DOI: 10.7860/IJARS/2026/80655.3104

Review Article

Anatomy Section

DNA Methylation Patterns in Down Syndrome and Congenital Heart Disease: A Short Review

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ABSTRACT

Congenital Heart Disease (CHD) is the most common structural birth defect. It arises from issues in heart formation during embryonic development. The estimated prevalence in the general population is about 1%. However, among individuals with Down Syndrome (DS), CHD affects nearly 40 to 60% of cases. DS, caused by trisomy 21, leads to genetic and epigenetic changes that affect many developmental pathways, including those important for heart formation. Epigenetic regulation, especially Deoxyribonucleic Acid (DNA) methylation, is crucial for controlling gene expression without changing the DNA sequence. Abnormal DNA methylation patterns can affect genes related to heart development such as GATA4, NKX2-5, and TBX5. This can result in structural and functional problems in the heart. In people with DS, there are widespread issues with DNA methylation, including both too much and too little methylation. These disturbances go beyond chromosome 21 and impact pathways connected to CHD. Understanding between the genetic effects of trisomy 21 and the epigenetic regulation through DNA methylation is important. Thus, present review looks at current evidence on DNA methylation patterns in DS and CHD. It highlights significant genes, regulatory mechanisms and findings specific to the Indian population.

Keywords: Chromosomal abnormality, Deoxyribonucleic acid, Epigenetics, Hypermethylation, Trisomy 21

INTRODUCTION

People with DS, a genetic condition resulting from the presence of an extra copy of chromosome 21 (trisomy 21), exhibit a significantly higher risk of developing CHDs. CHDs represent one of the most frequent and life threatening co-morbidities associated with DS. Epidemiological studies have shown that approximately 40 to 63% of individuals with DS are affected by one or more types of congenital heart malformations, in stark contrast to the estimated 1% prevalence observed in the general population [1]. These cardiac anomalies, which often include Atrioventricular Septal Defects (AVSD), Ventricular Septal Defects (VSD), and atrial septal defects, contribute notably to early morbidity and mortality in this population. Consequently, early diagnosis and timely surgical or medical management of CHDs are essential for improving survival rates and enhancing the quality of life among individuals with DS. The most common types of CHDs associated with DS include AVSD. This condition, which affects approximately 29% of individuals with DS, is characterised by a combination of ventricular and atrial septal defects (VSD and ASD). The prevalence of CHDs in people with DS has varied over time and across different geographical regions. For instance, a multi-site European study reported that among live-born infants with DS, the frequency of heart abnormalities was 43.6% [2]. Understanding the development of CHDs in people with DS requires an understanding of the function of epigenetic variables, specifically DNA methylation. One important epigenetic change is DNA methylation, which is the insertion of a methyl group to DNA that modifies gene expression without changing the underlying DNA sequence. Research has identified various Differentially Methylated Regions (DMRs) associated with CHDs in individuals with DS. For instance, a study reported 3,938 DMRs specifically in males with DS-CHD, indicating a potential male-specific epigenomic signature [3]. In the present study, the correlation between DS and CHD, along with the role of DNA methylation, will be analysed in a detailed manner.

The DS has been reported to occur in approximately one in every 700 live births. Among this population the severity of CHD is evident from its prevalence in approximately 50% of infants with DS, which is a major contrast from the general population that has been reported to occur among as low as 1% of the population [1]. In this regard, understanding the epigenetic modifications is integral as it has an influence on both the incidence of CHDs among individuals with DS and phenotypic variability associated with this chromosomal abnormality. Considering the role of epigenetic modifications in DS and CHDs, in present study, the role of DNA methylation patterns in DS and CHDs has been analysed in a detailed manner. Both global and locus-specific methylation changes can be observed in DS. Trisomy 21 results in epigenetic disruptions across the entire genome, which is not limited to chromosome 21 itself. The Nuclear Factor of Activated T-cells (NFAT) pathway is an integral regulator of heart valve and septal development. Overexpression of DYRK1A and RCAN1 results in disruption of this pathway, which leads to impaired cardiac morphogenesis [4].” COL6A1 and COL6A2 encode for collagen VI, which is associated with Extracellular Matrix (ECM) organisation. Overexpression results in abnormal ECM development in the endocardial cushions. The reviewed studies have revealed that GATA4, NKX2-5, TBX5, and NRG1 are some of the notable genes, hypermethylation of which has been associated with CHDs among individuals with DS [3]. This review comprehensively analyses existing evidence on DNA methylation and gene expression, its role in CHD in DS, the key genes involved, and the prevalence of CHD in the Indian DS population.

A considerable number of studies have been conducted on DS, CHD, and the role of DNA methylation in CHD. However, most of these investigations have been carried out independently, lacking an integrated approach. The present review aimed to bridge this gap by synthesising existing evidence to provide a comprehensive understanding of the epigenetic mechanisms linking DNA methylation, DS, and CHD.

DISCUSSION

DNA Methylation and Gene Expression

Epigenetics and DNA Methylation: Regarding the role of DNA methylation in gene regulation, in the study conducted by Moore LD

et al., the researchers reported that DNA methylation is an integral epigenetic mechanism, which is responsible for modifying DNA without changing its sequence [5]. It is done without changing the sequence, primarily by adding a methyl group to the cytosine base of DNA, it result in 5-methylcytosine. The mentioned process plays a pivotal role in regulating gene expression, which has an impact on different biological processes including differentiation, development, and disease conditions such as cancers, including colorectal, breast, lung, prostate, and liver cancers, where hypermethylation of tumour suppressor genes and global hypomethylation contribute to genomic instability and tumour progression [6,7]. In the context of gene regulation, Attwood JT et al., opined that methylation usually occurs at gene promoters, where it acts to repress transcription [8].

In the context of the role of DNA methylation in epigenetic modification, it has been established that DNA methylation induces gene silencing primarily by inhibiting transcription factor binding. To elaborate further, gene silencing occurs through the addition of a methyl group to the cytosine base of DNA, primarily at Cytosine-phosphate-Guanine (CpG) dinucleotides, leading to transcriptional repression and altered gene expression, primarily at CpG dinucleotides. The implication of DNA methylation can be observed across various biological processes including differentiation, development and diseases. This makes it further essential to discuss the mechanisms of gene silencing and the role of DNA methylation in this context. The way in which DNA methylation results in gene silencing is by inhibition of transcription factors. By blocking the binding sites for transcription factors, methylated DNA results in inhibiting gene expression. In the opinion of the researchers, when methylation occurs at or near gene promoters, it prevents these factors from accessing the DNA necessary for transcription initiation. Considering the fact that histone deacetylation is an essential process of gene regulation, its role in gene silencing is also needs to be discussed here. In the context of the role of histone deacetylation in gene silencing, it is associated with elevated Histone-DNA interaction. When histones are deacetylated it results in the restoration of the positive charge on the lysine residues. It in turn results in elevating the charge density, which is responsible for enhancing the electrostatic interactions between histones and the negatively charged DNA backbone. According to Kouzarides T, the increased positive charge on lysine residues following histone deacetylation enhances electrostatic interactions between histones and the negatively charged DNA backbone, resulting in a more condensed chromatin structure and transcriptional repression [9]. Furthermore, Histone deacetylation in context to its role in inhibiting gene expression, it is also associated with inhibition of transcription factor access. The compacted chromatin resulting from histone deacetylation makes it difficult for transcription factors along with other required proteins to access the DNA, Chen HP et al., reported that histone deacetylation creates a physical barrier that restricts the assembly of the transcriptional machinery, including RNA polymerase II, thereby suppressing gene expression [10]. Another important mechanism by which histone deacetylation contributes to gene silencing involves the recruitment of co-repressor complexes. Histone Deacetylases (HDACs) often act in conjunction with these complexes, which recognise deacetylated histones and attract additional regulatory proteins that stabilise the repressive chromatin structure, further reinforcing transcriptional inhibition. As an instance Asmamaw MD et al., stated that HDACs have the ability to interact with corepressors, for example SMRT/N-CoR, which contribute to maintaining transcriptional repression with the help of stabilising the compact chromatin structure [11].

The incorporation of methyl groups to these regions may result in physically blocking the binding of transcription factors that are integral for gene activation, effectively silencing the gene. Methylated DNA may also result in recruiting “methyl-CpG-Binding Domain proteins (MBDs)”, which are responsible for further attracting transcriptional corepressors for instance HDACs. The recruitment of these proteins results in a more compact chromatin structure, heterochromatin. Heterochromatin is less accessible for transcription. While discussing the role of DNA methylation in gene regulation, the major epigenetic modification also results in having significant impact on genomic stability through different mechanisms. The process mainly involves the addition of a methyl group to the cytosine base of DNA, mainly at CpG dinucleotides. Methylation patterns are significant for the effective functioning of DNA Damage Response (DDR) mechanisms. These pathways are essential for repairing DNA damage and maintaining genomic stability. The active nature of DNA methylation enables it to play both regulatory roles as well as protective roles in maintaining genome integrity [12]. An extensive of hypomethylation has been associated with an increase genomic instability in different types of cancer [13].

DNA methylation in developmental biology: While discussing the role of DNA Methylation in developmental biology, its impact on gene expression during cardiac development is of particular interest to the present study. In promoters, Methylation generally inhibits transcription, which is done by preventing the binding of transcription factors that result in gene silencing. In the opinion of Greco CM et al., it is of specific significance at the time of differentiation of cardiac cells where particular gene expression patterns are necessary for effective cardiac development [14]. At the time of cardiac development, various methylation patterns are established, which aid in regulating the gene expression that is associated with major processes, for instance, apoptosis and cell signaling, among other processes. Genes that are associated with muscle contraction and cardiomyopathies demonstrate differential methylation patterns that are associated with CHD phenotypes, such as “VSD”. As an instance, hypermethylation of specific GATA4, NKX2-5 and TBX5 has been associated with down-regulation of genes that are important for the cardiac functioning. “Altered methylation patterns” in CHDs may act as an epigenetic memory which results in impacting the long-term gene expression and phenotypic outcomes. Altered methylation levels of imprinted genes for example MEST and GRB10 have been associated with different types of CHDs, Tian J et al., opined that these genes are significant in growth regulation and embryonic development, which is indicative of the fact that the deregulation of these genes may result in having direct impact on cardiac development [15]. The mentioned downregulation may result in disrupting usual cardiac function and development. It has been established that particular methylation changes correlate with the type and severity of cardiac malformations. The findings of the study conducted by Grunert M et al., have established that alteration of DNA methylation has a significant role for CHDs [16]. Based on the analysis of DNA methylation and gene expression data it has been noted by the researchers that methylation alterations and “aberrant methylation of promoter CpG islands” are the mechanisms contributing to the phenotypic expression of CHDs that result in differential splicing [17]. In the opinion of the researchers majority of CHDs are based on a combination of heterozygous genetic mutations where each genetic mutations results in having small gene dosage effects at a functional level.

The polycomb group protein EZH2 has been identified as one of the important regulators of DNA methylation during cardiac development and diseases. Yuan JL et al., opined that it is responsible for mediating the repression of metabolic genes using promoter hypermethylation that results in impacting cardiac metabolism and function. KLF15 which is regulated by EZH2, has also been reported to play an important role in controlling gene expression [18]. It suppression through methylation, according to Pepin ME et al., may result in adverse remodeling in cardiac tissues under ischemic conditions [19]. Thus, based on the discussed findings of the existing studies, it can be inferred that the impact of DNA methylation in CHDs is significant, which may result in impacting gene expression, interactions with different genetic factors, and disease progression.

DNA Methylation in CHD in DS

Global Methylation Patterns in DS: When it comes to the correlation between DNA Methylation and DS it can be opined that DS which is primarily caused by an extra copy of chromosome 21 (trisomy 21) is associated with major alterations in DNA methylation across the genome. These epigenetic changes have major implications for gene expression and developmental outcomes in individuals with DS. In the studies conducted in this arena, a large number of Differentially Methylated Positions (DMPs) and Regions (DMRs) in different tissues of individuals with DS have been observed. In the study conducted by Henneman P et al., "Widespread domain-like perturbations of DNA methylation in whole blood of DS neonates", the researchers have found 121,953 significant DMPs where 49.8% have been estimated to be hypomethylated and the remaining have been hypermethylated [20]. In this alignment, in the study conducted by Naumova OY et al., more than 4,000 DMPs and 115 DMRs have been reported to be found in blood cells from toddlers with DS [21]. The mentioned findings are indicative of the widespread methylation alterations, which are observed in a consistent manner throughout various developmental stages. In the context of DS, the effect of trisomy 21 is specifically significant on chromosome 21 itself, where the number of DMPs is located. However, Mendioroz M et al., also opined in this regard that major changes can also be noted in other chromosomes, which is indicative of the fact that the epigenetic landscape in DS is not completely determined by the additional genetic material on chromosome 21, it also involves broader genomic interactions [22]. The observed methylation changes are associated with various developmental impairments characteristic of DS, which include various health issues and intellectual disabilities. In the opinion of Li E, genes associated with transcription regulation and chromatin remodeling undergo major methylation alterations, which may result in disrupting usual developmental pathways [23]. The findings determine the "genome-wide perturbations in the DNA methylation" of various tissues and cells of individuals with DS, neuronal tissue, buccal epithelial cells and blood cells [24]. It has also been observed in the study that methylation disturbances occur in the genes that are associated with cardiovascular system conditions, along with myeloid leukaemias, metabolic diseases, neuronal disorders, and haematopoietic disorders that have high prevalence among individuals with DS. It is also worth mentioning here that dysregulated gene expression during the significant developmental years may result in disrupting pathways that are essential for organ formation and development. The study associated with DS has indicated that inflammatory pathways are significantly impacted among individuals with neurodevelopmental disorders, including DS [25]. In the study conducted by Li T et al., it has been reported that GATA4 and NKX2-5, which are integral for cardiac development, may lack effective regulation that results in having an adverse impact on cardiac functioning and morphology [26]. Thus, based on the discussed findings based on the existing studies, it can be opined that genome-wide differences in DNA methylation that are noted in individuals with DS emphasise on the complex relationship between epigenetic factors and genetic factors in shaping the clinical manifestation of the disorder [27].

Hypomethylation and Hypermethylation in DS: In the study conducted by Naumova OY et al., in regard to the specific methylation patterns that are commonly observed in DS, it has been revealed in the study that the majority of DS-associated DNA methylation differences that are observed among children with DS are hypermethylation cases [21]. An 83.5% of the DMRs and 82% of the DMPs have been reported to be hypermethylated. It has also been stated in this context that trans-hypermethylation in DS because of trisomy 21 is indicative of the fact that hypermethylation may be because of elevation in chromosome 21 genes that are associated with methylation pathways, for instance, DNMT3L, PRMT2, CBS, and SLC19A1, among others, and/or, because of abnormal patterns of specific transcription factors binding sites, for instance, sites for RUNX1 and CTCF occupancies [21]. In addition to that, based on the findings from the study conducted by Mendioroz M et al., a trend of global hypermethylation have been observed in a consistent manner in DS foetal and adult brain tissue [22]. It has also been reported to be observed in placenta tissue. Thus, an equal amount of hyper- and hypomethylated cases have been noted in the blood of newborns with DS. In the study by Mendioroz M et al., [22], both hypermethylation and hypomethylation were observed in peripheral T lymphocytes of adults with DS. Altered methylation of genes involved in cardiac function may disrupt normal heart development and contribute to the increased incidence of CHDs in this group, It has been reported that NKX2-5 plays a pivotal role in the heart development. Alterations in its expression because of methylation changes may result in structural heart defects. GATA4's dysregulation through methylation can disrupt normal heart formation. TBX5 which has been associated with cardiac morphogenesis, is also affected by methylation patterns that can contribute to CHD. It is worth mentioning here that changes in methylation may result in impacting signaling pathways that are integral for heart development, for instance, Wnt/β-catenin and Notch, which are significant for effective cardiac cell proliferation and differentiation [22].

Differentially Methylated Regions (DMRs): The DMRs primarily refer to particular genomic areas that are characterised by variations in DNA methylation status across different biological samples [25].

DMRs serve as key epigenetic markers for distinguishing individuals with DS-associated CHD from those with DS without CHD, enabling the identification of disease-specific methylation signatures." In the study conducted by Mouat JS et al., the researchers have considered conducting a comprehensive analysis, where it has been revealed that 1,052 DMRs associated with have been observed specifically at promoter and enhancer regions that are associated with gene expression changes [3]. For comparing DMRs among healthy individuals, Dhar GA et al., considered comparing fetal heart DNA from individuals with congenital malformations to the control population [28]. In this context, Moore-Morris T et al., (2018) conducted a study demonstrating that hypermethylation at specific genomic loci can lead to transcriptional repression, thereby affecting developmental pathways critical for cardiac formation [29].Tanaka Y et al., further put forward the observation that hypermethylation can be noted in major regulatory genes for instance FLI1 and RUNX1 which are essential for haematopoiesis [30]. The observations associated with differential methylation patterns may result in impacting gene expression and in turn contribute to the pathogenesis of CHDs in DS [31]. In the study conducted by Tabish AM et al., the researchers on conducting pathway analyses of DMR-associated genes revealed that it demonstrates enrichment in biological processes that are associated with muscle contraction and cardiomyopathies, which highlights the functional relevance of these epigenetic alterations [32]. The findings revealed that there exists a major difference in methylation between the two groups of the population. According to researchers Yuan X et al., on average, approximately three regions per sample demonstrated aberrant methylation, with particular genes implicated in growth regulation and apoptosis being affected [33].

In a study analysing global CpG methylation patterns, several DMRs were found to be hypermethylated in individuals with DS-CHD compared to those without CHD [3]. Individuals with DS with CHD have also been reported to demonstrate global hypomethylation, which has been specifically observed among males, as compared to DS individuals without CHD. In the opinion of the researcher the hypomethylation is associated with increased levels of nucleated red blood cells, which is not observed among females. A considerable number of DMRs have been identified, 58 common DMRs have been reported among both the genders, wherein, based on DMR Gene Ontology PIK3CA, FGF12, TNNI3, TFAP2B among other genes, have been mentioned to be associated with cardiac muscle contraction, ductus arteriosus closure among the other issues.

Cardiac Development Genes: Another notable gene associated with cardiac development is Mef2c. According to the study conducted by Moustafa AM, it is involved in the development of cardiomyocytes and the regulation of muscle-specific genes [34]. Findings of the study by Clapham KR et al., have revealed that Mef2c plays a pivotal role in cardiac morphogenesis and organogenesis [35]. It has been opined in this context that Mef2c is associated with severe defects, including failure of looping morphogenesis and the inability to develop a right ventricle, leading to embryonic lethality. It has been further observed in this regard that in mouse models with a null mutation of Mef2c, there occurs a major disruption in cardiomyocyte differentiation and overall heart structure. Referring to the observations from previous studies Clapham KR et al., further stated that expression of Mef2c is integral for the effective development of the cardiac outflow tract and right ventricle, which is indicative of critical function during early heart development [35], while analysing the regulatory mechanisms of Mef2c opined that Mef2c interacts with other transcription factors for instance, GATA4 and NKX2-5 which are also vital for cardiomyocyte differentiation. In regard to its pathophysiological implications Wales SE further suggested that its expression increases in conditions for instance as hypertrophy and pressure overload, which indicates a dual role in both normal development and pathological states [36, 37]. Isl1 is another mentionable transcription factor which has been reported to be associated with cardiac development George RM and Firulli AB stated that Isl1 marks progenitor cells in the Second Heart Field (SHF), contributing to the formation of the right ventricle and outflow tract [38], Gao R while analysing the functioning of Isl1 stated that it is primarily associated with the SHF, which contributes to the formation of both atrial and ventricular structures in the heart [39]. Zhao K and Yang Z opined that Isl1 for SHF progenitors acts as a marker and is essential for the proper development of the venous pole of the heart, influencing the formation of the right ventricle and outflow tract [40]. Based on the study by Lu J et al., a trend of global hypermethylation has been observed in a consistent manner in DS fetal and adult brain tissue [41].

Genes implicated in DNA methylation and Congenital Heart Disease (CHD): GATA4 Gene Methylation

The GATA4 gene, plays a pivotal role in cardiac development. Among individuals with DS it is often noted to be hypermethylated. Its function can be significantly adversely impacted by hypermethylation of its promoter region. This epigenetic alteration has been associated with various cardiac anomalies, including CHDs. It has been established in the existing studies that hypermethylation of GATA4 is associated with lowering the expression levels of this transcription factor. According to the study conducted by Serra-Juhé C et al., hypermethylation of GATA4 can be observed in fetuses with CHDs including those with DS [42]. It is indicative of the fact that epigenetic alteration can contribute to the pathogenesis of CHDs. Impaired GATA4 function because of hypermethylation has also been associated with structural heart defects for instance atrial septal defects. Thus, based on the reported findings of the existing studies it can be inferred that hypermethylation of the GATA4 promoter is one of the major factors contributing to impaired heart formation. This epigenetic modification not only impacts the expression of GATA4 but also hinders the critical developmental pathways essential for normal cardiac development. An understanding of these mechanisms can contribute to providing insights into potential therapeutic targets for treating or preventing CHDs associated with GATA4 dysregulation.

Other than GATA4, NKX2-5, and TBX5 which are the major genes associated with cardiac development that have already been discussed in the above section, hypermethylation of the NRG1 gene has also been associated with the occurrence of heart defects among individuals with DS. According to the study conducted by Dobosz A et al., significant hypermethylation of the NRG1 gene promoter region can be observed among children with CHD with DS [43]. It is indicative of the fact that alterations in methylation patterns may result in the pathogenesis of CHDs in DS patients. MTHFR C677T Polymorphism has been observed to be significant in impacting LINE-1 DNA among mothers of children with DS and CHDs. The underlying reason behind this is a low-folate diet and the CT+TT genotype. DYRK1A ("dual-specificity tyrosine phosphorylation-regulated kinase 1A) and RCAN1("regulator of calcineurin 1") genes which are located on chromosome 21, have been reported to be implicated in abnormal heart development. Their deregulation may result in increasing the risk of CHDs in DS because of overexpression linked to trisomy.

Population-Specific Findings: DNA Methylation in India

Prevalence of CHD in Down Syndrome (DS) in India: The global prevalence of CHDs in DS has been reported to be upto 50%. However, in the Indian scenario, specific regional studies are limited. According to the study conducted by Fulse AC et al., which focused on Vidarbha Region Central India reported that among the 374 diagnosed cases of CHD considered in the study [44], four males and three females were confirmed with the DS, which represented 1.87% of the population. According to the study conducted by Kava MP et al., the prevalence of DS in the Indian scenario can be observed to have regional variations, wherein the highest prevalence has been reported in Madhya Pradesh, with one in 692 cases [45].

Methylation patterns in indian populations: In the study conducted by Muskens IS et al., the researchers considered conducting a multiethnic study [46]. The study identified more than 1,000 DMRs that correlate with gene expression changes in DS individuals across different populations. It is indicative of the fact that certain methylation patterns are consistent for instance those affecting haematopoiesis, while others may be impacted by local genetic backgrounds. In the Indian context, in the study conducted by Asim A and Agarwal S, it has been demonstrated that MTHFR hypermethylation and CRELD1 gene mutations may result in contributing to cardiac defects and AVCD respectively among DS patients [47,48].

FUTURE DIRECTIONS AND RESEARCH GAPS

Based on the reviewed studies it can be noted that there are limited studies conducted in the area of DNA methylation, DS and CHDs in the Indian context. In future studies, emphasising on understanding the specific DNA methylation patterns in Indian populations with DS is essential for developing targeted therapeutic strategies. In addition, comparative studies between the Indian and global population associated trends can also help in improving the existing knowledge on the ways in which environmental and genetic factors develop the epigenetic landscape in DS.

CONCLUSION(S)

It has been observed that GATA4, NKX2-5, TBX5, NRG1, DYRK1A, and RCAN1 are some of the notable genes, hypermethylation of which have been revealed to be associated with CHDs among individuals with DS. Thus, improved therapeutic approaches and diagnostic markers may result from knowledge of the roles played by DNA methylation and other epigenetic changes in the pathophysiology of CHDs in DS. In addition to that, it has also been observed in this study that an understanding of and data pertaining to the prevalence of DS and CHDs in the Indian scenario is lacking, which clearly highlights the need for further studies in this arena in the Indian scenario.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jun 12, 2025
- Manual Googling: Jan 20, 2026
- iThenticate Software: Jan 28, 2026 (9%)

ETYMOLOGY: Author Origin

EMENDATIONS: 8

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? No
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **May 30, 2025**
Date of Peer Review: **Sep 15, 2025**
Date of Acceptance: **Jan 29, 2026**
Date of Publishing: **May 01, 2026**