



## LONG NON-CODING RNAs AND CORONARY ARTERY DISEASE

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### ABSTRACT

Cardiovascular diseases are the leading cause of morbidity and mortality in the world. Atherosclerotic disease and its thrombotic complication lead to the development of Coronary artery disease, and if untreated, it progresses into myocardial infarction. Reducing plasma low-density lipoprotein, antiplatelet agents, anticoagulants, percutaneous coronary intervention, and coronary artery bypass surgery are available effective treatment measures for coronary artery diseases. However, the mortality remains unchanged. Modern advancement in genome-wide analyses has identified large number of long non-coding RNAs (lncRNAs), which are transcribed in human genome. LncRNAs are non-protein coding RNA, which are 200 to 10,000 nucleotides in length. Recent studies have shown several lncRNAs associated with initiation and progression of cardiovascular diseases. And they can serve as a novel biomarkers for diagnosis of diseases like heart failure, cardiac hypertrophy, atherosclerosis and coronary artery disease.

**Keywords:** Long non-coding RNA, Coronary Artery Disease, Biomarker, Review

## INTRODUCTION

Coronary artery disease (CAD) is caused by building-up of plaque in the wall of the coronary arteries, narrowing them over time and the process is called atherosclerosis. In the process, plaque first grows within the walls of the coronary arteries until the blood flow is limited. It may be chronic narrowing of the coronary artery over time giving stable or unstable CAD. Or it can be acute, resulting from a sudden rupture of a plaque and formation of a thrombus or blood clot, giving acute myocardial infarction (AMI). Therefore, it can be said that coronary heart disease is the result of coronary artery disease.

Long non-coding RNAs (lncRNAs) are non protein coding RNA with 200 to 10,000 nucleotides in length which are transcribed by RNA polymerase II.<sup>1</sup> They were previously taken as “transcriptional noises” or “genetic junks”, but, later researchers have discovered that they are responsible for many biological processes and play pivotal roles in gene expression and regulation, such as transcription, chromatin remodelling, RNA splicing and cellular apoptosis.<sup>2</sup> Numerous number of studies have shown that lncRNAs actively take part in development of several disease conditions including cancers, autoimmune diseases, cardiovascular disease and neurological disorders. Some lncRNAs are identified to be involved in development of heart failure, cardiac hypertrophy, atherosclerosis and coronary heart disease.

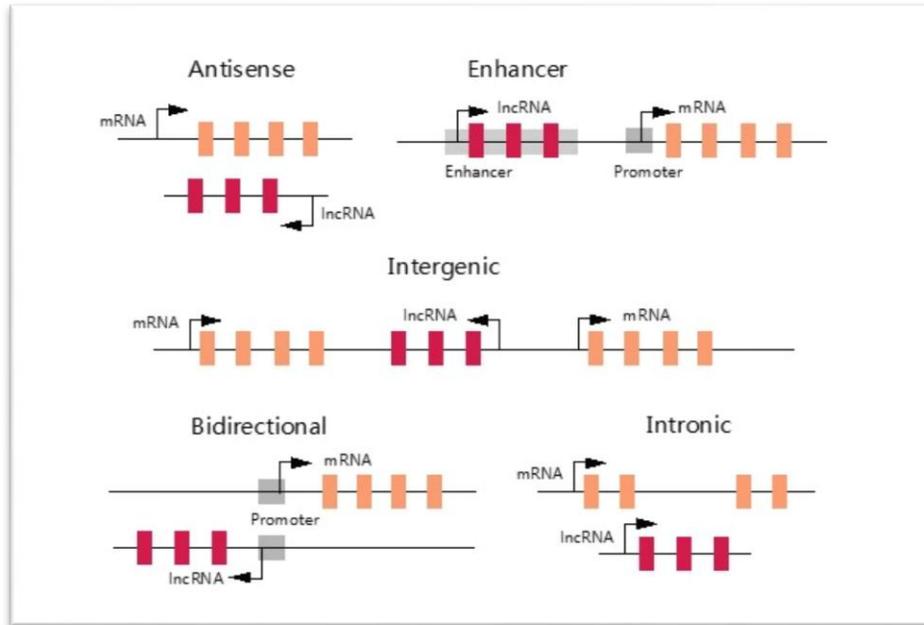
Biomarkers play a topmost role and are very important in the course of identifying disease and its severity. For example, NT-proBNP or BNP is also used as marker for the diagnosis and monitoring of severity of heart failure. A biomarker is a measurable and quantifiable biological parameter which can be used for screening, identifying, categorizing, monitoring disease, its risk and the therapy. Even though lncRNA is new to the research world, they have already been used as promising biomarkers for diagnosis of several diseases. lncRNA PCA3 is prostate specific lncRNA found in urine. It is already a well-established biomarker for prostate cancer.<sup>3</sup> Likewise, lncRNA AA17084 in gastric juice has found to be indicator of gastric cancer,<sup>4</sup> and same as lncRNA ATB can serve as biomarker to detect Coal Worker Pneumoconiosis.<sup>5</sup> In recent study lncRNA LIPCAR is shown to have potential to serve as a biomaker for heart failure after myocardial infarction,<sup>6</sup> and lncRNA Coromarker is validated novel biomarker for coronary artery disease.<sup>7</sup>

lncRNAs as biomarker is still convincing especially in the context of signature and network analysis for disease detection, prediction and progression. Therefore, the aim of this review is to summarise and evaluate the existing evidence for the analytic and predictive potential of different lncRNAs in Coronary Artery Disease.

### Classification of lncRNAs:

Long non-coding RNAs are non-protein coding RNAs with more than 200 nucleotides in length. On the basis of their genomic location relative to their neighbouring encoding regions, lncRNAs can be classified into five sub-classes (Fig 1): (1) sense (2) antisense (3) bidirectional (4) intronic (5) intergenic. Sense lncRNAs are formed within a sense strand of protein-coding genes. Likewise, antisense lncRNAs are formed from the transcription of anti-sense protein-coding genes whereas bi-directional lncRNAs are found on the opposite

strand of their neighbouring coding transcripts. Similarly, Intronic lncRNAs are transcribed by sequences of DNA that intercept a gene sequence. These are anticipated to modulate gene expression through various transcriptional mechanisms. In addition, Intergenic lncRNAs are located inside the introns of the coding transcript, or in the genomic interval between two protein-coding genes. However, it is considered that lncRNA classification will continuously change with the expansion and discoveries of new lncRNAs and their functions.



**Figure 1:** Schematic diagram of representative classes of lncRNAs.

### Biological mechanism of lncRNA functions:

lncRNAs are modulators of complex cellular biological regulatory networks rather than just the simple intermediate products of gene expression. The biological mechanism of lncRNAs can be explained to epigenetic, transcriptional and post-transcriptional regulation.

Studies have described a range of mechanisms by which lncRNAs regulate their targets; many seem to depend on specific features of primary sequence, secondary structure and genomic positioning of lncRNA effector transcripts. Many lncRNAs act as RNA decoys, adjusting the transcription factors away from their DNA targets by directly binding to them as target mimics. Others work at the post-transcriptional level as microRNA target site decoys, titrating microRNA effector complexes away from their mRNA targets. Several lncRNAs bind to specific regulatory proteins, scaffolding within ribonucleoprotein complexes. Recruitment of chromatin-modifying complexes to their DNA targets in cis has also emerged as a well-characterized function for several mammalian lncRNAs.<sup>8</sup>

### **lncRNA in atherosclerosis:**

Atherosclerosis is narrowing of artery due to formation of plaques in subendothelial spaces. Impairment of the endothelium attracts monocytes, causing them to leave the bloodstream, penetrate the arterial walls and transform into macrophages, then forming myogenic foam cells that triggers a cascade of complex immune responses producing an atheroma. After arterial damage, vascular smooth muscle cells proliferate and migrate from the tunica media to the intima where they replicate to form a fibrous cap, induced by growth factors and cytokines from endothelial cells, monocytes, and platelets. However, the mechanisms of each event in atherosclerosis are still unknown.

Recent studies have reveal the involvement of lncRNAs in etiology of plaque formation in arterial wall leading to atherosclerosis. Reddy et al. states that lncRNA called E33013P06 can up-regulate the expression of CD36 in macrophages and promote foam cell formation.<sup>9</sup> This data suggests that lncRNA may be involved in synthesis and uptake of lipids by macrophages leading to atherosclerotic disease. The exact cause and mechanism worth further study. Hu et al., found that lncRNA RP5-833A20.1 regulates atherosclerosis by inhibiting NFIA expression.<sup>10</sup> lncRNA ANRIL located in chromosome 9p21 regulates the expression of genes associated with fatty acid, glucose metabolism and inflammation, including adiponectin receptor1(ADIPOR1), vesicle associated membrane protein3 (VAMP3) and chromosome 11 open reading frame10 (C11ORF10).<sup>11</sup> Reverse cholesterol transport removes and mobilizes lipids and cholesterols that might cause a risk for plaque formation. The primary function of ATP binding cassette transporters called ABCA1, is to transport cellular cholesterols to its corresponding apolipoproteins. Hu et al. explains that the oxidized low-density lipoprotein (ox-LDL) stimulates a lncRNA DYN-LRB2-2, which then leads to ABCA1-mediated cholesterol efflux and up-regulates G protein-coupled receptor 119 (GPR119). GPR119 is found to be associated with glucose and lipid metabolism that regulates atherosclerotic plaque formation by decreasing cellular cholesterol levels and inflammation.<sup>12</sup> Both MAPK and NF- $\kappa$ B signalling pathways have been reported to be a central mediator and a key participant of the inflammatory process, and also involved in regulation of atherosclerosis,<sup>13</sup> J-K PAN found that lncRNA H19 regulates atherosclerosis by activating the MAPK and NF- $\kappa$ B signalling pathway.<sup>14</sup> In addition, Huang et al. reported that lncRNA HOXC-AS1 causes in down regulation in atherosclerosis, that could suppress ox-LDL-induced cholesterol accumulation in THP-1 macrophages by promoting HOXC6 expression.<sup>15</sup> However, Shan et al. suggests the down regulation of lncRNA RNCR3 would lead to decreased proliferation and migration in ECs and VSMCs, indicating lncRNA RNCR3 being a potential therapeutic target for treating atherosclerosis.<sup>16</sup>

### **lncRNAs in Coronary Artery Disease:**

Coronary Artery Disease (CAD) is major cause of sudden cardiac death and it usually progresses to myocardial infarction if remain untreated. Among the individuals with CVDs, coronary artery disease accounts for 22% of early deaths and 15% of late death.<sup>17</sup> Various studies have pointed several lncRNAs to be involved in development of coronary artery disease.

A case control study done by Yue et al. has revealed 86 different lncRNAs including lncRNA BATS5, lncRNA IL21R-AS1 and lncRNA OTTHUMT00000387022 (Coromarker) that were overexpressed in patients with CAD. Among them, Coromarker was found to be highly sensitive and specific for the diagnosis of CAD. The authors also validated it for its diagnostic accuracy in a prospective way. Individuals with overexpressed plasma Coromarker were later diagnosed with CAD during coronary artery angiogram. As a result, they proposed it as a novel biomarker for coronary artery disease.<sup>7</sup> However, the functions and mechanism of Coromarker is still unknown and warrants further investigation.

Genome-wide association studies have found that regions on chromosome 9p21 (Chr9p21) are associated with various diseases including CAD and other coronary heart diseases.<sup>18</sup> Likewise, Jianhui et al. has found the altered expression of an antisense noncoding RNA in the INK4 locus (ANRIL) on chromosome 9p21 mediates methylation of p15INK4b which is associated with the development of CAD.<sup>19</sup> In another study of Xiao et al., provided the clinical evidence that ANRIL regulates inflammatory responses as a novel component of NF- $\kappa$ B pathway.<sup>20</sup> They further suggested about the feasibility of ANRIL as specific target for drug development.

Guoliang et al. points Peroxisome proliferator activated receptor delta (PPAR $\delta$ ) modulates lipopolysaccharide-induced TNF $\alpha$  inflammation.<sup>21</sup> Earlier study conducted by Lingyao et al. have shown TNF- $\alpha$  associated with the risk of development of CAD.<sup>22</sup> According to Yue et al., the lncRNA associated with protein coding gene PPAR $\delta$  (lncRNAPPAR $\delta$ ) is widely expressed in patients with coronary artery disease.<sup>23</sup> And they proposed lncRNA PPAR $\delta$  as a biomarker of CAD with high sensitivity and specificity. According to Mingjiao et al., the research conducted in a small population size reveal lncRNA uc022bqs.1 being overexpressed in coronary heart disease and suggested as a potential biomarker for early diagnosis of coronary heart disease.<sup>24</sup>

Earlier study as shown that p53 is the key factor in the pathogenesis of atherosclerosis. In addition, Gengze et al. states that lincRNA-p21 regulates cell proliferation, apoptosis and atherosclerosis in vitro and in vivo by binding MDM2 and enhancing p53 activity.<sup>25</sup> In contrast one of the research article shows the association of G-A-A-G Haplotype gene of lincRNA p-21 in reducing the risk of coronary artery disease in Chinese Han population.<sup>26</sup> The mechanism is still unclear, and does need further investigation. In a recent study conducted by Asieh et al. found that over expression of lncDC and STAT3 in coronary artery disease with diabetic population. And lncDC was associated with the pathogenesis of diabetes-related CAD.<sup>27</sup>

### **lncRNAs Myocardial Infarction:**

Acute myocardial infarction is the most serious cardiovascular disease with high morbidity and mortality. Protein biomarkers like TnI/T, Creatine kinase MB (CK-MB) are widely used for diagnosis of AMI. Recently circulating lncRNAs emerged and reported to be promising biomarker for cardiovascular disease.

In a cohort based study, Vausort M et al., reported lncRNA KCNQ10T1, MIAT AND MALAT1 were reported high in acute MI patient in comparison to healthy volunteers. In the same study lncRNA ANRIL was

expressed lower and lncRNA HIF1a-AS2 levels varied with onset time of chest pain.<sup>28</sup> In one of the research conducted by Youyou et al., among 49 AMI and 15 non- AMI patients, the less number of lncRNA UCA1 was found in plasma in early state of AMI and tend to increase on third day after the onset of AMI.<sup>29</sup> Similarly, global transcriptomic analysis explains LIPCAR was down regulated early but up-regulated later in the plasma of patients after AMI.<sup>6</sup> Ying Z et al., in their study detected 15 cardiac relevant lncRNAs in blood samples of AMI patients. Among which, lncRNA ZFAS1 and lncRNA CDR1AS showed significant differences in their circulating level between AMI patients and control subjects.<sup>30</sup> In this study they found expression of lncRNA ZFAS1 is reduces and expression of lncRNA CDR1AS is escalated in AMI. In conclusion, the cause and mechanism is still unknown and worth further study. They suggested lncRNA ZFAS1 and lncRNA CDR1AS can predict AMI.

LncRNA	Significance	Reference
LIPCAR	Biomarker for coronary heart disease, predicts survival in patients with heart failure after acute myocardial infarction.	6,24
Coromarker	Novel biomarker for coronary artery disease	7
E33013P06	Promotes atherosclerosis	9
ANRIL	Associated with atherosclerosis and coronary artery disease. Expressed lower in Acute myocardial Infarction than in normal population.	11,19,20,28
H19	Promotes atherosclerosis by regulating NF-kB signalling pathways	14
lncRNA RNCR3	Could be a potential therapeutic target for treating atherosclerosis	16
LncPPAR $\delta$	Biomarker of coronary artery disease	23
LincRNA-21	Regulates atherosclerosis and associated coronary artery disease in a Chinese Han Population.	25, 26
Lnc DC	Overly expressed in coronary artery disease with type 2 diabetic population	28
HIF1A-AS2	Overexpressed in Acute Myocardial Infarction and expression varied with onset time.	
KCNQ10T1	Overly expressed in Acute Myocardial Infarction	28
MALAT1		
MIAT	Lower expression in Acute myocardial infarction than in normal population	28
UCA1	Novel biomarker for Acute myocardial infarction	29

ZFAS1	Decrease expression in Acute myocardial infarction, can serve as biomarker	30
CDR1AS	Increase expression in Acute myocardial infarction, can serve as biomarker	30

**Table:** LncRNA related with Coronary Artery Disease.

## CONCLUSION

Long non-coding RNAs are emerged from once considered to be 'genomic junks'. Now they have been identified as key regulators in process of several diseases including coronary artery disease. Current advanced research in this field has identified several lncRNAs potential to serve as biomarkers and therapeutic targets for diverse pathological changes involved in Coronary artery disease. However, large multicentred experimental research study in huge population size is needed to validate the biomarker potentiality of lncRNAs .

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## Conflict of interest:

The author declares that there is no conflict of interest.

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