health estimates that approximately 12,000 infants develop heart failure secondary to CHD each year, yet only about half receive adequate treatment (Ministry of Health of Indonesia, 2023). Hospital-based studies further highlight the severity of this issue. For example, a study conducted at Dr. Soetomo Hospital in Surabaya reported CHD-related hospital mortality rates of 11.64% in 2004, 11.35% in 2005, and 13.44% in 2006, with most deaths occurring within the first year of life and frequently associated with respiratory infections such as pneumonia and bronchiolitis (Yunis et al., 2008).

The prevalence of consanguineous marriage in Indonesia is not well documented; however, this factor is of particular importance because genetic causes play a major role in CHD development. Consanguinity increases the risk of autosomal recessive disorders, including congenital heart defects(Ooko et al., 2017). Certain cultural groups in Indonesia continue to practice consanguineous marriage, and a case–control study conducted in Indonesia demonstrated a significant association between parental consanguinity and increased CHD risk in offspring (Fazeriandy et al., 2018).

Strong evidence supports a genetic contribution to CHD. Studies have demonstrated higher concordance rates of CHD in monozygotic twins compared with dizygotic twins, as well as increased recurrence risk among siblings. For example, the recurrence risk is approximately 3.4-fold higher for atrial septal defects and up to 79-fold higher for heterotaxia (Shieh et al., 2012). Populations with high rates of consanguinity also exhibit increased CHD prevalence, further supporting the involvement of recessive genetic mechanisms.

Despite this, many severe CHD cases occur in families with no prior history of the disease, suggesting an important role for de novo genetic events. These include chromosomal abnormalities, copy number variations (CNVs), and single-nucleotide variants. Aneuploidy, which affects multiple genes simultaneously, is strongly associated with CHD and is observed in approximately 35–50% of individuals with trisomy 21, 60–80% with trisomy 13 or 18, and 33% with monosomy X (Zaidi & Brueckner, 2017). Advances in genomic technologies now allow simultaneous analysis of millions of single-nucleotide polymorphisms (SNPs) to detect genomic deletions and duplications.

Recent research indicates that a diverse range of genes and genetic variants contribute to CHD pathogenesis. With the use of whole-exome sequencing (WES), whole-genome sequencing (WGS), and advanced bioinformatic analyses, pathogenic variants including missense mutations, small insertions or deletions, loss-of-function mutations, and CNVs can be identified in nearly half of CHD patients. Key genes involved in cardiac development include transcription factors such as GATA4, TBX1, TBX5, HAND1, HAND2, NKX2.5, NKX2.6, and nuclear receptors such as NR2F2 (COUP-TFII), as well as structural genes including MYH6 and CITED2 (Dianatpour et al., 2020)(Khatami et al., 2018)(Suluba et al., 2020)(Khatami et al., 2022)(Nees & Chung, 2020)(Odogwu et al., 2023)(Tabrizi et al., 2024).

The NR2F2 gene, also known as COUP-TFII, encodes three isoforms of the COUP transcription factor II protein (UniProt: P24468), which plays a critical role in cardiovascular and vascular development (Ganapathi et al., 2023). COUP-TFII regulates epithelial-to-mesenchymal transition and promotes venous and lymphatic vessel specification while suppressing arterial gene expression (Polvani et al., 2020). analysed 17 individuals with rare NR2F2 variants, of whom 15 carried de novo mutations. Several loss-of-function variants were identified, which disrupted protein activity and impaired interaction with GATA4, thereby increasing susceptibility to congenital heart defects such as double outlet right ventricle (DORV). In addition, a study conducted in an Iranian CHD cohort identified five heterozygous single-nucleotide variants in exon 2 and exon 3 of the NR2F2 gene. These novel variants included four missense mutations and one synonymous variant, all absent from public databases, with in silico analyses predicting pathogenic effects on protein function(Mansoor et al., 2025).

Although significant progress has been made in understanding the genetic basis of CHD, the underlying cause remains unidentified in more than half of affected individuals. This gap highlights the need for population-specific genetic studies; Therefore, the present study aims to investigate coding variants in first exon of the NR2F2 gene in Indonesian pediatric patients with congenital heart disease using DNA sequencing, in order to clarify the potential contribution of NR2F2 to CHD pathogenesis in this population

Ethical considerations

All stages of this study were carried out in accordance with recognized ethical standards for research involving human participants. Throughout the review and data collection process, every effort was made to ensure transparency in the selection of studies and accuracy in data analysis. The review followed established ethical research guidelines and included feedback from relevant experts to keep the findings reliable and meaningful. Ethical approval for this research was granted by the Health Research Ethics Committee of RSUP Dr. M. Djamil Hospital, Padang, West Sumatra, Indonesia (Approval No: DP.04.03/D.XVI.10.1. /380/2027). The Committee waived the requirement for WHO 2011 Standards, Scientific Values, Equitable Assessment and Benefits, Risk, Persuasion/ Exploitation, Confidentiality and Privacy, and informed consent referring to the 2016 CIOMS Guidelines because the study used anonymized clinical data collected from hospital records, with no direct contact with patients. This research was conducted among children diagnosed with congenital heart disease (CHD) at RSUP Dr. M. Djamil General Hospital, Padang, Indonesia, between October 2025 to October 2026. Clinical information, including age, sex, CHD type, and ethnicity, was gathered for all patients and used solely for research purposes (Mansoor et al., 2026).

2. MATERIALS AND METHODS

2.1 Patients Sample collection

The Blood samples were collected from the cohort of individual congenital heart diseases patients with non-hereditary congenital heart disease (CHD) and a group of healthy patients as a control from each individual into K2-EDTA tubes at M. Djamil Heart Centre in west Sumatra Padang City, Indonesia. The replication cohort included 35 individuals from different Ethnics in Padang. Also inform consent was obtained from legal guardians or their Parents. All the individual replication group of patients were diagnosed with congenital heart diseases by both a pediatric surgeon and a cardiologist and were undergoing open-heart surgery. Furthermore, 30 healthy unrelated patients with no history of any congenital heart diseases were selected as control for this study from the same geographic area with similar age and gender, All procedures followed were in accordance with the ethical standards of the responsible committee of M. Djamil Heart Centre on human experimentation (Andalas University, Indonesia) and with the Helsinki Declaration of 1975, as revised in 2000 and Informed consent was obtained from all patients for being included in the study. The tubes were stored at -20°C in the Molecular Biology Laboratory of Health Research unit of Faculty Medicine universitas Andalas. The GeneJET Genomi DNA Purifiation Kit protocol, were utilize to extract whole genome DNA, and its quality and quantity were assessed by Electrophoresis, Followed by PCR Amplification to

screen for point mutations in coding regions of two Exon of NR2F2 gene by Using two Pair of PCR Primer that we design by Geneious Prime, Gene Runner and Primer3Plus.

2.2 Mutational Genomic analysis

The Polymerase chain reaction (PCR) was performed on the sequences of first Exon of the NR2F2 gene to screen for point mutations in coding regions of exons one of the NR2F2 gene. using two pairs of primers designed via primer design bioinformatic tools Primer3Pluse (https://www.primer3plus.com/index.html), Gene Runner (http://www.generunner.com/), Geneious Prime® 2025.2.2 (Website: www.geneious.com) and Oligo Analyzer .

this primer was design (Table 1). base on gene sequences in the National Centre for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/nuccore/NG_016753.1).

Polymerase chain reaction was accomplished in a final reaction of 25 µL with the 2X Taq DNA Polymerase Master Mix kit (Amplicon Co. Ltd., Denmark). For the 25 µL reaction mixture, 3 µL of DNA (100 ng of genomic DNA) was combined with 1.0 µL of each primer (10 mM/L), 7,5 0f the NFW and 12,5 µL of 1x PCR Master Mix. The process of amplifying for Exon one of the NR2F2 gene DNA fragments set up with specific PCR conditions. In the initial denaturation step of DNA fragments were at for 5 min. Followed by 35 cycles of denaturation at 95°C for 30 seconds, A combine annealing and extension step were carried out at 64°C for 50 sec and 72°C for 5 minutes, the final incubation step was carried out at 72°C for 5 minutes. Nanodrop spectrophotometer 1000 were used to analysis DNA Concentration and Purity, For the size of PCR, product was evaluated by running them on a 1.5% agarose gel containing GEL RED and subjecting them to UV-transillumination.

All the samples were analysed by using Sanger Sequencing the results were carefully studied to investigate homozygous or heterozygous single nucleotide variations in the Exon one of NR2F2 gene.

Table 1. The pair of primer for First Exon of NR2F2 gene

Exon	Primer Sequences	Product Size (986)
Exon-1	F1-(5'- ACCAACAAAGCTGAGCCGAGAG -3')	986 (bp)
	R1-(5'-GCAGAAAGGCAGACTCCAGT-3')	

2.3 Bioinformatics Analysis

The comprehensive bioinformatic analysis was employed to characterize and identified Variant in NR2F2 gene. The Human Single Nucleotide Poly morphism database, the 1000 Genomes Project database (http://browser.1000genomes.org/) and National Centre for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/nuccore/NG_016753.1 and Geneious Prime® 2025.2.2 (Website: www.geneious.com) were used to investigate the location of variant genomic sequence and nucleotide changes in the NR2F2 gene.

The Ensembl Variant Effect Predictor were used to Identified variants were annotated for genomic location and predicted consequence, which maps variants on to annotated transcripts and classifies them by functional category, such as coding, 5'-UTR, or intronic (https://www.ensembl.org/).

For In Silico Prediction of Transcription-Factor Binding JASPAR were utilized to identified the variant within a non-coding regulatory region rather than the coding sequence and localized in the predicted CTCF-binding motif and evaluated computationally potential effect on transcription-factor binding (https://jaspar.elixir.no/).

TO Determine variant Population Frequency was previously reported and to estimate its frequency in reference populations, we utilize the Genome Aggregation Database (https://gnomad.broadinstitute.org/).

We used FIMO bioinformatic tool with the human CTCF motif to assess whether the Variants substitution could alter transcription-factor recognition and show a substantial reduction in motif score (https://meme-suite.org/), the Fisher's Exact Test were perfume to compare allele frequency and genotype polymorphisms between patients and control.

2.4 Statistical Analysis

Statistical Analysis was utilized to measured allele frequency and genotype polymorphisms of the NR2F2 gene by Use the Fisher's Exact Test to determine whether or not there is a significant association between patients and control, When the (two-sided) P-value (the probability of obtaining the observed result or a more extreme result) is less than the conventional 0.05, the conclusion is that there is a significant relationship between the two classification factors Group and Category (Society & Society, 2013).

3. RESULTS AND DISCUSSION

The orphan nuclear receptor (NR) chicken ovalbumin upstream promoter transcription factor II (COUP-TFII) regulates many biological processes such as metabolic and developmental programs including cell proliferation, survival, migration, and differentiation (Al-rayyan et al., 2015). Previous work shows that's NR2F2 as an important developmental transcription factor in the cardiovascular system many works establish that's mutation in this gene may case cardiovascular diseases, supporting the importance of appropriate NR2F2 dosage and activity during cardiac development, in this study we investigate total of thirty-five (35) pediatric patients diagnosed with congenital heart disease (CHD) and twenty-seven (27) controls. The Patients were diagnosed by cardiac surgeons and pediatric cardiologists with different type of congenital heart defects including (VSD) ventricular septal defect, (PDA) patent ductus arteriosus, (ASD) atrial septal defect, (AVSD) atrioventricular septal defect and combined VSD with PDA (Table 2) We successfully extracted high-quality genomic DNA from all blood samples and analysed the target region of Exon one of the NR2F2(COUP-TFII) gene by using Sanger sequencing, by using design primers for Specific Exon of NR2F2 gene (Table 1) Polymerase chain reaction (PCR) amplification yielded clear and specific amplicons of the expected sizes (986 bp), confirming the technical reliability of the assay, Subsequent sequence alignment and variant analysis demonstrated that all examined sequences were identical to the reference NR2F2 sequence (Mansoor et al., 2026).

We investigated NR2F2 variation in Indonesian CHD patients and identified a 5'-UTR/non-coding regulatory variant whose sequence context is predicted to alter CTCF recognition, providing a candidate regulatory mechanism that warrants functional investigation. while the variant was not detected among the 27 ethnically matched controls in this Indonesian cohort. The variant was located in the non-coding region adjacent to the coding sequence in predicted to alter CTCF recognition and was therefore considered a potential regulatory variant rather than a protein altering mutation.

Its occurrence in a patient but absence from the control group prompted further in silico analysis to investigate whether the nucleotide substitution could affect transcription-factor binding, CCCTC-binding factor (CTCF) is a highly conserved transcription factor that plays many roles in regulating transcription, including acting as an insulator that blocks interactions between certain enhancers and promoters. In silico analysis predicted a substantial reduction in CTCF motif affinity following the A>G substitution, suggesting potential disruption of CTCF-mediated regulatory activity.

Although the variant was observed only once in the present cohort, its potential biological relevance was investigated because non-coding variants can influence gene expression through modification of transcription-factor binding sites and regulatory elements. This is particularly relevant for NR2F2, which encodes the nuclear receptor COUP-TFII and has an established role in cardiovascular development. Previous human studies have demonstrated that rare NR2F2 variants are associated with CHD, while experimental studies have shown that disruption of NR2F2 function can interfere with cardiac morphogenesis and vascular development, many studies have reported rare pathogenic NR2F2 variants in patients with congenital heart defects, particularly atrioventricular septal defects and complex cardiac phenotypes (Al Turki et al., 2014)(Bashamboo et al., 2018)(Qiao et al., 2018).

Notably, A study in an Iranian CHD cohort identified five heterozygous variants in exon 2 and exon 3 of NR2F2, including four missense variants predicted to be pathogenic by in-silico analysis(Mansoor et al., 2025). In addition, loss-of-function variants and copy number deletions involving NR2F2 have been implicated in CHD, supporting haploinsufficiency as a disease mechanism (Bashamboo et al., 2018).

The c.-97A>G variant is located within the NR2F2 5'-UTR and overlaps a predicted CTCF binding motif, where the alternate G allele markedly reduces the predicted motif score, A key factor that may explain the discrepancy between our findings and previous investigation about Exon 2 and 3 of NR2F2 gene in Indonesian patients with congenital heart disease identified the rare intronic variant rs753513812 (c.970+11G>C), which was present in 8 of 35 CHD patients and absent from 27 ethnically

matched controls (Mansoor et al., 2026). These findings provided evidence that non-coding variation within NR2F2 may contribute to genetic susceptibility to CHD. Building on this observation, the present study investigated an additional non-coding A>G variant located near the NR2F2 coding region and evaluated its potential effect on transcription-factor binding using in silico motif analysis. The variant was identified in one CHD patient and was not detected among the control individuals examined in the present cohort, The absence of similar variants in the Indonesian cohort suggests that NR2F2 coding variants may be rare or absent in certain populations.

Furthermore, Methodological considerations further influence interpretation. Only coding region of NR2F2 were analysed in this study, and variants were located in 5'-UTR/non-coding regulatory regions, the untranslated regions and two of the introns of NR2F2 gene would not have been detected. Moreover, structural variants such as copy number variations affecting NR2F2, which have been implicated in CHD, cannot be identified by Sanger sequencing in coding region. Given that more than half of CHD cases remain genetically unexplained, broader approaches such as full-gene sequencing, targeted CHD gene panels, or whole-exome sequencing may provide greater diagnostic yield (Jin et al., 2018)(Y. Wu et al., 2021).

Although the variant was not significantly associated with CHD in the present cohort, in silico analysis revealed a marked reduction in the predicted CTCF-binding motif score, together with increased FIMO p- and q-values, suggesting weakened CTCF recognition.

These findings support the variant as a potential regulatory candidate and highlight the importance of investigating non-coding NR2F2 variation in Indonesian CHD. Functional studies and larger independent cohorts are required to determine whether this variant alters CTCF binding, NR2F2 expression, and susceptibility to congenital heart disease.

Table 2 : General Characteristics of the Study Population

Characteristics	**Number**
Male	19
Female	16
Age	Age/; 20 days - 15 years
VSD	12
ASD	3
PDA	8
TOF	3
AVSD	1
TGA	1
VSD + PDA	6
TGA + VSD	1

3.1 Genotype–Phenotype Correlation of Variant

The identified NR2F2 variant was observed exclusively in the heterozygous state in one of the 35 Patients (Figure 1)with no homozygous carriers detected among one the study participants. Variant-positive patients presented with congenital heart defects, including ventricular septal defect (VSD), indicating that the variant may occur in association with specific cardiac phenotypes. Notably, the variant was detected in affected individuals but was not observed among the analysed control participants (Figure 2), suggesting a possible enrichment in the CHD cohort. However, the presence of the variant in a limited number of patients does not establish a causal relationship with CHD. The observed genotype–phenotype pattern may indicate a potential contribution of this rare variant to cardiac developmental abnormalities, particularly those involving ventricular

septation, because the Variant was not detected among all control sample larger population-based studies, segregation analysis, and functional assays are warranted to determine whether the variant alters NR2F2 regulatory activity and contributes to CHD susceptibility.

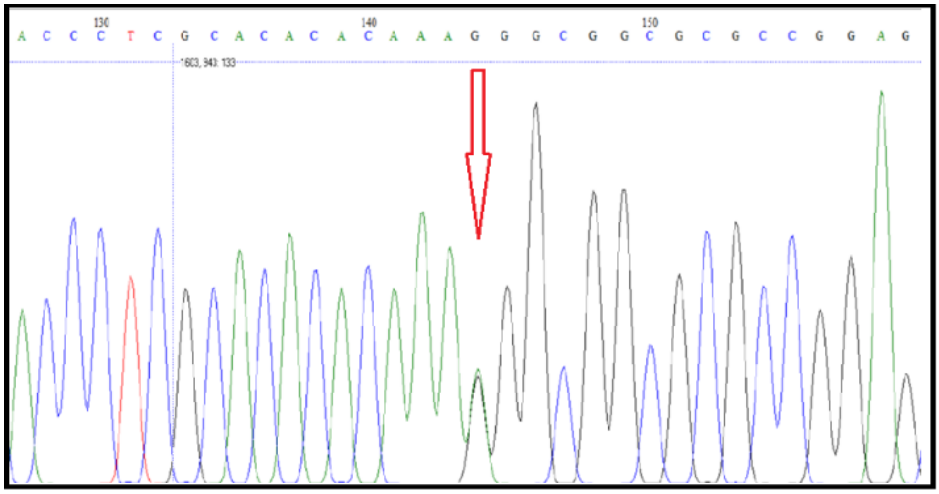

Figure 1: chr15:96332009 A>G; rs561241591

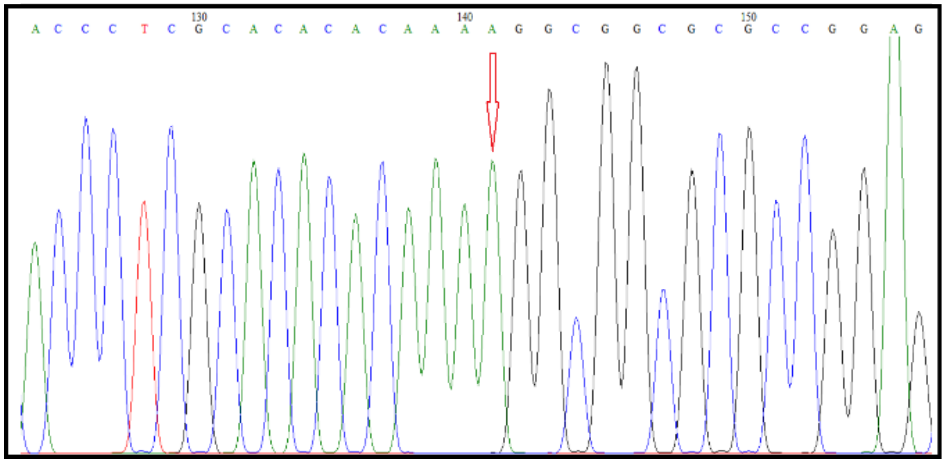

Figure 2: control - rs56124159

3.2 CTCF Binding Site Analysis of the NR2F2 5'-UTR Variant

In silico analysis using the JASPAR database identified the chr15:96332009 A>G variant within a predicted CTCF binding motif in the NR2F2 regulatory region. The predicted CTCF motif comprised 19 bp (positions 2–20) on the positive strand, with the variant nucleotide located at position 9 of the motif (Figure 3).

The reference sequence generated a motif score of 3.4021, corresponding to a relative score of 0.7424. In contrast, substitution of the reference A allele with G resulted in a markedly lower motif score of −1.0382, representing a decrease of 4.4403 points. This substantial reduction in the predicted binding score suggests that the A>G substitution may reduce the affinity or recognition of CTCF at this regulatory site. Because CTCF is an important chromatin-associated transcription factor involved in transcriptional regulation and three-dimensional genome organization, alteration of a CTCF binding site could potentially affect local regulatory activity of the NR2F2 locus. These findings therefore provide computational evidence that the chr15:96332009 A>G variant may alter transcription-factor binding within the NR2F2 regulatory region. However,

experimental assays such as electrophoretic mobility shift assays or reporter-based functional studies are required to confirm whether the variant directly affects CTCF binding and NR2F2 transcriptional regulation. (Mathelier et al., 2016).

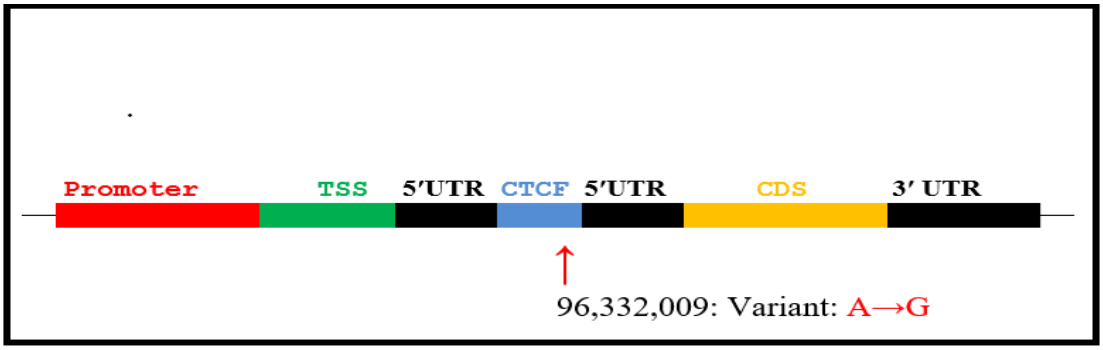

Figure 3: A>G Variant within a predicted CTCF binding motif in the NR2F2 regulatory region

3.3 POPULATION frequency and database analysis

Population frequency analysis was performed using the Genome Aggregation Database (gnomAD) v4.1.1. The NR2F2 5'-UTR variant (GRCh38: chr15:96332009 A>G; rs561241591) was observed in 3,451 of 1,210,828 alleles, corresponding to an overall allele frequency of 0.002850 (0.2850%). The variant was observed at a frequency of 0.003012 in the exome dataset and 0.001717 in the genome dataset (Table 3) Within the East Asian ancestry group, the variant was observed only once among 27,688 alleles (AF = 0.00003612; 0.003612%), with no homozygous individuals identified (Karczewski et al., 2020).

Database	Dataset/population	AC	AN	AF
gnomAD v4.1.1	Overall	3,451	1,210,828	**0.002850**
gnomAD v4.1.1	Exomes	3,191	1,059,422	**0.003012**
gnomAD v4.1.1	Genomes	260	151,406	**0.001717**
gnomAD v4.1.1	East Asian	1	27,688	**0.00003612**

Table 3: Population frequency analysis for 96332009 A>G variant

3.4 Ensembl VEP Functional Annotation of the NR2F2 Variant

Ensembl Variant Effect Predictor (VEP) analysis annotated the chr15:96332009 A>G single-nucleotide variant as a 5'-UTR variant of the NR2F2 gene (ENSG00000185551), affecting the protein-coding transcript ENST00000394166.8. The variant was therefore predicted to occur within the untranslated regulatory region rather than the protein-coding sequence. Localization of the variant to the NR2F2 5'-UTR prompted further investigation of its potential regulatory effects.

Subsequent analysis using the JASPAR database identified the variant within a predicted CTCF binding motif, in which the A>G substitution markedly reduced the predicted motif score. Together, these findings suggest that the variant may influence NR2F2 transcriptional regulation through alteration of a putative CTCF binding site, although experimental validation is required to establish its functional effect(McLaren et al., 2016).

3.5 Predicted effect of the A>G substitution on CTCF binding

To assess whether the A>G substitution could alter transcription-factor recognition, the wild-type and mutant sequences were analysed using FIMO with the human CTCF motif MA0139.1 from the JASPAR CORE database. MA0139.1 represents a human CTCF binding profile derived from ChIP-seq data (JASPAR, 2026). FIMO evaluates motif occurrences using position-

specific scoring matrices and converts motif scores into statistical significance values expressed as p-values, followed by false-discovery-rate correction to obtain q-values (Grant et al., 2011). A CTCF motif was identified at positions **2–20** on the positive strand in both the wild-type and mutant sequences. However, the A>G substitution produced a marked reduction in the motif score from 3.4021 in the wild-type sequence to −0.95082 in the mutant sequence (Table 4).

The relative score also decreased from 0.7424 to 0.6909. Consistent with this reduction in motif strength, the FIMO p-value increased from 0.0005 in the wild-type sequence to 0.00209 in the mutant sequence. The corresponding q-value increased from 0.037 to 0.154, Using the predefined FIMO reporting threshold of $p \leq 0.01$, both the wild-type and mutant sequences remained within the reported significance threshold. Nevertheless, the mutant sequence showed substantially weaker statistical support for CTCF recognition than the wild-type sequence. Importantly, the wild-type prediction had a q-value below 0.05, whereas the mutant prediction had a q-value above 0.05. Together, these findings indicate that the A>G substitution weakens the predicted CTCF-binding motif and may reduce the likelihood of CTCF occupancy at this sequence.

The approximately 4.46-unit decrease in motif score together with the increase in p- and q-values provides computational evidence that the A>G substitution alters the sequence context recognized by CTCF.

	Wild type	
Motif	MA0139.1	MA0139.1
Transcription factor	CTCF	CTCF
Position	2–20	2–20
Strand	+	+
Score	3.4021	−0.95082
Relative score	0.7424052	0.69085485
P-value	0.0005	0.00209
Q-value	0.037	0.154

Table 4: Summary of FIMO analysis for 96332009 A>G variant

3.6 Fisher's exact test

The NR2F2 5'-UTR variant (GRCh38: chr15:96332009 A>G; rs561241591) was identified in 1 of 35 CHD patients (2.86%) and was absent in the 27 controls (0%). Assuming heterozygosity in the affected individual, the corresponding allele frequency was 1.43% in the CHD group and 0% in controls.

Fisher's exact test showed no statistically significant difference in variant carrier frequency between CHD patients and controls (P = 1.00), Despite the absence of pathogenic variants, this study provides valuable evidence from an underrepresented population (https://www.medcalc.org/en/calc/fisher.php).

DISCUSSION

The present study provides preliminary evidence that a non-coding NR2F2 A>G variant identified in an Indonesian patient with CHD may have regulatory significance through alteration of a predicted CTCF-binding site, this observation is noteworthy because genetic studies of NR2F2 have predominantly focused on coding variants, whereas regulatory variation in or near NR2F2 remains less extensively investigated. Previous work established NR2F2 as an important developmental transcription factor in the cardiovascular system and demonstrated that rare human variants can contribute to CHD susceptibility, in contrast, no pathogenic or likely pathogenic variants were identified within the coding region in the first exon of NR2F2 gene in this

cohort. This finding differs from previous reports describing pathogenic or likely pathogenic missense, a loss-of-function and nonsense variants elsewhere in NR2F2 gene in patients with CHD, among CHD patients from other populations, including cohorts from Iran and Saudi Arabia, further supporting the importance of appropriate NR2F2 dosage and activity during cardiac development (Mansoor et al., 2025)(Al Turki et al., 2014)(Qiao et al., 2018).

The potential regulatory relevance of the present variant is supported by its predicted effect on CTCF recognition. CTCF is a highly conserved zinc-finger transcription factor with functions extending beyond conventional transcriptional activation or repression. It participates in chromatin organization, enhancer-promoter communication, insulation, and regulation of long-range genomic interactions(Grant et al., 2011). Consequently, a nucleotide substitution that weakens a CTCF motif could theoretically influence local chromatin architecture or transcriptional regulation without changing the amino acid sequence of the encoded protein.

The relevance of CTCF to cardiac development has also been demonstrated experimentally. Developmental inactivation of CTCF in cardiac progenitor cells causes severe cardiac abnormalities and disrupts the expression of genes involved in cardiomyocyte differentiation and maturation. These effects were associated with altered chromatin interactions and impaired enhancer-promoter communication, demonstrating that CTCF-dependent genome organization is important for normal heart development(Gomez-Velazquez et al., 2017). More recent experimental work has likewise shown that disruption of CTCF function can interfere with promoter-enhancer interactions and cardiac development(Ren et al., 2025). Therefore, the predicted alteration of a CTCF-binding site near NR2F2 is biologically plausible as a mechanism through which a non-coding variant could influence a gene involved in cardiac development.

The FIMO analysis showed a pronounced decrease in the CTCF motif score following the A>G substitution. The increase in p-value from 0.0005 to 0.00209 indicates reduced statistical support for the mutant sequence as a CTCF motif. More importantly, the q-value changed from 0.037 to 0.154, meaning that the wild-type prediction met a conventional 5% false-discovery-rate criterion whereas the mutant prediction did not. These findings are consistent with a weakening of CTCF motif recognition. However, because the mutant p-value remains below the selected FIMO threshold of 0.01, the result should not be interpreted as complete loss of the CTCF-binding site. Rather, the most appropriate interpretation is that the A>G substitution reduces the predicted strength and statistical significance of CTCF binding.

This distinction is important because motif prediction alone cannot establish physical binding of CTCF in vivo. FIMO identifies sequence patterns that resemble known transcription-factor motifs, but transcription-factor occupancy is also influenced by chromatin accessibility, DNA methylation, nucleosome positioning, cellular context, cofactors, and the three-dimensional organization of the genome(Grant et al., 2011). Thus, the present computational result should be regarded as a hypothesis-generating finding that requires experimental validation.

The location of the variant close to the NR2F2 coding region provides an additional rationale for investigating a possible cis-regulatory effect. Regulatory elements can occur within promoters, untranslated regions, introns, and other non-coding regions and may influence transcription through interactions with promoters or distal regulatory elements. Changes in transcription-factor occupancy at such sites can alter gene expression without producing a conventional coding mutation. This is particularly relevant to CHD, which has a complex genetic architecture involving coding variants, regulatory variants, structural variation, and interactions among multiple developmental pathways (Zaidi & Brueckner, 2017).

The observation of the A>G variant in one of 35 CHD patients and none of 27 controls should nevertheless be interpreted cautiously. The small sample size limits the statistical power of a case-control comparison, and the absence of the variant in controls cannot by itself establish pathogenicity.

In particular, the observation is compatible with either a rare susceptibility allele, a rare benign variant, or a variant with effects that depend on additional genetic or environmental factors. Large population datasets and independent CHD cohorts will therefore be necessary to determine whether the variant is recurrently enriched in affected individuals.

The biological plausibility of the finding is strengthened by the established relationship between NR2F2 and cardiac development. Experimental studies have shown that COUP-TFII deficiency affects atrioventricular septation, endocardial cushion formation, epicardial development, and coronary vessel morphogenesis (Pereira et al., 1999)(S. pin Wu et al., 2013). In humans, rare NR2F2 variants have been identified in patients with different forms of CHD, and functional assays have demonstrated that several coding variants can alter NR2F2 transcriptional activity(Al Turki, 2013). These observations provide

a biologically coherent framework in which a regulatory variant capable of modifying NR2F2 transcription could contribute to abnormal cardiac development. Taken together, the findings support the hypothesis that the identified NR2F2 A>G variant may influence transcriptional regulation by altering a predicted CTCF-binding motif. The substantial reduction in FIMO motif score and the increase in both p- and q-values are consistent with weakened CTCF recognition. Nevertheless, the present evidence is computational and should not be interpreted as proof of altered CTCF occupancy or NR2F2 expression. Functional studies such as electrophoretic mobility shift assays (EMSA), allele-specific ChIP-qPCR/ChIP-seq, reporter assays containing the wild-type and mutant sequences, and expression analysis of NR2F2 would be valuable for determining whether the predicted motif alteration produces a measurable regulatory effect. Such experiments would provide a stronger basis for assessing whether this rare non-coding variant contributes to CHD susceptibility.

4. CONCLUSION

The present study contributes to the understanding of genetic variation in NR2F2 among Indonesian pediatric patients with congenital heart disease, with particular attention to non-coding variation that may influence gene regulation. Unlike conventional studies that mainly focus on protein-altering mutations, our findings emphasize the potential importance of regulatory regions surrounding NR2F2. The identified c.-97A>G variant is located in the 5'-UTR of the gene and does not alter the encoded amino acid sequence. Nevertheless, its position within a predicted transcription-factor binding site provides a possible mechanism through which a non-coding nucleotide change could influence NR2F2 activity.

The biological relevance of this observation is supported by the established role of NR2F2/COUP-TFII in cardiovascular development. Appropriate regulation of NR2F2 expression is important for several developmental processes, including cardiac morphogenesis, septation, vascular development, and differentiation of cardiovascular tissues. Therefore, genetic changes that affect the regulation of this gene may have biological consequences even when they are located outside the protein-coding region. In the present study, the c.-97A>G substitution was predicted to occur within a CTCF-binding motif. The substantial decrease in the FIMO motif score after the nucleotide substitution, together with the increased p- and q-values, these findings suggest that the variant may weaken or disrupt the binding of CTCF, a regulatory protein involved in chromatin organization, enhancer–promoter interactions, and transcriptional regulation. This finding provides a plausible regulatory hypothesis and supports further investigation of the relationship between non-coding NR2F2 variation and cardiac development.

However, computational predictions alone cannot demonstrate that the variant has a functional effect. Additional studies are required to determine whether c.-97A>G alters NR2F2 transcription, chromatin accessibility, transcription-factor binding, or downstream gene regulation. Luciferase reporter assays, electrophoretic mobility shift assays, chromatin immunoprecipitation, and expression analyses in relevant cardiac or endothelial cell models could help clarify its biological significance. The small study population also limits the ability to determine its true frequency and clinical significance, Future research should also include larger case-control studies involving diverse populations are also necessary to assess the frequency and clinical relevance of this variant more accurately. Family-based analyses may further determine whether the variant segregates with congenital heart disease or occurs independently of the phenotype. More comprehensive approaches, such as whole-gene sequencing, whole-exome and analysis of structural variation, may also identify additional genetic factors that cannot be detected by targeted Sanger sequencing.

Overall, this study identified a rare **NR2F2 5'-UTR A>G variant** in one Indonesian pediatric patient with congenital heart disease, while the variant was not detected in the 27 control individuals examined. The variant was annotated within the 5'-UTR of NR2F2 and was located within a predicted CTCF-binding motif, the finding provides a potential regulatory mechanism that may affect NR2F2 transcription. However, it highlights the potential importance of non-coding NR2F2 variation and provides a foundation for future functional and population-based studies in Indonesian and other underrepresented populations.

Acknowledgements

We are deeply grateful to all patients and their parents for their cooperation to made this study Possible, we thank Prof. Dr. Syaifullah, Prof. Dr. Mansyurdin, MS, and Dr. Putra Santoso for their guidance, constructive comments, and continued support throughout this research Examiners from Biology department. We also thank the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Andalas, for its institutional support.

This research was supported by Universitas Andalas through the Penelitian Disertasi Doktor (PDD) scheme (No. 5/UN16.19/PT.01.03/PDD/2026), administered by the Rector and the Institute for Research and Community Service (LPPM) of Universitas Andalas, we are grateful from (LPPM) and Universitas Andalas for its institutional support.

Author Contributions

Wahidullah Mansoor: Conceptualization, Data curation, Methodology, Writing – review & editing.

Dewi Imelda Roesma: Methodology, Project administration, Writing – review & editing.

Djon Hon Tjon: Writing – review & editing, Project administration.

Hirowati Ali: Project administration, Writing – review & editing.

Kino, M.D and Didik Hariyanto, M.D as Pediatric cardiologist in Dr. M. Djamil Hospital, West Sumatra for kind courtesy in this research as cardiologist.

Conflict Of Interest

All authors of this manuscript have viewed and approved the manuscript. There is no conflict of interest is existed between the authors.

Ethics Statement

Ethical approval was collected for this study from Research Ethics Committee of RSUP Dr. M. Djamil Hospital, Padang, West Sumatra, Indonesia (Approval No: DP.04.03/D.XVI.10.1. /380/2027).

Data Availability Statement

The datasets used and/or analysed during the current study are available from the correspond author on reasonable request.

REFERENCES

[1] Al-rayyan, N., Litchfield, L. M., Ivanova, M. M., Radde, B. N., Elbedewy, A., & Klinge, C. M. (2015). NIH Public Access. 347(1), 139–150. https://doi.org/10.1016/j.canlet.2014.02.001.5-Aza-2-deoxycytidine

[2] Al Turki, S. (2013). Integrated approaches to elucidate the genetic architecture of congenital heart defects.

[3] Al Turki, S., Manickaraj, A. K., Mercer, C. L., Gerety, S. S., Hitz, M. P., Lindsay, S., D'Alessandro, L. C. A., Swaminathan, G. J., Bentham, J., Arndt, A. K., Low, J., Breckpot, J., Gewillig, M., Thienpont, B., Abdul-Khaliq, H., Harnack, C., Hoff, K., Kramer, H. H., Schubert, S., … Semple, R. (2014). Rare variants in NR2F2 cause congenital heart defects in humans. American Journal of Human Genetics, 94(4), 574–585. https://doi.org/10.1016/j.ajhg.2014.03.007

[4] Bahado-Singh, R., Friedman, P., Talbot, C., Aydas, B., Southekal, S., Mishra, N. K., Guda, C., Yilmaz, A., Radhakrishna, U., & Vishweswaraiah, S. (2023). Cell-free DNA in maternal blood and artificial intelligence: accurate prenatal detection of fetal congenital heart defects. American Journal of Obstetrics and Gynecology, 228(1), 76.e1-76.e10. https://doi.org/10.1016/j.ajog.2022.07.062

[5] Bashamboo, A., Eozenou, C., Jorgensen, A., Bignon-Topalovic, J., Siffroi, J. P., Hyon, C., Tar, A., Nagy, P., Sólyom, J., Halász, Z., Paye-Jaouen, A., Lambert, S., Rodriguez-Buritica, D., Bertalan, R., Martinerie, L., Rajpert-De Meyts, E., Achermann, J. C., & McElreavey, K. (2018). Loss of Function of the Nuclear Receptor NR2F2, Encoding COUP-TF2, Causes Testis Development and Cardiac Defects in 46,XX Children. American Journal of Human Genetics, 102(3), 487–493. https://doi.org/10.1016/j.ajhg.2018.01.021

[6] Chhatwal, K., Smith, J. J., Bola, H., Zahid, A., Venkatakrishnan, A., & Brand, T. (2023). Uncovering the Genetic Basis of Congenital Heart Disease: Recent Advancements and Implications for Clinical Management. CJC Pediatric and Congenital Heart Disease, 2(6), 464–480. https://doi.org/10.1016/j.cjcpc.2023.10.008

[7] Chowdhury, D. (2007). Pathophysiology of congenital heart diseases. Annals of Cardiac Anaesthesia, 10(1), 19–26. https://doi.org/10.4103/0971-9784.37920

[8] Dianatpour, S., Khatami, M., Heidari, M. M., & Hadadzadeh, M. (2020). Novel Point Mutations of CITED2 Gene Are Associated with Non-familial Congenital Heart Disease (CHD) in Sporadic Pediatric Patients. Applied Biochemistry and Biotechnology, 190(3), 896–906. https://doi.org/10.1007/s12010-019-03125-8.

[9] Fazeriandy, A., Ali, M., Saing, J. H., Tobing, T. C. L., & Adriansyah, R. (2018). Consanguinity and congenital heart disease in offspring. Paediatrica Indonesiana, 58(2), 75–79. https://doi.org/10.14238/pi58.2.2018.75-9.

[10] Ganapathi, M., Matsuoka, L. S., March, M., Li, D., Brokamp, E., Benito-sanz, S., White, S. M., Lachlan, K., Ahimaz, P., Sewda, A., Bastarache, L., Thomas-wilson, A., & Stoler, J. M. (2023). Heterozygous rare variants in NR2F2 cause a recognizable multiple congenital anomaly syndrome with developmental delays. (July). https://doi.org/10.1038/s41431-023-01434-5.

[11] Gomez-Velazquez, M., Badia-Careaga, C., Lechuga-Vieco, A. V., Nieto-Arellano, R., Tena, J. J., Rollan, I., Alvarez, A., Torroja, C., Caceres, E. F., Roy, A., Galjart, N., Delgado-Olguin, P., Sanchez-Cabo, F., Enriquez, J. A., Gomez-Skarmeta, J. L., & Manzanares, M. (2017). CTCF counter-regulates cardiomyocyte development and maturation programs in the embryonic heart. PLoS Genetics, 13(8), 1–25. https://doi.org/10.1371/journal.pgen.1006985.

[12] Grant, C. E., Bailey, T. L., & Noble, W. S. (2011). FIMO: Scanning for occurrences of a given motif. Bioinformatics, 27(7), 1017–1018. https://doi.org/10.1093/bioinformatics/btr064

[13] Jin, S. C., Homsy, J., Zaidi, S., Lu, Q., Morton, S., Depalma, R., Zeng, X., Qi, H., Chang, W., Sierant, M. C., Haider, S., Zhang, J., Knight, J., Bjornson, R. D., Tikhonoa, I. R., Bilguvar, K., Mane, S. M., & Sanders, S. J. (2018). HHS Public Access. 49(11), 1593–1601. https://doi.org/10.1038/ng.3970.Contribution

[14] Karczewski, K. J., Francioli, L. C., Tiao, G., Cummings, B. B., Alföldi, J., Wang, Q., Collins, R. L., Laricchia, K. M., Ganna, A., Birnbaum, D. P., Gauthier, L. D., Brand, H., Solomonson, M., Watts, N. A., Rhodes, D., Singer-Berk, M., England, E. M., Seaby, E. G., Kosmicki, J. A., … Xavier, R. J. (2020). The mutational constraint spectrum quantified from variation in 141,456 humans. Nature, 581(7809), 434–443. https://doi.org/10.1038/s41586-020-2308-7

[15] Khatami, M., Ghorbani, S., Adriani, M. R., Bahaloo, S., Naeini, M. A., Heidari, M. M., & Hadadzadeh, M. (2022). Novel Point Mutations in 3'-Untranslated Region of GATA4 Gene Are Associated with Sporadic Non-syndromic Atrial and Ventricular Septal Defects. Current Medical Science, 42(1), 129–143. https://doi.org/10.1007/s11596-021-2428-9

[16] Khatami, M., Mazidi, M., Taher, S., Heidari, M. M., & Hadadzadeh, M. (2018). Novel point mutations in the NKX2.5 gene in pediatric patients with non-familial congenital heart disease. Medicina (Lithuania), 54(3). https://doi.org/10.3390/medicina54030046

[17] Mansoor, W., Heidari, M. M., Khatami, M., Hadadzadeh, M., Tabrizi, F., & Darvand Araghi, M. H. (2025). Rare pathogenic NR2F2 (COUP-TFII) variants as potential etiological causes in pediatric patients with congenital heart diseases (CHDs). Hellenic Journal of Cardiology, D. https://doi.org/10.1016/j.hjc.2025.02.005

[18] Mansoor, W., Roesma, D. I., Tjong, D. H., & Ali, H. (2026). Molecular Characterization of two NR2F2 Exons identifies a Rare Intronic Variant Enriched in Indonesian Congenital Heart Disease Patients. Natural Resources for Human Health, 6(1S), 660–672.

[19] Mathelier, A., Fornes, O., Arenillas, D. J., Chen, C. Y., Denay, G., Lee, J., Shi, W., Shyr, C., Tan, G., Worsley-Hunt, R., Zhang, A. W., Parcy, F., Lenhard, B., Sandelin, A., & Wasserman, W. W. (2016). JASPAR 2016: A major expansion and update of the open-access database of transcription factor binding profiles. Nucleic Acids Research, 44(D1), D110–D115. https://doi.org/10.1093/nar/gkv1176

[20] McLaren, W., Gil, L., Hunt, S. E., Riat, H. S., Ritchie, G. R. S., Thormann, A., Flicek, P., & Cunningham, F. (2016). The Ensembl Variant Effect Predictor. Genome Biology, 17(1), 1–14. https://doi.org/10.1186/s13059-016-0974-4

[21] Nees, S. N., & Chung, W. K. (2020). Genetic basis of human congenital heart disease. Cold Spring Harbor Perspectives in Biology, 12(9), 1–40. https://doi.org/10.1101/cshperspect.a036749

[22] Octavius, G. S., Daleni, V. A., & Sagala, Y. D. S. (2024). An insight into Indonesia's progress for newborn screening program: What is currently going on. Heliyon, 10(13), e33479. https://doi.org/10.1016/j.heliyon.2024.e33479

[23] Odogwu, N. M., Hagen, C., & Nelson, T. J. (2023). Transcriptome studies of congenital heart diseases: identifying current gaps and therapeutic frontiers. Frontiers in Genetics, 14(December), 1–13. https://doi.org/10.3389/fgene.2023.1278747

[24] Ooko, E., Kadioglu, O., Greten, H. J., & Efferth, T. (2017). Pharmacogenomic Characterization and Isobologram Analysis of the Combination of Ascorbic Acid and Curcumin — Two Main Metabolites of Curcuma longa — in Cancer Cells. 8(February), 1–17. https://doi.org/10.3389/fphar.2017.00038

[25] Pereira, F. A., Qiu, Y., Zhou, G., Tsai, M.-J., & Tsai, S. Y. (1999). The orphan nuclear receptor COUP-TFII is required for angiogenesis and heart development. www.genesdev.org

[26] Pierpont, M. E., Brueckner, M., Chung, W. K., Garg, V., Lacro, R. V., McGuire, A. L., Mital, S., Priest, J. R., Pu, W. T., Roberts, A., Ware, S. M., Gelb, B. D., & Russell, M. W. (2018). Genetic Basis for Congenital Heart Disease: Revisited: A

Scientific Statement from the American Heart Association. In Circulation (Vol. 138, Number 21). https://doi.org/10.1161/CIR.0000000000000606

[27] Polvani, S., Pepe, S., Milani, S., & Galli, A. (2020). COUP-TFII in health and disease. In Cells (Vol. 9, Number 1). MDPI. https://doi.org/10.3390/cells9010101

[28] Qiao, X. H., Wang, Q., Wang, J., Liu, X. Y., Xu, Y. J., Huang, R. T., Xue, S., Li, Y. J., Zhang, M., Qu, X. K., Li, R. G., Qiu, X. B., & Yang, Y. Q. (2018). A novel NR2F2 loss-of-function mutation predisposes to congenital heart defect. European Journal of Medical Genetics, 61(4), 197–203. https://doi.org/10.1016/j.ejmg.2017.12.003

[29] Ren, H., Zhong, H., Zhang, J., Lu, Y., Hu, G., Duan, W., Ma, N., & Yao, H. (2025). CTCF Point Mutation at R567 Disrupts Mouse Heart Development via 3D Genome Rearrangement and Transcription Dysregulation. Cell Proliferation, 58(4). https://doi.org/10.1111/cpr.13783

[30] Shieh, J. T. C., Bittles, A. H., & Hudgins, L. (2012). Consanguinity and the risk of congenital heart disease. American Journal of Medical Genetics, Part A, 158 A(5), 1236–1241. https://doi.org/10.1002/ajmg.a.35272

[31] Society, R. S., & Society, R. S. (2013). FISHER 1922 - On the Interpretation of χ2from Contingency Tables, and the Calculation of P. 85(1), 87–94.

[32] Suluba, E., Shuwei, L., Xia, Q., & Mwanga, A. (2020). Congenital heart diseases: genetics, non-inherited risk factors, and signaling pathways. In Egyptian Journal of Medical Human Genetics (Vol. 21, Number 1). Springer. https://doi.org/10.1186/s43042-020-0050-1

[33] Wu, S. pin, Cheng, C. M., Lanz, R. B., Wang, T., Respress, J. L., Ather, S., Chen, W., Tsai, S. J., Wehrens, X. H. T., Tsai, M. J., & Tsai, S. Y. (2013). Atrial Identity Is Determined by a COUP-TFII Regulatory Network. Developmental Cell, 25(4), 417–426. https://doi.org/10.1016/j.devcel.2013.04.017

[34] Wu, Y., Jin, X., Zhang, Y., Zheng, J., & Yang, R. (2021). Genetic and epigenetic mechanisms in the development of congenital heart diseases. World Journal of Pediatric Surgery, 4(2), 1–14. https://doi.org/10.1136/wjps-2020-000196

[35] Yunis, K., El Rafei, R., & Mumtaz, G. (2008). Consanguinity: Perinatal outcomes and prevention - A view from the Middle East. NeoReviews, 9(2). https://doi.org/10.1542/neo.9-2-e59

[36] Zaidi, S., & Brueckner, M. (2017). Genetics and Genomics of Congenital Heart Disease. Circulation Research, 120(6), 923–940. https://doi.org/10.1161/CIRCRESAHA.116.309140

[37] Zych-Krekora, K., Sylwestrzak, O., Grzesiak, M., & Krekora, M. (2023). Impact of Prenatal and Postnatal Diagnosis on Parents: Psychosocial and Economic Aspects Related to Congenital Heart Defects in Children. Journal of Clinical Medicine, 12(18). https://doi.org/10.3390/jcm12185773.