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FELLOWS FORUM

WILEY Congenital Heart Disease

Epigenetics for the pediatric cardiologist

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ABSTRACT

A genetic basis of congenital heart disease (CHD) has been known for decades. In addition to the sequence of the genome, the contribution of epigenetics to pediatric cardiology is increasingly recognized. Multiple epigenetic mechanisms, including DNA methylation, histone modification, and RNA-based regulation, are known mediators of cardiovascular disease, including both development and progression of CHD and its sequelae. Basic understanding of the concepts of epigenetics will be essential to all pediatric cardiologists in order to understand mechanisms of pathophysiology, pharmacotherapeutic concepts, and to understand the role of epigenetics in precision medicine.

KEYWORDS

congenital heart disease, DNA methylation, Epigenetics, histone modification, microRNA, precision medicine

1 | INTRODUCTION

A genetic basis of congenital heart disease (CHD) has been recognized for more than 30 years, but the significant genetic contribution to CHD was underestimated until 10–15 years ago.[1] Despite this recognition, we identify a specific genetic etiology in only a minority of cases of sporadic CHD.[2] Epigenetics is being increasingly acknowledged as a key mediator of cardiovascular disease, including both development and progression of CHD and its sequelae. Specifically, epigenetics is known to play a role in the pathophysiology of multiple aspects of cardiovascular health including, but not limited to, development of CHD, aortic valve stenosis, heart failure, cardiac hypertrophy, and cardiac fibrosis. Pediatric cardiologists should be familiar with the basic concepts of epigenetics in order to understand mechanisms of pathophysiology, pharmacotherapeutic concepts, and to recognize the role of epigenetics in precision medicine.

2 | OVERVIEW OF EPIGENETICS

Beyond the strict sequence of the genome, epigenetics refers to a set of mechanisms that regulate gene expression without changing the underlying nucleotide sequence. Epigenetic inheritance is a necessary process as progenitor cells differentiate into more specialized cells. Thus, epigenetic mechanisms maintain stable gene expression in differentiated cells to define their cellular identity. Epigenetic modifications are also dynamic and responsive to external stimuli. Epigenetics may help explain genetic heterogeneity and complex physiologic processes. Epigenetic regulation is achieved through multiple mechanisms, and the principle mechanisms in humans include DNA methylation, histone modification, and RNA-based regulation.

For the clinician to understand the various types of epigenetic modifications, a brief review of DNA structure is helpful. DNA is stored and packaged in the nucleus as tightly wrapped chromatin in one of two forms: (1) euchromatin or (2) heterochromatin. Euchromatin, which makes up the majority of the genome, is less tightly packed DNA and is thus more readily transcribed into RNA. Heterochromatin, on the other hand, is more tightly packed DNA, which makes the DNA less available for RNA polymerase to transcribe RNA.

Chromatin itself is made up of nucleosomes, which consists of a DNA strand wrapped twice around a histone core—more specifically, an octamer of 4 histone proteins. The components of a nucleosome—DNA and histone—are the primary targets of epigenetic modifications. Generally, DNA is modified by methylation, and histones are modified by methylation, acetylation, phosphorylation, ubiquitylation, and sumoylation. Importantly, a single epigenetic modification does not regulate gene expression alone. Rather, expression is influenced by the complex interplay of multiple epigenetic modifications.

In addition to these structural regulations of transcription, various RNA molecules can direct epigenetic modifications. Noncoding RNAs

Abbreviations: BAV, bicuspid aortic valve; CHD, congenital heart disease; HDAC, histone deacetylase; HLHS, hypoplastic left heart syndrome; ncRNA, noncoding RNA; mRNA, messenger RNA; 5mC, 5-methylcytosine; miR, microRNA; lncRNA, long noncoding RNA; TOF, tetralogy of Fallot.

(ncRNAs) can regulate DNA transcription, or more commonly, can regulate RNA translation (i.e., post-transcriptional regulation). That is, they can inhibit or stabilize messenger RNA (mRNA) prior to translation into protein.

3 | DNA METHYLATION

DNA methylation is the most studied and well-understood mechanism of epigenetics. During this process, a methyl group is covalently added to the 5 position of a cytosine nucleotide preceding a guanosine nucleotide to create 5-methylcytosine (5-mC). A cytosine nucleotide preceding a guanosine nucleotide is referred to as a CpG dinucleotide. CpG dinucleotides are enriched in CpG islands, which are defined as a segment of DNA of ≥ 200 base pairs with CpG frequencies $> 60\%$.[3] Most gene promoter regions are located within CpG islands. Methylation of CpG islands in the promoter region of genes leads to decreased gene expression.

DNA methylation is not restricted to CpG islands.[4] More recent studies describe DNA methylation within so-called CpG shores and within the gene body itself.[5] DNA methylation in these regions can lead to varying degrees of gene regulation and transcription, not necessarily gene silencing.

4 | HISTONE MODIFICATION

Histone octamers consist of two copies of each of the four core histone proteins: H2A, H2B, H3, and H4. A strand of DNA then wraps twice around a histone octamer to form a nucleosome, which serves as the basic structural unit of chromatin.

While DNA methylation is traditionally the addition of a methyl group to the promoter region of a target gene causing decreased transcription, histone modifications are more complex. Modifications to histone occur on amino acid residues on the N-terminal tail of the histone protein. There are numerous types of histone modifications with variable effects on DNA transcription. That is to say that histone modifications fine-tune gene expression to either increase or decrease transcription. Briefly, histone modifications usually include changes to lysine (via methylation, acetylation, ubiquitylation, and sumoylation), arginine (via methylation), and serine and threonine (via phosphorylation).[6] The specific effect of each modification depends on the location and degree of modification.

In contrast to the steric hindrance that DNA methylation causes, histone modifications alter how DNA wraps around histones and thus the availability of DNA for transcription. For example, acetylation of lysine can neutralize the negative charge of lysine, loosen DNA binding to histone, and activate transcription. Histone modifications can also create binding sites for specific proteins that alter transcription. For example, acetylation of lysine may create a binding site for chromatin modifying enzymes with subsequent activation of transcription.[7]

Histone modifications are described with a sort of "alphabet soup" to the clinical pediatric cardiologist. It is worth briefly elaborating on the standard nomenclature for specifying histone modifications. In the order they are written: (1) the specific histone protein; (2) the modified amino acid residue; and 3) the type of modification.[8] Example: H3K4me3 = histone protein H3, lysine in the position of the fourth amino acid residue (K4), and the modification is addition of three methyl groups (tri-methylation of lysine 4 of histone H3); H3K27ac = acetylation of lysine 27 of histone H3; and H3K9me1 = mono-methylation of lysine 9 of histone H3.

5 | RNA-MEDIATED REGULATION

Noncoding RNA (ncRNA) includes both RNA involved in "housekeeping" cellular processes (i.e., transfer RNA, ribosomal RNA, etc.) and RNA involved in gene regulation. Gene regulatory ncRNA primarily includes microRNA (miR), long noncoding RNA (lncRNA), small interfering RNA, antisense RNA, and piwi-interacting RNA.[9] MiRs are the best studied group of ncRNAs. For simplicity, this review will focus on miRs.

MiRs are small, evolutionally conserved noncoding RNA strands ~20–22 base pairs long. More than 1000 miRs are expressed in humans, and they regulate up to 30% of human genes with each miR potentially regulating multiple genes.[10] MiRs mostly decrease gene expression by targeting mRNAs for cleavage or translational repression.[11] Less frequently, though, they can enhance gene expression by interacting with other epigenetic modifiers or transcription factors.[12]

The study of miRs has been a growing focus over the past decade, and studies demonstrate their vital role in development, including heart development, cardiac remodeling, and cardiac disease.[13–17]

6 | IMPACT OF EPIGENETICS IN CONGENITAL AND ACQUIRED PEDIATRIC HEART DISEASE

Before reviewing disease-specific epigenetic changes, it is worth highlighting the landmark article from the Pediatric Cardiac Genomics Consortium in 2013, which underscores the critical role that epigenetics plays in congenital heart disease.[18] They evaluated exome sequencing from 362 parent-offspring trios comprising a child with severe CHD and unaffected parents compared to 264 control trios without CHD. In these cases, they found a high rate of de novo variants in histone-modifying genes. Specifically, they found de novo variants contributing to approximately 10% of severe CHD and many of these variants were in genes associated with histone modifications. Five of the genes identified are even involved in a single specific histone modification (H3K4me3), which is associated with transcriptional up-regulation. As previously mentioned, epigenetic modifications fine-tune gene expression. These findings reinforce the concept that "gene dosage" is vital to the development of CHD, and further raises the possibility of the interaction between environmental stimuli, epigenetic changes, and critical developmental pathways.

Multiple excellent reviews focus on epigenetics and cardiovascular disease in adults.[9,19–21] These mechanisms similarly apply to pediatric cardiovascular disease, and, in fact, some are unique to pediatric cardiology. Here, we will highlight several mechanisms that relate to pediatric heart disease, including both congenital and acquired heart disease.

6.1 | CHD

The list of syndromes and single gene defects associated with CHD is well reviewed and continues to grow.[14] Abnormal transcriptional regulation of these same genes is also associated with CHD. For example, Sheng et al. recently reported aberrant DNA methylation of *NKX2–5* and *HAND1* in patients with tetralogy of Fallot (TOF).[22] This aberrant DNA methylation was associated with decreased mRNA expression of these two transcription factors, suggesting that DNA methylation plays a key role in transcriptional regulation and pathophysiology of TOF. Similarly, the histone methyltransferase G9a is well-known to regulate the expression of *MEF2C*, a transcription factor associated with outflow tract anomalies.[23–25] While we are learning the importance of transcription factors in the development of CHD, it will be vital to understand the epigenetic regulation of transcription factors.

6.2 | Aortic valve stenosis

Bicuspid aortic valve (BAV) is the most common type of congenital heart disease and a significant risk factor for development of aortic stenosis due to acquired calcific aortic valve disease.[26] Initial work of the genetics of BAV showed that *NOTCH1* variants caused calcific aortic valve disease, both familial and de novo variants.[27,28] Interestingly, further study also showed that *NOTCH1* variants are involved in other forms of CHD including tetralogy of Fallot.[29]

Study of the epigenetic control of *NOTCH1* shows that hypomethylation of the promoter of a lncRNA (lncRNA *H19*) is associated with increased expression and subsequent dysregulation of *NOTCH1*.[30]

Additionally, previous work identified specific miR profiles of human BAV leaflets compared to human controls with tricuspid aortic valves. Tissue was obtained from patients undergoing elective valve replacement for aortic stenosis. They found 35 differentially expressed miRs, including miR-141, which was down-regulated 14.5-fold in patients with BAV.[31] Further, using porcine aortic valvular interstitial cells, they reversed calcification with miR-141 transfection. While this study does not show causality with these miRs, it shows the therapeutic potential of miRs.

6.3 | Heart failure

Heart failure may serve as the prototypical example for the role of epigenetics. Heart failure has long been viewed as a complex interaction of genetic and environmental factors. Epigenetics serves as a link between environmental exposures and genetic predisposition to the cardiac phenotype of heart failure. As reviewed by Papait et al. multiple epigenetic mechanisms accompany the gene expression changes associated with pathologic cardiac remodeling and clinical syndrome of heart failure.[32]

For example, *Brg1*, a chromatin-remodeling protein, has a critical role in regulating cardiac hypertrophy and failure. *Brg1* interacts with two other classes of chromatin-modifying enzymes, histone deacetylase (HDAC), and poly-ADP-ribose polymerase (PARP), to regulate gene expression during cardiac growth, differentiation, and hypertrophy in mice. Cardiac stress (increased afterload via transverse aorta constriction) activates this complex in adult mice to induce pathologic myosin heavy chain isoform switching, which is associated with cardiomyopathy and heart failure.[33,34] Further, they showed that patients with hypertrophic cardiomyopathy had a decreased ratio of α-MHC:β-MHC expression and increased *Brg1* expression, and that the level of *Brg1* expression correlated well with the α-MHC:β-MHC ratio in both controls and hypertrophic cardiomyopathy subjects.

Studies have shown not only association of epigenetic changes with heart failure, but also epigenetic changes associated with reverse remodeling after treatment with a beta blocker or left ventricular assist device.[35,36] Miyamoto et al. also showed that circulating miRs are differentially expressed between children with dilated cardiomyopathy who recover versus those requiring a transplant.[37] These differences may represent a valuable biomarker for risk stratification, as well as a potential molecular therapeutic target.

6.4 | Cardiac fibrosis

Multiple miRs are associated with development and/or progression of interstitial fibrosis. Van Rooij et al. demonstrated that mice with knockout of miR-208 did not develop fibrosis in response to aortic banding, whereas wild-type mice developed left ventricular fibrosis.[38] Additionally, inhibition of miR-208 by antisense oligonucleotide (anti-miR) during hypertension-induced heart failure in rats prevented cardiac fibrosis.[39] While miR-208 is associated with pro-fibrotic changes, other miRs are associated with anti-fibrotic changes. For example, *CTGF* (connective tissue growth factor), a pro-fibrotic growth factor, is a target gene of miR-133. Knockout of miR-133a in mice was associated with severe cardiac fibrosis and heart failure, indicating that miR-133a is potentially associated with anti-fibrotic changes by down-regulation of *CTGF*.[40]

6.5 | Cardiac hypertrophy

In addition to pro-fibrotic changes, miR-208 is associated with pro-hypertrophic changes. MiR-208 is known to target *Mstn* (myostatin) and *Thrap-1* (thyroid hormone associated protein (1), which both suppress cardiac hypertrophy.[38] Inhibition of miR-208a by anti-miR in hypertensive rats, as described earlier, prevented cardiac hypertrophy and pathologic myosin switching.[39]

6.6 | Hypoplastic left heart syndrome and single ventricle physiology

Recent investigation in patients with hypoplastic left heart syndrome (HLHS) shows that while some miRs have differential expression based on stage of palliative surgery (miR-99a, miR-100, and miR-145a), others were consistently dysregulated independent of palliative stage (miR-208 and miR-378).[41] While these differences do not yield insight into development of HLHS, miR expression may help provide insight into myocardial changes during single ventricle palliation, or perhaps provide therapeutic targets in a vulnerable population. For example, inhibition of miR-208 is associated with decreased cardiac fibrosis, hypertrophy, and myosin switching. Mitchell et al. recently

demonstrated that myosin heavy chain 6 (*MYH6*) variants are common in patients with HLHS and that patients with *MYH6* variants had a decreased ratio of α-MHC: β-MHC expression with decreased transplant-free survival.[42]

Blakeslee et al. also recently showed that patients with single ventricle physiology with a systemic right ventricle had elevated protein levels of multiple histone modifying proteins (HDACs class I, IIa, and IIb) at time of transplant compared to the right ventricle of controls (pediatric organ donors with structurally normal hearts and normal function but were not donated for heart transplant).[43] Further, they showed similar changes to hypoxic neonatal rats compared to control rats. They concluded that myocardial HDAC changes in patients with a systemic right ventricle could represent a novel therapeutic target.

7 | BIOMARKERS

The list of studies exploring epigenetics, and especially miRs, as biomarkers is growing considerably. Clinicaltrials.gov currently has 203 studies, including 50 pediatric studies, registered that specifically include miRs as biomarkers. Recent studies specific to pediatric cardiology include already mentioned studies of dilated cardiomyopathy and HLHS, as well as evaluating miRs after cardiopulmonary bypass, long-term in patients after atrial switch, and even for potential fetal diagnosis.[37,41,44–46] Thus, miRs represent an opportunity for developing more specific biomarkers compared to traditional brain natriuretic peptide and troponin.

8 | THERAPEUTICS

HDAC inhibitors are very well studied for therapeutic use in multiple types of cancer, and two HDAC inhibitors are already approved by the Food and Drug Administration to treat lymphoma.[47] There is growing evidence that HDAC inhibitors are therapeutic in adult heart failure.[48–51] Little is known for pediatric heart disease, but as discussed above, HDAC inhibitors may have a therapeutic role in patients with a systemic right ventricle.[43]

Multiple studies already discussed show the therapeutic potential of targeting miRs.[31,38,39] Van Rooij et al. describe the safe and well-tolerated use of miR-based therapy in animal models and human patients for hepatitis C virus, various cancers, and several cardiovascular diseases, including cardiac fibrosis and atherosclerosis.[52] Because miRs are ubiquitously expressed in different tissues, miR-based treatment has substantial risk for adverse effects. Current efforts are focused on development of stable miR modulators to specific cell types, or bypassing this difficulty with creation of device-based delivery methods. For example, anti-miR-eluting stents are being studied similarly to drug-eluting stents.[53]

In addition to new therapies specifically developed to target epigenetic mechanisms, several commonly used medicines, including statins, hydralazine, procainamide, have known effects on DNA methylation and histone modification.[54,55] As we further our understanding of cardiovascular epigenetics, we may find that more medications and naturally occurring compounds have pleiotropic effects that utilize epigenetic mechanisms.

9 | PRECISION MEDICINE IN PEDIATRIC CARDIOLOGY

Patients with congenital heart disease continue to face multiple challenges throughout their lifetime, whether their specific defect was repaired or palliated. As outlined by Touma et al., a multilayered approach to precision medicine for this population will help modify the disease course and potentially attenuate disease development.[56] In their review, they outline a methodology for phenotypic assessment, genetic diagnosis, and analysis of modifying environmental factors through a multidisciplinary approach for precision medicine. The use of Next-Generation Sequencing, including analysis of the epigenome, will play a critical facet in precision medicine.

Additionally, there is considerable interest in computational fluid dynamics, which aims to model hemodynamic flow and structural properties of blood vessels in a clinically relevant manner. As described in a recent review, integration of molecular data into computational fluid dynamics can further complement the technology for predicting vascular phenomena.[57] Data on gene expression and epigenetic modifiers could assist computational modeling and synergistically amplify the clinical benefit.

10 | CONCLUSION

Epigenetics is a growing field with potential valuable contributions to the understanding and treatment of pediatric heart disease. Basic understanding of the concepts of epigenetics will be essential to all pediatric cardiologists in order to understand mechanisms of pathophysiology, developing pharmacotherapy, and to understand the role of epigenetics in precision medicine to better care for patients with pediatric heart disease.

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Andrew Spearman, MD is currently a third year pediatric cardiology fellow at Children's Hospital of Wisconsin. After completion of pediatric cardiology fellowship, he plans to pursue a fourth year of fellowship in basic science research with a special interest in the epigenetic mechanisms of pediatric cardiovascular disease. He completed medical school at New York University of School of Medicine and pediatric residency training at University of Chicago.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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