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# The Role of Long Non Coding RNA on Cardiac Hypertrophy and their Possible Therapeutic Usage

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**Abstract**

Cardiac hypertrophy is a major cause of heart failure and a common symptom of congenital heart disease (CHD). Cardiac hypertrophy has usually been studied through the lens of coding genes, but a promising new field of research has emerged regarding the critical role long non-coding RNA (lncRNA) plays with cardiac hypertrophy. Utilizing next-generation sequencing (NGS), scientists have been able to uncover some of these lncRNAs, such as Chaer, Chast, H19, Lipcar, and Hotair, as well as their newly-established association with cardiac hypertrophy. These lncRNAs have potential for use as epigenetic regulators, biomarkers, or even sponges for microRNAs. With 68.4% of non-syndromic cases of cardiac hypertrophy associated with various lncRNAs, understanding the role they play could transform the landscape of cardiac treatments in acute and chronic illnesses with new treatments with lncRNAs, discovered with NGS, pushing forward the front in the fight against heart failure. Epigenetic regulators can either inhibit or promote cardiac hypertrophy. For example, Mhrt and H19 inhibit hypertrophy while Chaer and Chast promote hypertrophy. Targeting specific lncRNAs for upregulation or downregulation could provide insights into developing new treatments for cardiac hypertrophy. However, effective delivery mechanisms for lncRNAs must be established and human trials must be undergone before lncRNA therapies are considered for clinical use.

*Keywords: Cardiac hypertrophy, lncRNA, Chaer, Chast, H19, Lipcar, Hotair*

## 1. Introduction

This literature review aims to provide a basic understanding of the epigenetic roles of lncRNA, and subsequently delve deeper into the therapeutic potential for the use of lncRNAs in the treatment of cardiac hypertrophy and propose future research directions. We hypothesize that given their epigenetic functions within the human genome, the right lncRNAs could have beneficial effects in potentially reducing cardiac hypertrophy through prevention or reducing overall scarring.

There are two predominant types of RNA, non-coding RNA (ncRNA) and coding RNA. ncRNA refers to a functional RNA molecule that is not translated into a protein and coding RNA refers to sequences of RNA that do code for proteins. There are various sub-types of coding RNA, including, but not limited to, messenger RNA (mRNA), ribosomal RNA (rRNA), and transfer RNA (tRNA). mRNA directs the synthesis of proteins, rRNA makes up the cores of different ribosomes and helps catalyze protein synthesis, and tRNA acts as an adapter between mRNA and the amino acids that make up proteins (B et al., 2002). This section will focus on the genetic factors of cardiac hypertrophy.

Within the human genome, 90% of genes are transcribed into ncRNA (Mercer et al., 2009). There are multiple types of ncRNA: microRNA (miRNA), small interfering RNA (siRNA), Piwi-interacting RNA (piRNA), and long non-coding RNA (lncRNA) (Zhang et al., 2019). miRNA refers to sequences of ncRNA around 30 nucleotides and lncRNA

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refers to sequences of ncRNA around 200 nucleotides (Mercer et al., 2009). Another way to classify ncRNA is by their characteristics. They can be stratified into: sense; being transcribed from the same strand of DNA and in the same orientation as the nearby coding RNA, antisense; being transcribed from the opposite strand of DNA, intronic; being found in intronic regions of coding RNA, intergenic; being found in between two introns, bidirectional; being found within one kilobase from the promoter region of a coding gene, but being transcribed from the opposite strand of DNA (Hermans-Beijnsberger et al., 2018) (Figure 1).

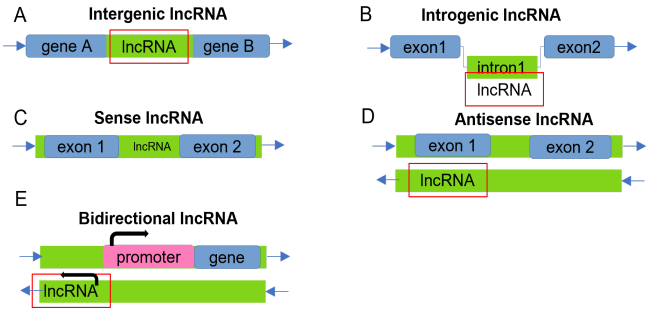


Figure 1: Types of lncRNA. (A) Intergenic lncRNA is located in between genes. (B) Introgenic lncRNA can be found within genes. (C) Sense lncRNA spans exons on and is transcribed from the same strand (D) Antisense lncRNA spans multiple exons on and is transcribed from the opposite side (E) Bidirectional lncRNA is transcribed on the opposite strand of a promoter region and is transcribed in the opposite direction. Made with Microsoft PowerPoint.

There is still much research needed on lncRNA, as estimates on the amount of lncRNAs in the human genome range from 16,000 to 100,000 genes (Fang et al., 2018; Uszczynska-Ratajczak et al., 2018). Although the functions of lncRNA are still debated, next-generation sequencing (NGS) can give us a new idea of what they do (Yasuhara & Garg, 2021). It has been suggested that lncRNA could epigenetically regulate the transcription of nearby genes, as well as DNA synthesis and repair (Statello et al., 2021). A change in lncRNA has been observed to affect the emergence of several diseases (Statello et al., 2021). As such, different sequences of lncRNA could be used as warning signs for different diseases, and the upregulation and downregulation of different lncRNA sequences could affect whether a disease emerges or not (Hermans-Beijnsberger et al., 2018). The objective this review article will be focusing on the impacts different sequences of lncRNA have on the development and treatment of cardiac hypertrophy.

## 2. Effects of lncRNA on Congenital Heart Diseases

### 2.1 Cardiac Hypertrophy

Cardiac hypertrophy occurs when cardiomyocytes are overgrown due to scarring or cardiac pressures (Lai et al., 2017). Cardiac hypertrophy is usually indicated by increased cardiomyocyte size, increased protein synthesis, and greater thickness of ventricular walls (Frey et al., 2004; Tham et al., 2015) (Figure 2). There are various types of cardiac hypertrophy (Shimizu & Minamino, 2016). Physiological hypertrophy is associated with normal or enhanced contractile function. As such, physiological hypertrophy is not considered to be detrimental to health (Shimizu & Minamino, 2016; Weeks & McMullen, 2011). Physiological hypertrophy is usually caused by exercise, growth, or pregnancy (Shimizu & Minamino, 2016). Pathological Hypertrophy is associated with cardiomyocyte cell death and fibrotic remodeling, and is associated with heart failure. Cardiac hypertrophy can also be classified by the geometry of the heart (Shimizu & Minamino, 2016): eccentric (volume-overload) or concentric (pressure-overload) (Sugden & Clerk, 1998). Eccentric hypertrophy is characterized by cardiomyocytes increasing in length as well as width while concentric hypertrophy is characterized by cardiomyocytes increasing in width only (Heineke & Molkentin, 2006; Selby et al., 2011). Concentric hypertrophy is typically observed in hypertensive states, while eccentric hypertrophy is usually associated with valvular incompetence (Heineke & Molkentin, 2006) as

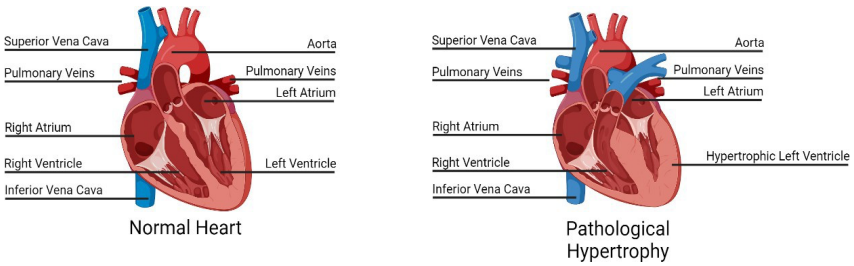


Figure 2. A hypertrophic heart (right) exhibits more swelling and scarred heart material than a normal heart (left). Made with BioRender.

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well as cardiac diseases such as myocardial infarctions and dilated cardiomyopathy (Shimizu & Minamino, 2016). Heart failure is characterized by a failure of the heart to adequately pump oxygenated blood around the body. Heart failure could be caused by various factors, such as genetic factors, environmental factors, and cardiac stressors, such as myocardial infarction (Grossman et al., 1975).

### 2.2 Long Non-Coding RNAs Associated with Cardiac Hypertrophy

One relevant sequence of genes pertaining to cardiac hypertrophy is a cluster of lncRNA on the Myh7 locus, dubbed myosin heavy-chain-associated RNA transcripts (Mhrt). (Han et al., 2014). In an experiment by Han et al, researchers permanently downregulated Mhrt in mice and observed an isoform switch from Myh6 to Myh7, a telltale sign of cardiomyopathy, where the heart cannot pump blood effectively Han et al., 2014). The researchers also observed that an increased expression of Mhrt is conducive to reduced fibrosis as well as hypertrophy of the heart in mice when compared to mice whose Mhrt expression have been permanently downregulated (Han et al., 2014). Mhrt has also been suggested as a predictive biomarker for heart failure in humans Xuan et al., 2017). Terminal differentiation-induced ncRNA (Tincr) is another lncRNA that affects the emergence of cardiac hypertrophy. Increased expression of Tincr has shown decreased cardiac hypertrophy in mice (Shao et al., 2017). However, there are multiple more lncRNAs that have been associated with cardiac hypertrophy (Hobuß et al., 2019).

Another lncRNA associated with cardiac hypertrophy is Cardiac-hypertrophy-associated epigenetic regulator (Chaer) (Wang et al., 2016). In mice where Chaer was downregulated, the test subjects exhibited reduced fibrosis and hypertrophy of the heart while mice with upregulated Chaer exhibited increased hypertrophy Wang et al., 2016).

Another significant lncRNA observed in mice is cardiac hypertrophy-associated transcript (Chast) (Viereck et al., 2016). The upregulation of Chast was observed to induce hypertrophic growth of heart both in vivo and in vitro. To the contrary, the downregulation of Chast prevented hypertrophic cell growth in the heart and also helped preserve cardiac function (Viereck et al., 2016). Chast in humans was also able to induce hypertrophic cell growth in the heart, and upregulated Chast was also found in patients with aortic stenosis, demonstrating the usefulness of lncRNA as a preventative diagnostic for cardiac hypertrophy (Viereck et al., 2016).

In addition, another lncRNA associated with cardiac hypertrophy is cardiac hypertrophy related factor (Chrf). (Wang et al., 2014). In an experiment by Wang et al conducted in vitro and in vivo, researchers used a microarray to analyze miRNAs. They discovered an miRNA—miR-489—that helps combat cardiac hypertrophy (Wang et al., 2014). miR-489 is able to regulate the myeloid differentiation primary response gene 88 (Myd88). Myd88 is targeted by miR-489 for expression. Knockdown of Myd88 showed a decrease in hypertrophy. However, miR-489 itself is regulated by Chrf. The researchers discovered that Chrf acts as a sort of sponge to downregulated Myd88. Therefore, Chrf directly contributes to increased cardiac hypertrophy (Wang et al., 2014).

Furthermore, Liu et al discovered that the lncRNA H19 could inhibit cardiac hypertrophy (Liu et al., 2016). Male mice underwent the same procedure as the mice performed by Han et al, with the hearts being dissected to examine hypertrophic growth (Liu et al., 2016; Han et al., 2014). In hypertrophic mice that underwent transverse aortic construction (TAC) surgeries, the researchers found that H19 was upregulated to combat the sudden growth post mortem (Liu et al., 2016). To determine the role of H19 in regulating cardiac hypertrophy, the researchers overexpressed H18 in neonatal cardiomyocytes with the help of an adenovirus containing H19. The upregulation of H18 was observed to directly reduce cardiac hypertrophy in the neonatal cardiomyocytes (Liu et al., 2016).

In a similar experiment and with the same surgery as Han et al and Liu et al, (Lai et al., 2017; Liu et al., 2016; "A long noncoding RNA protects the heart from pathological hypertrophy", 2014), Lai et al used TAC surgeries to create a model of cardiac hypertrophy (Lai et al., 2017). The researchers also isolated cardiomyocytes from neonatal mouse hearts. They discovered the lncRNA Hotair also inhibits cardiac hypertrophy through similar means of Han et al and Liu et al (Lai et al., 2017; Liu et al., 2016; Han et al., 2014).

Another type of lncRNA that is relevant to cardiac hypertrophy is mitochondrial long noncoding RNA (mtlncRNA). One particular mtlncRNA is Lipcar, a biomarker for cardiac remodeling (Chen et al., 2021). Kumarswamy et al conducted an experiment where 246 patients with a variety of cardiac syndromes were chosen to have their blood sampled for lncRNA. The results showed that Lipcar is upregulated in patients with chronic heart

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failure and were associated with a higher risk of death caused by cardiovascular complications (Kumarswamy et al., 2014). The experimental team conducted follow-up experiments with similar results.

## 3. Possible Therapeutic Usages of lncRNA

As of now, most therapies concerning cardiac hypertrophy are symptomatic by nature (Liu et al., 2020), meaning that instead of addressing the root cause of the problem, therapies focus on alleviating symptoms instead. For example, some current medication intended to alleviate cardiac hypertrophy lowers the workload of the heart, leading to less work done and less tissue scarring (Kamisah & Che Hassan, 2023). The result is damage-control, instead of prevention or curative. With increased knowledge on lncRNAs such as Mhrt, Chaer, Chast, Chrf, H19, Lipcar, and Hotair could give valuable understanding into the etiologies of various hypertrophic conditions, and can also give insight into novel treatments addressing the root causes of cardiac hypertrophy.

lncRNA can be inserted into cells through a process called transfection (Chong et al., 2021). Transfection is when nucleic acids which are not part of the original cell are used to modify the cell's genes and create genetically modified cells (Chong et al., 2021). Transfection can be divided into two types, transient and stable (Kim & Eberwine, 2010). The classification depends on the genetic material used. Stably transfected genes integrate themselves into the cell, while transiently transfection is more temporary. The most used method of transfection is known as transduction (also known as viral-based transfection) (Chong et al., 2021), where viruses are used to inject genes (Pfeifer & Verma, 2001). However, this method can potentially damage the cell (Kim & Eberwine, 2010). Transfection can also occur through chemical methods, exploiting the negatively-charged cell membrane.

Many studies concerning lncRNAs in a therapeutic sense are still in preclinical stages, but the research on mtlncRNA has progressed to a certain extent (Chen et al., 2021). Mitochondrial energy provided to functions related to apoptosis, inflammation, and metastasis are regulated by mtlncRNA (Olavarria et al., 2018). One mtlncRNA related to cardiac inflammation is Lipcar, a biomarker for cardiac remodeling that can anticipate survival in patients with heart disease (Kumarswamy et al., 2014).

Even though lncRNAs offer many targets for research due to the cellular processes affected by them, there have been few practical applications of lncRNAs so far (Gomes et al., 2017). Most research has been performed with the upregulation or downregulation of specific sequences through the use of adenoviruses or lentiviruses—chosen for their low pathogenicity. (Gray and Samulski, 2008; Nathwani et al., 2014; White et al., 2007; Zsebo et al., 2014). Another way to use lncRNAs is through adeno-associated viral (AAV) vectors due to their lower pathogenicity. This method has shown potential in clinical studies (Kota et al., 2009; Montgomery et al., 2014; Quattrocelli et al., 2013).

For example, identifying pathogenic variants in patients suffering from non-syndromic has been challenging, even in familial cases (Yasuhara & Garg, 2021). In addition, to properly characterize pathogenic variants and elucidate pathogenic mechanisms, genetic models are required. There are a number of in vitro and in vivo models available for use, but each model has strengths and weaknesses when evaluating their similarity to the human genome. Mice and rats have been used to study cardiovascular development since their degree of sequence conservation to humans is similar. However, the relationship between genotypes and phenotypes could be different (Yasuhara & Garg, 2021). Another novel method of in vitro experimentation includes human induced pluripotent stem cells (IPSCs) (Lin et al., 2021), where multipotent stem cells are chemically induced to become pluripotent, allowing them to express the genes of any human body part apart from the placenta.

## 4. Conclusion

lncRNAs pose an exciting new field of research for scientists looking to uncover the causes of cardiac hypertrophy as well as the means to combat it. Notably, scientists were able to observe solid observations due to the upregulation or downregulation of various lncRNAs causing increased or decreased cardiac hypertrophy in in vivo test subjects, establishing a direct link between lncRNAs' role within the human genome and cardiac hypertrophy. Since current treatments for cardiac hypertrophy are focused on alleviating the symptoms of the hypertrophy, lncRNA therapies offer an enticing alternative by striking at the cause of the hypertrophy itself, although

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clinical trials in humans have not begun in force yet, and much more research is needed as a result. lncRNAs can be used to prevent, cure, or diagnose cases of cardiac hypertrophy.

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