https://doi.org/10.48048/tis.2024.8576

Understanding the Complex Interplay of Epigenetic Factors in Atherosclerosis: A Review

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Received: 15 June 2024, Revised: 28 June 2024, Accepted: 5 July 2024, Published: 1 October 2024

Abstract

This review focuses on the epigenetic factors that play a vital role in the formation of plaques and lead to atherosclerosis and related diseases in humans during current times. Apart from the epigenetics there are plenty more factors that influence the atherosclerotic risk in the older population of the current era. Lack of physical activities has become one of the most commonly found traits that causes obesity in people especially the middles aged adults. People working 9 - 5 in an office, or an IT firm tend spend idle time with their computer for 4 - 5 consecutive hours with barely changing their position. Lack of exercise tends to affect not only the physical health but also stress a person mentally. Chronic inflammatory diseases like atherosclerosis have a major role in the morbidity and mortality from cardiovascular disease worldwide. Atherosclerotic plaques are created by the pathophysiological process of lipids, inflammatory cells and fibrous components gradually accumulating within artery walls. Endothelial dysfunction signals the start of atherosclerosis and allows low-density lipoproteins (LDL) to enter the subendothelial region. Oxidative stress-induced changes in retained low-density lipoprotein (LDL) set off an inflammatory cascade that draws T cells and monocytes to the site of the lesion. The interaction of these immune cells with the local vascular cells encourages the production of foam cells, which fills the plaque's centre with lipids. Smooth

muscle cells multiply and help produce fibrous caps as the lesion ages, which affects the stability of the plaque. Studies shows how the brain responds to physical labour and how differently the effectively the enzymes and hormones are secreted, and their actions are vastly guided based on the physical stress an individual undergoes. Among the most prominent causes of atherosclerosis epigenetics has been focussed majorly in this review, where we focus on the underlying genetic factors that play a vital role in atherosclerosis and related cardiovascular diseases and also the genetic approach to a treatment is also elaborated. A number of miRNAs are involved in the cause/cure for the atherosclerosis, the following tables give an outlook regarding the same. This review aims to focus on the causes, risk factors, pathophysiology and the treatment and therapies that are currently available all the way concentrating on the genetic approach in the above-mentioned perspective.

Keywords: Atherosclerosis, Dyslipoproteinaemia, Hyperhomocysteinaemia, Dyslipidemia, Hypermethylation, Epigentics

Abbreviations

miRNA:	Micro RNA
LPGAT1:	Lysophosphatidylglycerol Acyltransferase 1
ABCA1:	ATP binding cassette transporter A1
ABCG1:	ATP binding cassette sub-family G member 1
PGC-1α:	Peroxisome proliferator activated receptor gamma coactivator 1 α
PDK4:	Pyruvate dehydrogenase lipoamide kinase isozyme 4
DNMT:	DNA methyltransferases
RXR α:	Retinoid X receptor alpha
LPL:	Lipoprotein lipase
SREBP1:	Sterol regulatory element binding protein 1
SCD1:	Stearoyl-CoA desaturase 1
ACLY:	ATP citrate lyase
KLF:	Kruppel-like factor
SOCS:	Suppressor of cytokine signalling
BCL-6:	B-cell lymphoma 6
SHIP-1:	SH-2 containing inositol 5’ polyphosphatase 1
IL-13 Rα:	Interleukin 13 receptor alpha
TAG:	Triacylglycerol
HDL:	High density lipoprotein
LDL:	Low density lipoprotein
eNOS:	Endothelial nitric oxide synthase
iNOS:	Inducible nitric oxide synthase
Fads2:	Fatty acid desaturase 2
c-fos:	Cellular oncogene c-fos;
15-LO:	15 lipoxygenases;

EC-SOD: Extracellular superoxide dismutase
Igh2: Insulin like growth hormone 2
MMP: Matrix Metalloproteinase
TIMP: Tissue inhibitor of metalloproteinase
IFN: Interferon
PDGF-A: Platelet derived growth factor A
ICAM-1: Intercellular adhesion molecule 1
HHcy: Hyperhomocysteinemia
SAHA: Suberoylanilide hydroxamic acid
TET2: Tet methylcytosine dioxygenases 2
HDAC: Histone deacetylases
DNMT: DNA methyltransferase
SIRT1: Sirtuin 1
EGCG: Epigallocatechin gallate
EZH2: Enhancer of zeste homolog 2

Introduction

Atherosclerosis is the most important risk factor for cardiovascular disease, the leading cause of death worldwide. Atherosclerosis begins with endothelial activation and progresses through a series of events (lipid build-up, fibrous elements and calcification) to cause artery narrowing and the activation of inflammatory pathways. Along with these processes, the resulting atheroma plaque causes cardiovascular problems [1]. Epigenetics can be defined as the changes made in the gene expression without altering the actual DNA sequence. These changes can be inherited. Epigenetic modifications can be referred to as the memory of past cellular states and perturbations a cell retains without changing the sequence or altering the same but by bringing about changes in gene expression and chromatin structure [2]. This induces a phenotypical change without making any modification in the genotype of an organism or a cell. Atherosclerosis is a complicated vascular disease majorly noticed in arteries that are mid to large in size. This can be a cause initiated from more than 1 type of factors including genetic instability, mutations or physical injury etcetera. Atherosclerosis is a characteristic outcome of endothelial dysfunction, inflammation in vasculature and lipid, cholesterol, calcium deposition and the deposition of cellular debris in the microenvironment [3]. As the lipid and other debris deposits on the inner wall of the arteries it starts accumulation furthermore waste resulting is the narrowing of the arteries which fundamentally results in blockage of blood flow increasing blood pressure and cutting of the oxygen supply for the target vital organs [4].

Recent studies and molecular techniques have proven that the genetic factors play a major role in the risk of atherosclerotic cardiovascular disease and other organ malfunctions even though environmental and behavioural factors such as smoking may seem like potential treats [5]. Genetic factors also influence the underlying metabolic risk factors primarily including the diabetes mellitus, dyslipidaemia (abnormal levels

of lipids for example tri-glycerol, cholesterol) and hypertension. Nanoparticles are used as theranostic strategies to visualise and interpret atherosclerotic plaques [6].

Recent studies suggest that microRNA and circular RNA also have their role in causing atherosclerosis, yet the underlying genetics and environmental factors remain as one of the prominent causes. Atherosclerosis has been proven to increase mortality in the present population in-turn increasing the need for improvement in cautious eating and mobility habits [7].

Causes of atherosclerosis

Some environmental factors such as environmental toxins, maternal obesity, stress, hypoxia at sleep can be a cause of epigenetic change that develops into atherosclerosis [8]. Stroke is a leading cause of death and Ischemic stroke (IS) represents 80 % of strokes. The most common subtype of Ischemic stroke is Large-artery atherosclerotic (LAA) stroke. This study has also found an evidential change in MTRNR2L8 methylation can be used as a potentially biomarker to clinically diagnose LAA stroke [9].

Histones are composed of C-terminal globular domain and N-terminal tail domain. The histone modifications are found to occur majorly in the tail domain which alters the gene expression by changing electrostatic charges, docking sites for transcription and remodelling of chromatin structures. DNA methylation is a process of addition of methyl group to the 5^{th} position of pyrimidine ring of cytosine or the 6^{th} group of purine ring of adenine, these biochemical reactions occur at gene promoter site and typically deregulates gene expression [10]. Recent research stated that it was observed that DNA methylation levels were high on experimenting cohort specified coronary artery disease (CAD) patients which was a risk factor for atherosclerosis [11]. Therapies including statin therapy remains the cornerstone for the medical prevention and treatment of atherosclerotic disease since it is effective and typically safe. Cholesterol absorption inhibitors, particularly ezetimibe, should be considered in high-risk individuals with statin intolerance or in high-risk patients who do not achieve the desired LDL-C level with rigorous statin treatment. Bile acid sequestrants, fibrates, and niacin should be avoided. Future PCSK-9 inhibitors, whether monoclonal antibodies or other methods, appear to be potent medicines for dyslipoproteinaemia [12].

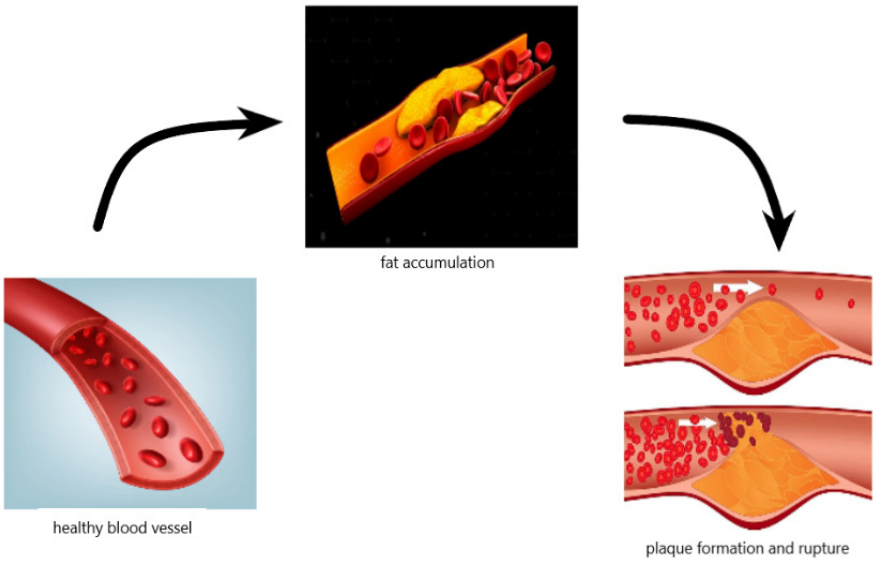

Figure 1 Steps involved in atherosclerosis.

The regulation of vascular, immune and gene specific expressions which possibly leads to atherosclerotic plaque deposition involves epigenetic modulators. It was importantly noticed that the epigenetic modifications are reversible and hence they also help in aiding as potential therapeutic targets and biomarker [13]. The regulation of lipoprotein metabolisms is carried out by many hepatic enriched miRNAs. An important function of this lipoprotein was stated to function as a carrier of cholesterol to be delivered and removed from cells and tissues medicating cholesterol homeostasis. Therefore, studies revealed that imbalances of cholesterol homeostasis such as accumulation of high levels of LDL-cholesterol and low levels of HDL-cholesterol leads to the formation of atherosclerosis [14].

Epigenetic causes of atherosclerosis

There are 3 biological processes playing their major roles in Atherosclerosis that are: Non-coding RNA mechanism, DNA methylation and histone modification.

Non-coding RNA mechanism

The development of atherosclerosis is significantly influenced by tiRNA (tRNA derived stress induced RNAs) and tRFs (tRNA related fragments). miRNAs (Micro RNAs) and ncRNAs (small non-coding RNAs) plays an important role in the regulation of vascular function and cell-cell communication which leads to the development of atherosclerosis [15]. When compared to samples from healthy patients, it was found that the RNA sequencing of atherosclerotic arterial samples detected up-regulated and down-regulated expressions of atherosclerotic patients [16]. LncRNAs (Long Non-Coding RNAs), such as MANTIS (metastasis-associated lung adenocarcinoma transcript 1), have been found to regulate inflammation by affecting transcription factors. MANTIS, a secondary structure, can limit inflammation in human endothelial cells by inhibiting ICAM-1 (intercellular adhesion molecule 1) expression. Statin therapy may also have anti-inflammatory properties by promoting MANTIS expression. Another lncRNA, RNCR3 (retinal noncoding RNA 3) affects atherosclerosis by downregulating KLF-2(Kruppel-like factor-

2) mRNA, leading to increased lesions in atherosclerotic mice. Future studies will need clarity on RNCR3's role. It was found that the endothelial-enriched lncRNA SNHG12 is crucial for DNA damage repair and cellular senescence in the vessel wall. Knockdown of SNHG12 in Ldlr−/− mice increased atherosclerosis lesion formation by over 2-fold. This senescence enhances permeability, leading to transcytosis, which accelerates atherosclerotic lesions [17]. A study investigates the role of miRNA-30d-5p in hyperhomocysteinemia (HHcy) induced atherosclerosis. It was found that miRNA-30d-5p is up-regulated in patients with HHcy and venous thrombosis, and TIMP3 (Tissue Inhibitor of Metalloprotease-3) is a predicted target. The study found that miRNA-30d-5p downregulates TIMP3 and increases MMP (Mouse peritoneal macrophages) activity, suggesting that HHcy influences atherogenicity. The results suggest that miRNA-30d-5p may play a role in atherosclerosis pathophysiology [18]. ANRIL (Antisense Noncoding RNA in the INK4 Locus), a 3.8-kb lncRNA, is a key element in the chromosome 9p21 CAD locus and has been linked to the Chr9p21 genotype [19]. Nucleotide polymorphisms (SNPs) in this region significantly affect ANRIL expression levels. Genetic variants associated with atherosclerosis lie within ANRIL codons, affecting cis- and trans-gene regulation [20]. Certain ANRIL polymorphisms, such as rs4977574, rs1333040, rs1333042 and rs10757274, are associated with coronary atherosclerosis but doesn't primarily involve Myocardial infarction and Acute coronary syndrome. Existing alleles affect DNA methylation and transcription factor binding, affecting ANRIL expression [21,22]. The following table represents the RNA, their target and the induced effects.

Table 1 miRNAs with potential effect on atherosclerosis [23].

Sl. No	ncRNAs	Target	Effect
1)	miRNA-30 c	LPGAT1	Low total-c and LDL
2)	miRNA-33	ABCA1/ABCG1	Low HDL
2.1)	Isoforms	PGC1-α/PDK4	Low ATP production
3)	miRNA-148a	ABCA1/ABCG1	Low HDL
		DNMT	High atherosclerosis
4)	miRNA-128-1	ABCA1/ABCG1/RXR α	High Ch efflux
5)	miRNA-27	LPL	Low lipid uptake Low inflammatory response Low atherosclerotic lesions
6)	miRNA-122	SREBP1, SCD1, ACLY	High total-c and TAG
7)	miRNA-92a	KLF-2, KLF4, SOCS5	Endothelial cell activation
8)	miRNA-155	BCL-6, SOCS1, SHIP-1, IL-13 Rα	Shift toward a pro-inflammatory macrophage profile

Abnormal DNA methylation

A study found that DNA hypermethylation that occurs in specific gene locations is a leading cause of atherosclerosis. It also shows that atherosclerosis is an underlying cause of cardiovascular diseases such as

coronary heart disease and stroke [24]. Considering the long-term accumulation of fat in arteries give rise to the formation of plaques which ruptures leading to clinical complications such as myocardial infarction and Ischemic stroke [25]. The regulation of vascular, immune and gene specific expressions which possibly leads to atherosclerotic plaque deposition involves epigenetic modulators. It was importantly noticed that the epigenetic modifications are reversible and hence they also help in aiding as potential therapeutic targets and biomarker [26]. According to research, abnormal DNA methylation, including abnormal hypermethylation and hypomethylation, plays a role in atherosclerosis. CpG islands in the promoter region of genes are often hypomethylated in healthy individuals, but CpG islands in the non-promoter area are hypermethylated. Global DNA hypomethylation (also known as DNA hypomethylation of non-promoter regions) can cause structural alterations and chromosome instability due to transcriptional initiation at erroneous areas and high transcriptional activity in normally silent places. Global DNA hypomethylation causes the expression of potentially dangerous genes as well as the overexpression of genes that are supposed to be silent. Global DNA hypermethylation, on the other hand, results in the deactivation of disease-suppressor or protective genes, gene mutation and allelic loss. DNA methylation is catalysed by DNMT proteins (DNMT1, DNMT3A and DNMT3B) and reversed by TET proteins (TET1, TET2 and TET3) [27]. It is speculated that hypermethylation has a dominant effect on atherosclerosis, as it tends to accelerate aging. The methyl transfer that causes hyperhomocysteinemia and the impact of oxidative stress on the hydroxyl-methylation of TET (10;11 translocation) proteins during the demethylation process are both considered to be directly related to hypermethylation [28]. The association between DNA methylation and inflammation related to cardiovascular disease due to the expression of Cytochrome C oxidase subunit II (Cox-2) gene. TNF-α (Tumour necrosis factor), a proinflammatory cytokine linked to the development of atherosclerosis, strongly induces the Cox-2 gene. However, DNA methylation can also lead to inflammation via DNA methyltransferase, which may hasten the onset of atherosclerosis [29]. According to a study, laminar shear stress affects PCSK9 expression in vascular smooth muscle cells and endothelial cells, which may control lipid deposition in regions that are susceptible to atherosclerosis. Instructions for producing the protein that controls blood cholesterol levels are provided by the PCSK9 gene. The aorta of wildtype mice was examined in different regions. It has also been reported that PCSK9 induces LDLr degradation, which causes LDL to accumulate in blood vessels and the bloodstream. This, in turn, aggravates LDL to oxLDL, which exhibits elevated PCSK9 gene expression [30].

1) DNA methyltransferase inhibitors

The FDA granted approval for the non-nucleoside DNMT inhibitor hydralazine to be administered as an anti-hypertensive drug. It has been demonstrated to hasten the development of atherosclerosis and decrease angiotensin II-induced hypertension in Apoe-/-mice [31]. Hydralazine has the potential to cure atherosclerosis because it also reactivates tumour suppressor genes and prevents DNA methylation. The impact of antisense oligonucleotides, like MG98, on different types of malignancies has been studied. Foods also include natural DNA methylation inhibitors, which have been investigated extensively [32].

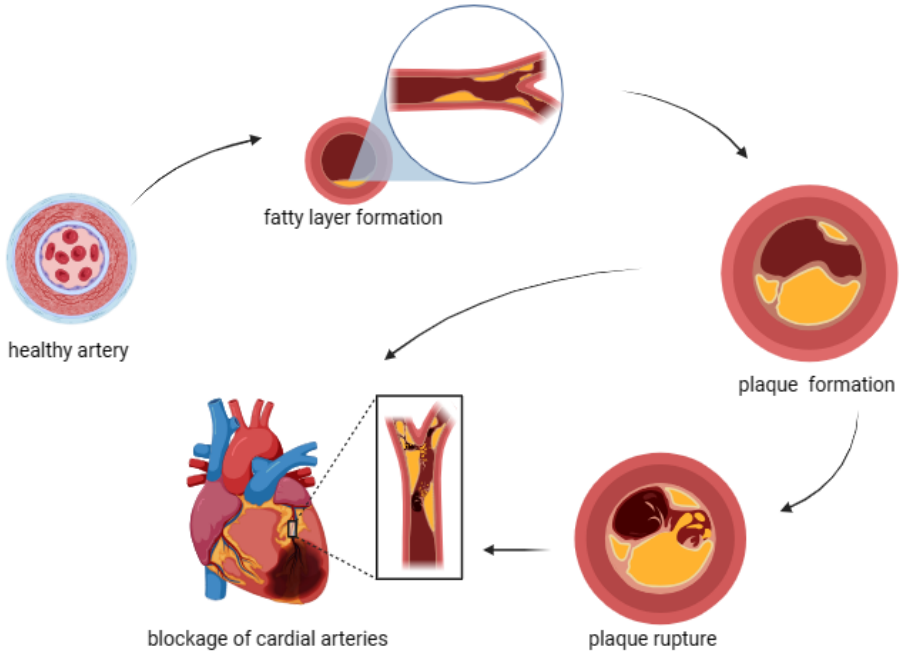

Figure 2 Representing pathway of atherosclerosis induced cardiac blood clot.

Histone modification

Histones are a group of essential proteins. These proteins are crucial in the regulation of gene expression. They exercise their power by altering the accessibility of DNA. When DNA is tightly coiled around histones, it blocks cellular machinery's access to specific genomic areas, suppressing gene expression. In contrast, when DNA is relaxed, genes become more accessible for transcription and subsequent cellular functions [33]. Histone's precise management of DNA compaction and accessibility is critical for a wide range of cellular functions, including developmental processes, cellular differentiation and responses to numerous stimuli. Understanding the subtle interactions between histones and DNA structure is essential for unravelling the complexity of genetic control and its consequences for health and illness. In eukaryotic systems, DNA often stays coiled to the histone proteins (H1, H2A, H2B, H3 and H4). Accurate and efficient regulation of gene expression can be achieved through chromatin compaction, signalling, or other signalling events including phosphorylation, acetylation, ubiquitination and methylation on histones [33].

Histone acetyl transferases (HATs) acetylate the lysine residue of histones [34]. In a study using carotid samples, it was discovered that the severity of plaque development in atherosclerosis was correlated with increased histone acetylation of H3K9 (9^{th} lysine residue of H3 protein) and H3K27 (27^{th} lysine residue of H3 protein) in smooth muscles and macrophages [35]. HDAC3 has been shown to play a crucial role in regulating and maintaining endothelium integrity, and its knockout would result in atherosclerosis and arterial rupture [36]. It was found that H3K9 ac is involved in the regulation of gene expression and histone modification plays an important role in the atherosclerosis pathology [37]. Dysfunction of vascular epithelial cells was found to be the initiation of atherosclerosis. Several studies showed that histone acetylation relates to MMP expression Research found that MMPs (matrix metalloproteinases) are vital in

the development of atherosclerosis, and that an imbalance in MMP-1 causes plaque instability and may have increased expression in the early stages of the disease [38].

1) Histone methylation and acetylation

A study revealed that levels of H3K27Me3 levels in the arteries was observed during different stages of plaque development [39]. Atherosclerotic lesions show unaltered H3K4 di-methylation, decreased H3K9 and H3K27 di-methylation, increased H3K4 appearance in smooth muscle cells, decreased H3K9 and H3K27 expression in inflammatory cells and increased histone methyltransferases [40]. Histone acetylation is catalysed by HATs, which acetylate conserved lysine residues on histone proteins [41]. Methylation on H3K4 has been linked to the stage-specific advancement of atherosclerosis, which is carried out by the proteins MYST1, MLL2/4 and GCN5L. High homocysteine (Hcy) levels were found to increase EZH2 expression in ApoE-/-mice, which resulted in H3 at lysine 27 (H3K27) tri-methylation. One of the components of the PRC1-like complex, the polycomb complex protein BMI-1, is necessary to prevent target gene expression by histone modification, such as mono-ubiquitination on H2AK119. BMI-1 stimulates the activity of E3 ubiquitin-protein ligase as a component of the PRC1 complex [42,43].

In atherosclerotic plaques, a global rise in H3K27 trimethylation was seen late in the pathogenesis. On the other hand, neither BMI-1 nor the H3K27-targeting histone methyltransferase EZH2 or demethylase JMJD3 were linked to reduced trimethylation of H3K27 [44].

2) Histone acetyl transferase inhibitor

Compounds known as histone acetyltransferase inhibitors (HATi) interfere with HATs, which are essential for the development of atherosclerosis and inflammation. Natural HAT inhibitor garcinol selectively inhibits p300 HAT and controls the gene EGR1 (Early Growth Response Protein 1), which is important in the development of atherosclerosis [45,46]. Another HAT inhibitor, anacardic acid (6-nonadecyl salicylic acid), targets PCAF and p300. The new anacardic acid analogue MG149 inhibits the NF-κB pathway, which is linked to pro-inflammatory cytokines and atherosclerosis, and is a strong inhibitor of the MYST family of HATs. It also showed a correlation with the severity of atherosclerosis [47,48].

3) Histone deacetylase inhibitors

The 4 groups of histone deacetylase inhibitors (HDACi) are benzamides, cyclic peptides, hydroxamates and aliphatic [49]. The FDA has approved some HDAC inhibitors for use as cancer therapies. One such inhibitor is vorinostat (also known as SAHA), which at nanomolar concentrations inhibits both HDACs and cell proliferation. Another HDAC inhibitor is trichostatin A (TSA), yet research has indicated that TSA negatively impacts the development of atherosclerosis [50]. It has been demonstrated that the selective HDAC inhibitor valproate reduces pro-atherogenic ER stress signalling pathways and attenuates atherogenesis in hyperglycaemic Apoe-/-mice [51]. In patients who have received prior systemic therapy, romidepsin, a structurally distinct inhibitor against HDAC1, HDAC2, HDAC3 and HDAC8, is licensed for the treatment of cutaneous T cell lymphoma [52]. HDAC1/2 and 3 are important contributors in the development of atherosclerosis; HDAC2 increases the histone deacetylation of SM22α (markers of differentiated SMCs), which accelerates the development of atherosclerosis. Atherosclerotic conditions can be suppressed by targeting these HDACs [53].

Table 2 Genes related to atherosclerotic diseases which are at least partly regulated by epigenetic mechanisms.

Sl. No	Gene	Target	Epigenetic mechanism	Reference
1)	eNOS	Endothelium	DNA methylation and Histone modification	[54,55]
2)	iNOS	Inflammation, macrophages	DNA methylation and Histone modification	[56]
3)	Fads2	Pathology of HHcy	DNA methylation	[57]
4)	c-fos	Shear stress	Histone modification	[58]
5)	Estrogen receptor α	Cardiovascular atherosclerotic tissues	DNA methylation	[59]
6)	Estrogen receptor β	Cardiovascular atherosclerotic tissues	DNA methylation	[60]
7)	15-LO	Pathogenesis of atherosclerosis	DNA methylation	[61]
8)	EC-SOD	Pathogenesis of atherosclerosis	DNA methylation	[62]
9)	H19/Igh2	Regulated by HHcy	Imprinting	[63]
10)	MMP-2	Extracellular matrix	DNA methylation	[64]
11)	MMP-7	Extracellular matrix	DNA methylation	[64]
12)	MMP-9	Extracellular matrix	DNA methylation	[64]
13)	TIMP-3	Extracellular matrix	DNA methylation	[65]
14)	IFN-ɣ	Inflammatory response	DNA methylation	[66]
15)	PDGF-A	Cell proliferation	DNA methylation	[67]
16)	ICAM-1	Inflammatory reactions	DNA methylation	[68]
17)	p53	Apoptosis	DNA methylation	[69]

Risk factors in atherosclerosis

Various pathological factors and processes like lipid metabolism disorder, the proliferation of smooth muscle cells and monocyte macrophages. Formation and aggregation of foam cells, impaired vascular endothelial cells and increased oxidative stress [70]. Genes that promote and prevent atherosclerosis are improperly regulated by the methylation level, which also affects gene expression. The enzyme AMP-activated protein kinase (AMPK), which is made up of the subunits and influences cellular homeostasis and metabolism [71]. Numerous others factor influences the atherosclerotic vascular diseases. Apart from the genetic and physiological factors that guide the fatty plaque formation by deposition of fats, lifestyle habits and practices also have similar roles to play in the atherosclerosis and related diseases.

Among the major role players homocysteine, fibrinogens, platelet reactivity, hypercoagulability, lipo-protein(a) and numerous infective agents are highlighted by recent studies.

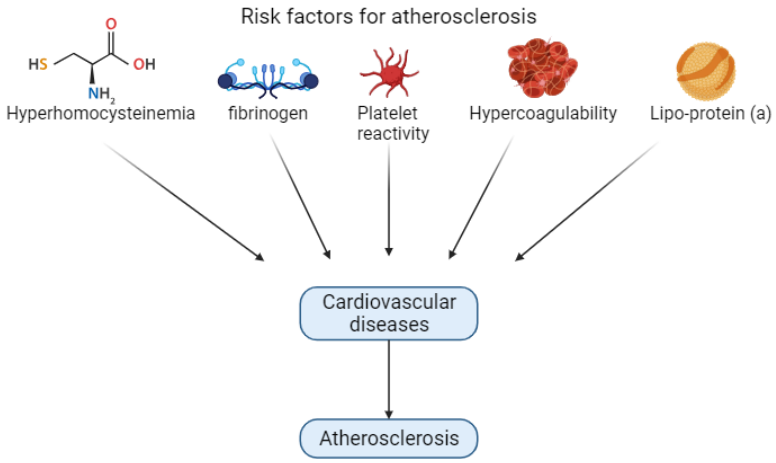

Figure 3 Risk factors in atherosclerosis.

Homocysteine

Homocysteine is an intermediate compound formed midst the metabolism of methionine, homocysteine is a thiol-containing amino acid. Studies and results suggest heightened plasma-homocysteine levels in-turn results in the higher risk factors for atherosclerotic vascular problems [72]. Along with the atherosclerotic risk high homocysteine levels are also co-related with menopause, aging and low plasma levels of vitamin B cofactors. Hyperhomocysteinemia has long been recognised as a risk factor for systemic atherosclerosis, cardiovascular disease (CVD) and stroke, with several epidemiologic and case-control studies demonstrating its link to these consequences. Furthermore, treating hyperhomocysteinemia with folic acid B vitamins helps to reduce the development of atherosclerosis, CVD and strokes. However, later prospective, randomised, placebo-controlled trials have not found a link between high homocysteine levels or their reduction with treatment and the risk of atherosclerosis, CVD, or strokes, probably due to folic acid fortification of food. As a result, there is currently debate on the importance of homocysteine as a risk factor for CVD and stroke, as well as whether patients should be routinely checked for homocysteine [73]. The amino acid homocysteine (Hcy), which contains sulfhydryl groups, is produced as an intermediate metabolite in the methionine cycle rather than being obtained through diet [74].

The formation of homocysteine (Hcy) occurs when the necessary amino acid methionine is converted to cysteine. Genetics, diet, age, sex, medications and illness status are among factors that influence the amount of Hcy in plasma. Numerous cellular biological processes, including cellular methylation status, cell metabolism and cell damage, are impacted by Hcy, an intermediate amino acid [75]. Because of elevated plasma homocysteine levels, progressive arteriosclerotic plaques in arteries were discovered because of cobalamin C disease. This conclusion was confirmed by observing arteriosclerotic plaques in a child with homocystinuria caused by methylenetetrahydrofolate reductase deficiency [76].

Fibrinogen

Numerous researches and work highlight fibrinogen's significance as a risk factor for atherosclerotic vascular disease. Increased fibrinogen levels have been linked to subsequent MI or stroke [77], according to a meta-analysis of 6 prospective epidemiological investigations. Those in the upper tertile showed a 2.3-fold increased relative risk of developing cardiovascular disease in the future compared to those in the lowest tertile. Only the Framingham Study included women out of these 6 investigations [78]. Fibrinogen is a clotting agent and an acute phase protein. It also has various other activities, such as being a necessary cofactor for platelet aggregation, determining blood rheology, and stimulating smooth muscle cell migration and proliferation. All these occurrences could play a part in fibrinogen's role in predisposing to atherothrombosis [79]. After accounting for cardiovascular risk variables, fibrinogen was positively correlated with the existence and severity of coronary atherosclerosis [80]. Fibrinogen expression is predominantly observed in hepatocytes and is subjected to transcriptional and post- transcriptional regulation. In the liver, fibrinogen is mostly produced persistently. Acute-phase proteins control the manufacture of fibrinogen. IL-6, which is produced by monocytes, macrophages and vascular endothelial cells, stimulates the synthesis of fibrinogen in the liver, while IL-1β and tumour necrosis factor-alpha (TNF-α) inhibit it [81].

Platelet reactivity

There may be a correlation between platelet quantity, size and function and the chance of atherosclerotic cardiovascular events. Platelet aggregation was measured in approximately 2,000 men who had no history of recent aspirin ingestion. The Caerphilly Collaborative Heart Disease Study provided cross-sectional data that showed patients with CAD had significantly higher adenosine diphosphate-induced platelet aggregation than controls [82]. Among a group of patients, spontaneous platelet aggregation proved to be a reliable indicator of survival and subsequent coronary events [83]. Clinical investigators have used a range of laboratory assays assumed to represent *in vivo* platelet behaviour to assess platelet reactivity in response to smoking. Platelet survival, bleeding time, platelet aggregation in response to various agonists, platelet aggregation ratio, plasma concentrations of platelet-specific proteins, serum thromboxane B2 (TXB2) production and serotonin (5-HT) release are all included.

Only the standardised template bleeding time, however, may be considered an accurate portrayal of *in vivo* occurrences. A correctly executed bleeding time provides a good indicator of platelet participation in primary homeostasis that is sensitive and reliable enough to serve as the best clinical screening test for platelet function [84].

Hypercoagulability

Congenital deficiencies of antithrombin III, protein C and protein are not linked to an increased incidence of arterial thrombosis or CAD, despite the predisposition to venous thrombosis. This is an intriguing aspect of the hypercoagulable states. Except in young women who smoke, the factor V Leiden mutation is related with venous thrombosis but not with atherosclerotic arterial disease.82.83G20210A

prothrombin gene mutation predisposes to venous thrombosis but may possibly be a risk factor for MI.85 [85,86].

Antiphospholipid antibodies have been linked to both arterial and venous thrombosis. A link to CAD has also been described [87].

Lipoprotein(a)

Lipoprotein(a) is a lipoprotein particle family that is comparable to LDL in core lipid content and has apo B-IOO as a surface apolipoprotein. Furthermore, Lp(a) has a unique glycoprotein, apo(a), which is linked to apo B-100.89Lipoprotein(a) is linked to an increased risk of cardiovascular disease, although its physiological purpose is unknown. Plasma levels vary substantially (approximately 1,000-fold) between individuals but are constant within individuals, indicating a high heritable component [88].

The levels are independent to those of other lipoproteins and apolipoproteins. The precise mechanisms governing Lp(a) levels are unknown. Lipoprotein(a) levels rise in renal failure, the nephrotic syndrome, following kidney transplantation, and in patients undergoing haemodialysis or peritoneal dialysis. Lp(a) fluctuations appear to occur in situations of hormonal change, such as diabetes, oestrogen therapy and pregnancy. When compared to white people, black people have higher amounts of LP(a) [89].

Other infectious agents

Recently, there has been a surge of interest in the viral explanation of atherosclerosis. The hypothesis was first offered in the first 2 decades of the 20th century [90]. That interest was revived when researchers demonstrated the development of atherosclerosis in chickens infected with the avian herpesvirus. Chlamydia pneumoniae, Helicobacter pylori, Herpesvirus hominis and CMV have since been implicated as key etiologic agents or cofactors in atherosclerosis pathogenesis. So far, evidence has consisted of immunocytochemistry or molecular biology indicating the presence of the agent in atherosclerotic lesions or correlating atherosclerotic disease and positive serologic test findings for an infectious agent [91].

Intranasal C pneumoniae infection exacerbated atherosclerosis in cholesterol-fed rabbits, according to a recent study. Furthermore, treatment with azithromycin after infectious exposure slowed the progression of atherosclerosis [92].

Pathophysiology

Hypercholesterolaemia is thought to be one of the primary causes of atherosclerosis. Increased plasma cholesterol levels cause changes in arterial endothelial permeability, allowing lipids, particularly LDL-C particles, to migrate into the artery wall. Circulating monocytes bind to endothelial cells that express adhesion molecules such as vascular adhesion molecule-1 (VCAM-1) and selectins and move in the subendothelial area via diapedesis. Once in the subendothelial region, monocytes acquire macrophage properties and transform into foamy macrophages. LDL particles in the subendothelial region oxidise and become potent chemo-attractants. These activities only increase the accumulation of large intracellular cholesterol via the development of scavenger receptors (A, B1, CD36, CD68, for phosphatidylserine and oxidised LDL) by macrophages, which bind native and modified lipoproteins and anionic phospholipids.

As a result, a series of vascular changes occur [93]. The most common mechanism responsible for the majority of acute coronary syndromes and sudden coronary mortality is atherosclerotic plaque rupture with luminal thrombosis. A thin cap fibroatheroma (TCFA) or "vulnerable plaque" is hypothesised to be the precursor lesion of plaque rupture. The necrotic core of TCFA is surrounded by a thin fibrous cap (65 m) that is invaded by macrophages and T-lymphocytes. Intraplaque haemorrhage is a primary cause of necrotic core expansion. The leaky vasa vasorum that invades the intima from the adventitia as the intima enlarges is thought to cause haemorrhage. Other causes of thrombosis include plaque erosion, which is less prevalent than plaque rupture but is a common cause of thrombosis in young people, particularly women under the age of 50. In plaque erosion, the underlying lesion shape is PIT or a thick cap fibroatheroma. Calcified nodule is the least common cause of thrombosis in older people with severely calcified and tortuous arteries [94]. However, unstable plaques are more likely to rupture, which could result in the formation of thrombus and acute cardiovascular events. They are identified by thin fibrous caps and inflammatory activity. Comprehending the complex biology of atherosclerosis is essential for formulating focused treatment approaches intended to slow down the disease's advancement and lessen the related cardiovascular load.

Table 3 Epigenetic drugs that delay atherosclerosis [95].

Drug name	Epigenetic drug category	Epigenetic targets
Vitamin C	TET2 activator	DNA methylation
5-Aza-2'- deoxycytidine	DNMT inhibitor	DNA methylation
Statins	EZH2 inhibitor	Histone modification
SAHA	HDAC inhibitor	Histone modification
Quercetin	DNMT inhibitor	DNA methylation
Curcumin	Broad-spectrum epigenetic modulator	-
EGCG	DNMT inhibitor	DNA methylation
Resveratrol	SIRT1 activator	Histone modification

After analysing data from the Framingham research, LDL-C, TG and HDL-C emerged as strong independent predictors of atherosclerotic disease. While other markers are being studied, TC, LDL-C and HDL-C remain the cornerstones in risk estimate for future atherosclerosis events. Low HDL-C levels have been demonstrated to be a powerful independent predictor of early atherosclerosis [96], and are included in the majority of risk estimation scores. Extremely high levels of HDL-C, on the other hand, have never been linked to athero-protection. The mechanism by which HDL-C protects against atherosclerosis is still being debated, although growing data suggests that the proportion of defective HDL vs functional HDL, rather than the levels, may be important. HTG has been demonstrated to be an independent risk factor for cardiovascular disease (CVD).

Furthermore, high TG levels are frequently linked to low HDL-C and high amounts of tiny dense LDL particles. The prevalence of HTG is considerable, with approximately 1/3 of adult adults having TG

levels greater than 1.7 mmol/l (150 mg/dL). Lp(a) is a subtype of LDL composed of an LDL-like particle and the apolipoprotein (apo) A.

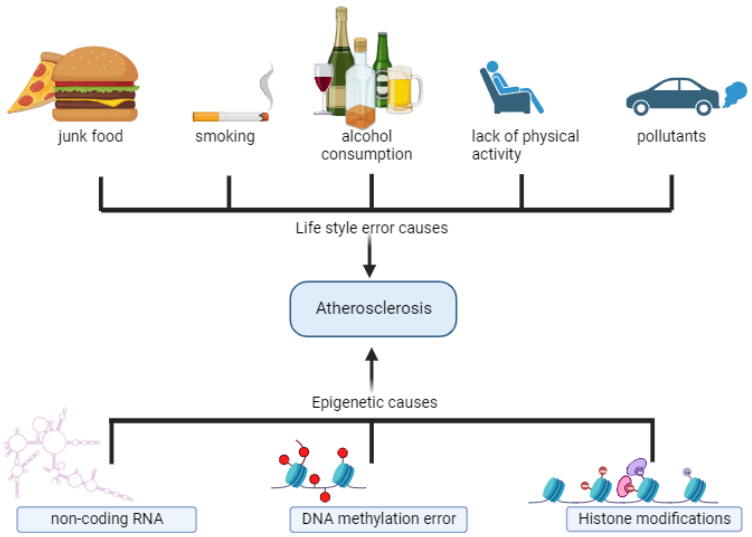

Figure 4 Causes of atherosclerosis.

Elevated Lp(a) is another independent risk marker, and genetic evidence suggests that it is causal in the pathophysiology of atherosclerotic vascular disease and aortic stenosis [97].

Therapy and treatment

Anti-cytokine therapies targeting specific IL signalling pathways could be powerful adjuncts to lipid lowering in the prevention and treatment of cardiovascular disease, as atherosclerosis is a complex chronic inflammatory disorder. Proinflammatory and proatherogenic cytokines [Like IL-1, IL-6 and TNF (tumour necrosis factor)] and anti-inflammatory and antiatherogenic cytokines (like IL-10 and IL-1rA) are the 2 main categories of cytokines linked to human atherosclerosis [98]. The RESCUE trial found that the IL-6 ligand monoclonal antibody ziltivekimab significantly reduced systemic inflammation associated with atherosclerosis in individuals with chronic kidney disease were studied. This finding suggests that large-scale cardiovascular outcomes trial could assess the effectiveness of this novel anti-inflammatory approach in reducing recurrent vascular events [99].

Studies have indicated that the equilibrium of DNA methylation in AS has been lost, highlighting the importance of developing medications for bidirectional modulation of DNA methylation in AS. A growing number of research have revealed that Chinese Herbal Medicines (HMs) may have a regulatory effect on DNA methylation in AS. Chinese HMs have the ability to (i) regulate methylation levels across the genome; (ii) regulate methylation of candidate genes; and (iii) allow methylation to interact with miRNAs. Single drugs and formulations, 2 typical HM components, offer clinical potential for treating abnormal DNA methylation in AS [100].

Cardiac Hospitalization Atherosclerosis Management Programme (CHAMP)

CHAMP was a successful strategy for increasing treatment utilisation of clinical trial evidence-based medicines. It is also the first trial to look at the feasibility, safety and influence on adherence of starting lipid-lowering drugs before discharge in patients with acute myocardial infarction who are hospitalised [101]. Prior research has evaluated the effectiveness of programmes to enhance risk factor reduction in patients with coronary artery disease. A home-based case management system led by a physician and maintained by a nurse was developed, compared to standard medical care in 585 men and ladies discharged from a hospital following a myocardial infarction Organisation for Health Care Maintenance [102]. Other studies have shown improved treatment rates in specialty lipid clinics and cardiac rehabilitation programmes, but these systems were applied to a subset of patients with coronary artery disease being cared for in the health care delivery system from which the patients were drawn [103].

When risk reduction by lifestyle modifications such as dietary changes, physical activity stimulation, and smoking cessation is insufficient, medication to appropriately regulate lipoprotein levels must be initiated. Medical therapy, in addition to lifestyle changes, is virtually always required in secondary prevention.

HDL-C increasing therapy

Even though lifestyle adjustments may raise HDL-C levels to some extent, many patients will require medication if a significant increase in HDL-C is deemed necessary. So far, there is no clear evidence that increasing HDL-C artificially leads to a better CV outcome If HDL-C boosting therapy is investigated, the following methods are available. Major study areas have included the role of HDL-C in atherosclerosis and the therapeutic modulation of HDL-C levels. The Framingham trial and several that followed could prove that HDL-C is an independent cardiovascular risk factor and that increasing HDL-C by just 10 mgL1 reduces risk by 2 - 3 %. While statin therapy and thus LDL-C decrease could significantly reduce coronary heart disease, cardiovascular morbidity and mortality still occur in a considerable proportion of subjects currently undergoing therapy. As a result, new tactics and medicines are required to minimise the risk even further. This was supposed to be accomplished via increasing HDL-C levels [104].

Bile acid sequestrate

At the maximum dose, cholestyramine, colestipol or the recently discovered colesevelam can reduce LDL-C by 18 - 25 %. The use of cholestyramine and colestipol is restricted due to gastrointestinal side effects and significant medication interactions with other commonly prescribed drugs. Colesevelam looks to be better tolerated, has fewer medication interactions, and can be used with statins. Large clinical trials for this class of medications have yielded relatively little hard evidence [105].

Several clinical investigations on colsevelam monotherapy were conducted. Colesevelam improves lipid profiles in hypercholesterolemic patients. Davidson *et al.* gave colesevelam to hypercholesterolaemia people [LDL-C > 160 mg/dL (4.14 mmol/L)] at 4 different doses (1.5, 2.25, 3.0 or 3.75g/day) with breakfast and dinner for 6 weeks. The accomplishment of LDL-C goal values is clinically significant [106].

Randomised controlled trials back up the efficacy regarding the safety of colesevelam in both monotherapy and combination therapy in conjunction with statins. Some smaller studies have also been conducted. The combination with fenofibrate or ezetimibe was evaluated. Colesevelam appears to have 'pleiotropic characteristics' as well. Anti-inflammatory and hypoglycaemic effects, for example. The negative effects of colesevelam consumption are as follows: This agent is often mild, making it considerably more tolerable than traditional BASs. On the other hand, the comparatively high price, the difficult dosing schedule, and a scarcity of long-term studies on CVD outcomes potential disadvantages [107].

Cholesterol absorption inhibitors

It has long been assumed that dietary cholesterol has a role in atherogenesis. Although this may be related to the increase in plasma cholesterol caused by dietary cholesterol consumption, a plasma cholesterol-independent impact of dietary cholesterol has been hypothesised. The metabolic syndrome, which is associated with insulin resistance, type 2 diabetes and an elevated risk of atherosclerosis, includes intra-abdominal obesity [108]. Ezetimibe lowers LDL-C via decreasing cholesterol absorption. In clinical trials, ezetimibe reduced LDL-C levels by 15 - 22 % when used alone, and it reduced LDL-C levels by 15-20 % when coupled with a statin. There have been no frequent serious adverse effects documented. Results from studies such as PRECISE-IVUS and IMPROVE-IT support the use of ezetimibe in conjunction with statins as second-line therapy when the therapeutic aim is not met at the highest tolerated statin dose, in statin-intolerant patients, or in patients with statin contra indications [109]. The degree of atherosclerosis was assessed in pinned aortas using the previously established face approach. Standard techniques were used to segment and stain formalin-fixed, paraffin-embedded adipose tissue and frozen OCT-embedded aortic sinuses with Movat's penta-chrome histochemical stain. A rat monoclonal antibody (Mac2; titre 1:2,500, Cedarlane Laboratories, Burlington, NC) was used to detect macrophages in adipose tissue and aortic root slices. The aortic root lesion area was quantified as previously described. Aortic root lesions were stained with Oil Red-O, photographed and then removed and stained with Movat's penta-chrome. Mac2 staining of aortic root lesions was conducted on an adjacent segment, photographed and then removed and stained with Movat's penta-chrome [110].

Bempedoic acid

Bempedoic acid is a first-in-class inhibitor of adenosine triphosphate (ATP) Citrate Lyase. The method of action involves inhibiting cholesterol production and increasing LDL-R, which reduces plasma LDL-C levels. Because of its tiny size and quick intestine absorption, bempedoic acid has a high bioavailability. Statins and bempedoic acid both work through the liver's machinery to exert their effects. The receptors used by the 2 medicines to enter the liver, however, differ. This characteristic of bempedoic acid guarantees that it does not compete with statin uptake in the liver [111]. A phase 3 clinical trial (CLEAR Harmony) is now being done in individuals who have a high CV risk and excessive LDL-C that is not being properly controlled by their current treatment. Almost 2,000 people will be randomly assigned to receive bempedoic acid or a placebo and will be observed for 52 weeks [112]. The biosynthetic property of bempedoic acid distinguishes it from statins, and the liver-specific nature of the mechanism of action may

explain the lack of muscle-related side effects found with this pro-drug [113]. Two important enzymes that control the metabolism of fats, carbohydrates and energy are modulated by bempedoic acid, which has positive effects on inflammation and metabolism. First, the enzyme hepatic ATP-citrate lyase (ACLY), which controls the flow of extra-mitochondrial citrate during lipid synthesis, is inhibited by bempedoic acid. Secondly, it increases the expression of AMP-activated protein kinase (AMPK), an essential enzyme that regulates inflammation and energy homeostasis across the entire organism. Together, these 2 molecular processes have a significant positive impact on systemic inflammation and LDL-C production, as well as the metabolic syndrome and diabetes [114].

Discussion

Although numerous factors and underlying genetic conditions play a major role in atherosclerosis recent research and study suggest that the ill habits and practices lead to fat deposition and cholesterol accumulation that have similar effects. Understanding epigenetics helps researchers explore how environmental factors influence our genes and contribute to diseases like atherosclerosis. It opens up possibilities for developing new treatments or preventive strategies by targeting these epigenetic changes to manage or even reverse the progression of atherosclerosis. Life style changes have influenced the obesity in the teen and adult population of the present times. There are a lot of prescribed and suggested medicine that are available abundantly in the market yet the solution for the root cause is not been developed. Research on solving the epigenetic errors that may cause atherosclerosis or induce the fat deposition and plaque formation. Synthetic drugs have been proven to have significant effect on reducing plaque formation and some drugs are designed to target in balancing the low-density cholesterol (LDL) and high-density cholesterol (HDL) thus, a need for a naturally available drug or a drug formulated by less toxic ingredients has been emerged. Genetic modifications have been proven to be a better alternative for not only the atherosclerosis but also to other similar health issues and disorders. Research is being enthusiastically conducted to improve and modify the therapies and treatments for the cure of atherosclerosis and also to find out the proper genetic cause to help the cure. Research holds the key to unlocking the ways and practices that may reduce or in fact have the potential to avoid being a victim to fat deposition. Therapies include cardiac hospitalization atherosclerosis management programme (CHAMP), HDL-C increasing therapy, bile acid Sequestrant, cholesterol absorption inhibitors and bempedoic acid as a synthetic cure that have been proven to have positive effect in reducing the bad cholesterol and plaque formation in adults which further decreases the risk of atherosclerosis. Like mentioned above the epigenetics plays a vital role in increasing or decreasing the risk for Atherosclerosis, thus a genetic approach for the prevention and rather a cure is of major interest among the researchers of the current era. The genetic approaches for the same include the narrowing down on the root cause and the main factor playing a vital role and treating the root cause which suits as a more effective way of approach.

Acknowledgements

Our study was supported by grants from the JSS AHER DBT-BUILDER: Sanction order no BT/INF/22/SP43045/2021. The authors would like to thank the Department of Science and Technology -

Fund for Improvement of Science and Technology Infrastructure (DST-FIST) and Promotion of University Research and Scientific Excellence (DST-PURSE) for the infrastructure provided.

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