Role of Epigenetics in the Development of Metabolic Disorders: A Review

By

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing a degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

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Ethics Statement

The project does not involve any clinical trial or human participants, no animals were used or harmed.

Abstract

Metabolic disorders such as diabetes, obesity, cardiovascular diseases and cancer have become a major global health concern accounting for significant morbidity and mortality rates. Although genetic factors largely contribute to these disorders, emerging evidence suggests that epigenetic modifications also play a crucial role in the development and progression of metabolic disorders. This review paper discusses the concept of epigenetics and provides an insight on the role of epigenetics in the development of metabolic disorders.

Keywords: Epigenetics; Metabolic disorders; DNA methylation; DNA acetylation; Histone modifications

Dedication

Dedicated to my faculty members, family and friends

Acknowledgement

In the beginning, I would like to thank Allah (SWT) in the sincerest way possible for giving me the good wellness, patience, strength, and wisdom without which I might not have gotten to where I am today.

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Table of Contents

List of Tables

List of Figures

List of Acronyms

5-hmC	5-hydroxymethylcytosine
5-mC	5-methylcytosine
BBB	Blood Brain Barrier
CpG	5'-Cytosine-phosphate-Guanine-3
DNMT	DNA Methyltransferases
DNMTi	DNA Methyltransferase Inhibitor
HAT	Histone Acetyltransferases
HATi	Histone Acetyltransferases Inhibitor
HDAC	Histone Deacetylase
HDACi	Histone Deacetylase Inhibitor
miRNA	MicroRNA
mRNA	Messenger RNA
ncRNA	Noncoding RNA
piRNAs	Piwi-interacting RNAs
DNA	Deoxyribonucleic Acid
TET	Ten eleven translocation methylcytosine
SAM	Cofactor S-adenosyl-L-methionine
ATP	Adenosine Triphosphate
BMI	Body Mass Index
STAT	Signal Transducer and Activator of Transcription
BAT	Brown adipose tissue
WAT	White adipose tissue

AMPK	AMP-activated Protein Kinase
LEP	Leptin
ADIPOQ	Adiponectin
TNF	Tumor necrosis factor
POMC	Proopiomelanocortin
NPY	Neuropeptide Y
H3K9	Histone 3 Lysine 9
H3K18	Histone 3 Lysine 18
T1D	Type 1 Diabetes
T2D	Type 2 Diabetes
CD4 T	Cluster of Differentiation 4 T cells
TFH T	Follicular Helper cells
IFNγ	Pro-inflammatory cytokines interferon gamma and
TNFα	Tumor necrosis factor alpha
ROS	Reactive Oxygen Species
GSK-3	Glycogen Synthase Kinase 3
PIP3	Phosphatidylinositol (3,4,5)-trisphosphate
AKT	Protein Kinase B (also known as Akt)
PI3K	Phosphoinositide 3-Kinase
MZ	Monozygotic
GAD2	Glutamate Decarboxylase 2
HLA-DQB1	Human Leukocyte Antigen - DQB1
RFXAP	Regulatory Factor X-Associated Protein

NFKB1A	Nuclear Factor Kappa B Subunit 1A
PDX1	Pancreatic and Duodenal Homeobox 1
H3K9Ac	Histone 3 Lysine 9 Acetylation
H4K16Ac	Histone 4 Lysine 16 Acetylation
H3K4me3	Histone 3 Lysine 4 Trimethylation
H3K9me2,3	Histone 3 Lysine 9 Dimethylation, Trimethylation
H3K27me3	Histone 3 Lysine 27 Trimethylation
HMOX1	Heme Oxygenase 1
MMP10	Matrix Metalloproteinase 10
SLC7A11	Solute Carrier Family 7 Member 11
MMP1	Matrix Metalloproteinase 1
MCP-1	Monocyte Chemoattractant Protein-1
ICAM	Intercellular Adhesion Molecule
CDKN2B-AS1	Cyclin-Dependent Kinase Inhibitor 2B Antisense RNA 1
KCNQ1OT1	KCNQ1 Opposite Strand/Antisense Transcript 1
CDKN2A/B	Cyclin-Dependent Kinase Inhibitor 2A/B
CVDs	Cardiovascular Diseases
PLC	Phospholipase C
VSMCs	Vascular Smooth Muscle Cells
EZH2	Enhancer of Zeste Homolog 2
PRC2	Polycomb Repressive Complex 2
CSE	Cystathionine -lyase
BMP2	Bone Morphogenetic Protein 2

WHO	World Health Organization
TSGs	Tumor Suppressor Genes
VHL	Von Hippel-Lindau (VHL) gene
MLL	Mixed Lineage Leukemia
MLL	Mixed Lineage Leukemia - EP300 (E1A-Binding Protein, p300)
MOZ	MOZ (Monocytic Leukemia Zinc Finger Protein)
MOZ	MOZ (Monocytic Leukemia Zinc Finger Protein)
HSP90	Heat shock protein 90
NPA	Non-polyadenylated
FDA	Food and Drug Administration

Chapter 1

Introduction

1.1 Background

Conrad Waddington was a British biologist who was curious about the methods by which embryonic cells develop into distinct cell types and also how environmental factors may affect the procedures. The word “epigenetics” is founded on Conrad Waddington's research (1905 – 1975). He came up with the term “epigenetics” by combining “epigenesis” which refers to the development of a complicated organism from a single unit (cell, spore, or similar), as well as “genetics”. By these two words he was hoping to emphasize the role that genetics plays in the growth and development of a person by fusing (Ospelt, 2022).

The field of epigenetics determines ways cells regulate gene function without altering the DNA sequence. The Greek word “epi” means on or above, the term "epigenetic" refers to influences other than the genetic code. Epigenetic alterations regulates the phenotypes without change in genotype by influencing the expression of genes. These alterations are connected to DNA and do not alter the order in which the DNA basic elements are arranged. The epigenome, which consists of the alterations that control how genes are expressed inside a cell's entire set of DNA, is referred to by the term genome. The major epigenetic modifications are DNA methylation, histone modification, and non-coding RNA. Some other type of epigenetic modification are DNA hydroxymethylation, DNA demethylation, chromatin remodeling, RNA editing, Nuclear reorganization, Polycomb group proteins Epigenetic modifications affect the synthesis of proteins in cells by influencing the decision of which genes are transcriptionally activated or inactivated. Each cell only generates the proteins required for it to operate because of the epigenetic regulation, such as, muscle cells do not create the proteins which support bone formation. The epigenome can be impacted by environmental factors like a person's nutrition and exposure to contaminants. When cells divide, epigenetic alterations can be preserved from cell to cell and, in some situations, passed down to next generations (*What Is Epigenetics?*, 2021).

Human diseases may develop and grow in part as a result of epigenetic alterations. Abnormal gene expression can result from problems in the epigenetic process, which can often lead to genetic disorders. Cancers, metabolic disorders, cardiovascular diseases, neurological disorders, and autoimmune diseases are a few examples of these conditions.

The occurrence of metabolic disorders have increased in the developed countries, hence biological research is concentrated on the study of its origin. Epigenetics play an important role in the development of metabolic disorders. The investigation of exact causes is particularly challenging because both hereditary and environmental influences contribute to the occurrence of the disease. The potential of environmental influences to promote phenotypic alteration by epigenetic DNA modifications was revealed to have an essential role in the development and propensity to distinct symptoms of metabolic disorder (KUNEŠ, 2015). Obesity, diabetes, cardiovascular diseases, liver diseases, cancer, hyperthyroidism, cystic fibrosis, hypothyroidism are some of the common metabolic disorders.

Metabolic disorders are a significant public health issue worldwide, with a high prevalence in both developed and developing countries. The World Health Organization (WHO) estimates that over 1 billion adults worldwide are overweight or obese, which is a major risk factor for metabolic disorders such as type 2 diabetes, cardiovascular disease, and some cancers. In the United States, for example, more than 34 million people have diabetes, with type 2 diabetes accounting for the majority of cases. In addition, it is estimated that more than 88 million American adults have prediabetes, which is a condition in which blood glucose levels are higher than normal but not yet high enough to be diagnosed as diabetes. The available evidence indicates that in most countries between 20% and 30% of the adult population has metabolic syndrome deteriorating people's quality of life. Because of this relatively high prevalence, metabolic syndrome accounts for an increasing proportion of cardiovascular risk worldwide.

This review paper provides a comprehensive insight on different metabolic disorders, the role of epigenetics in the development of those metabolic disorders and scopes of using epidrugs in their treatment.

1.2 Objectives of the Study

Several studies indicate that metabolic disorders like obesity, diabetes and cardiovascular disease may be influenced by epigenetic modifications. To correlate with the development of metabolic disorder in the field of epigenetics, the objectives of this literature review are:

- To provide an overview on the concept of epigenetics
- To discuss the role of epigenetics in the development of metabolic disorders

1.3 Rationale of the Study

It is now widely accepted that the disruption of the epigenetic marks is a frequent biological occurrence that contributes to the development and progression of metabolic diseases. The significance of epigenetic changes in metabolic disease is becoming clearer. We may learn more about the epigenetic and its importance by knowing its concept also, we may be capable of identifying the origins of metabolic problems by using the epigenetics concept. Learning more regarding the nature of the disease and possibly identifying new therapeutic targets will become easier by understanding the concept of epigenetics and how epigenetic modifications play a role in the development of metabolic disorders. Moreover, a comprehensive insight on the contribution of epigenetic modifications in the development of metabolic disorders will make it more convenient to diagnose metabolic syndromes.

Chapter 2

Methodology

An outline was created to organize the information to look for in a systematic manner. The primary and secondary research articles were collected from databases such as PubMed, Scopus, Google Scholar, and other authentic sources. Some of the keywords used for searching articles include: Epigenetics, metabolic disorders, DNA and histone modifications, cancer, obesity, diabetes, cardiovascular diseases, epigenetic drugs, chromatin remodeling, signaling pathways, treatment, etc. The articles were then compiled, paraphrased, and cited after being reviewed to gather the necessary information in accordance with the outline. The figures in this review were created by using software called BioRender, while the in-text citations and bibliography were generated using Mendeley Reference Manager.

Chapter 3

Epigenetics

3.1 Epigenetic Modifications

The complicated interaction between the genotype and environmental variables that controls cell and organ division and growth was the original definition of epigenetics. Nowadays, this phrase is used to describe heritable features that do not result from modifications to the DNA sequence. These characteristics are the outcome of altered gene expression that is controlled by modifications to the chromatin structure or accessibility of the DNA. DNA methylation, post-translational modification of histone proteins, and nuclear activities of noncoding RNA can all result in epigenetic alterations (Figure 1). Exogenous influences and environmental exposures can have an impact on epigenetic modifications, establishing a mechanistic connection between genes and environment in characterizing phenotype and delivering a description for phenotypic variations between monozygotic twins (Loscalzo & Handy, 2014).

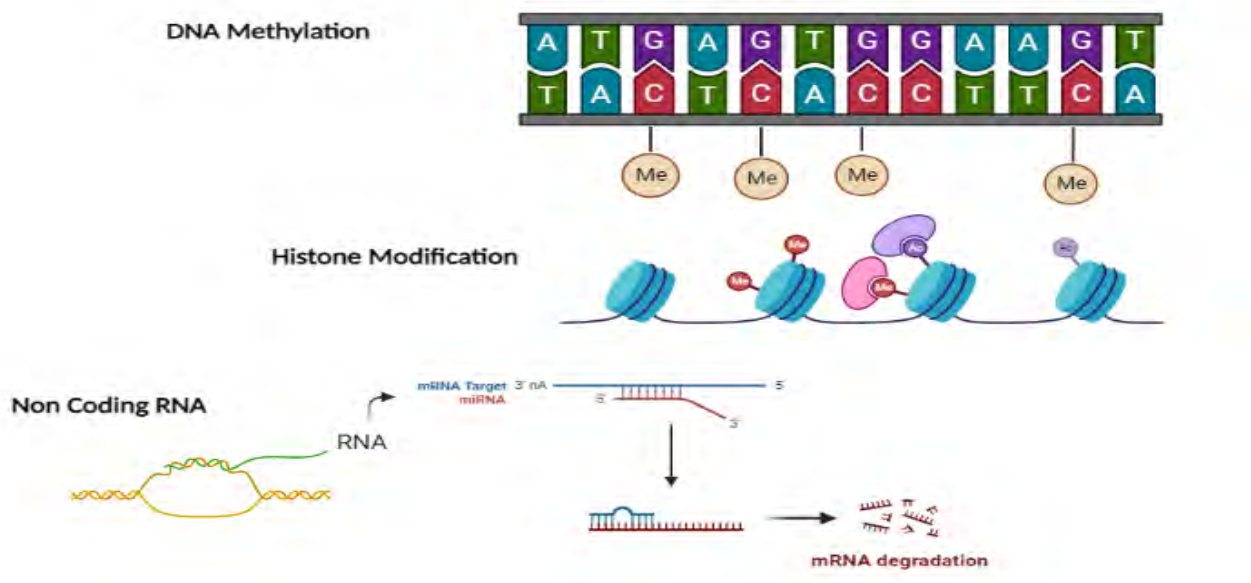

Figure 1: Schematic representation of the epigenetic modifications (Adapted from Rotondo et al., 2021; Zakhari, 2013)

3.2.1 DNA Methylation and DNA Demethylation

DNA Methylation

The binding of the transcription factors to the promoter regions of the DNA to initiate the transcription process are usually blocked by methyl group when it is introduced to certain locations on the DNA. The DNA methylation is carried out by an enzyme known as DNA methyltransferase (DNMT). With a procedure known as DNA demethylation caused by the enzyme demethylase, this methyl group can be eliminated. Usually, methylation turns off the genes and demethylation turns on the genes (Figure 2).

A methyl group at position of C5 is covalently added to the cytosine which is the primary epigenetic change of DNA. This alteration generally occurs in regions with CpG dinucleotides, frequently to maintain sequences that inhibit gene expression. For transcriptional suppression of transposons, imprinting and X-chromosome inactivation, tissue-specific gene expression throughout growth and differentiation also depends on CpG methylation. In order to control the expression of genes in embryonic stem cells, methylation of cytosines outside of CpG sequences can also take place.

A family of DNA methyltransferase DNMT, including DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L, controls the methylation of DNA. DNMT1 selectively methylates hemimethylated DNA, also during S phase it is confined to replicating the target sites. As a result, throughout DNA replication, DNA methylation patterns are copied to the daughter strands by the suggested control of methyltransferase (Jin et al., 2011). The correct cytosine methylation pattern is established throughout growth by two active enzymes, DNMT3A and DNMT3B that are members of the de novo DNA methyltransferase family. The pathophysiology and development of monogenic illnesses and malignancies are associated with abnormalities in DNMT3 function (Leppert & Matarazzo, 2014).

DNA Demethylation

Demethylation of DNA refers to the elimination of a methyl group from DNA. DNA demethylation can occur passively, actively, or in a hybrid manner. DNMT1 typically participates

in passively DNA demethylation on produced DNA strands throughout replication cycles. It is true that DNMT1 is widely recognized for its function in methylating DNA by introducing methyl groups. However, there is proof that DNMT1 may potentially be engaged in a procedure known as "passive demethylation." Whenever DNMT1 failed to add methyl groups to the freshly produced DNA strand during DNA replication, passive demethylation relates to the progressive loss of DNA methylation which results. This may cause DNA methylation levels to gradually drop over time due to the progressive loss of DNA methylation signals.

Active DNA demethylation is primarily accomplished by the successive alteration of cytosine bases which have undergone TET enzyme-mediated oxidation in order to remove 5-methylcytosine. TET1, TET2, and TET3 are 5-mC hydroxylases that belong to the ten-eleven translocation (TET) family. By attaching to CpG-rich areas to inhibit undesired DNA methyltransferase activity and by transforming 5-mC to 5-hmC, 5-hmC to 5-fC (5-formylcytosine), and 5-fC to 5-caC (5-carboxylcytosine), such proteins can induce DNA demethylation (Olinski et al., 2021).

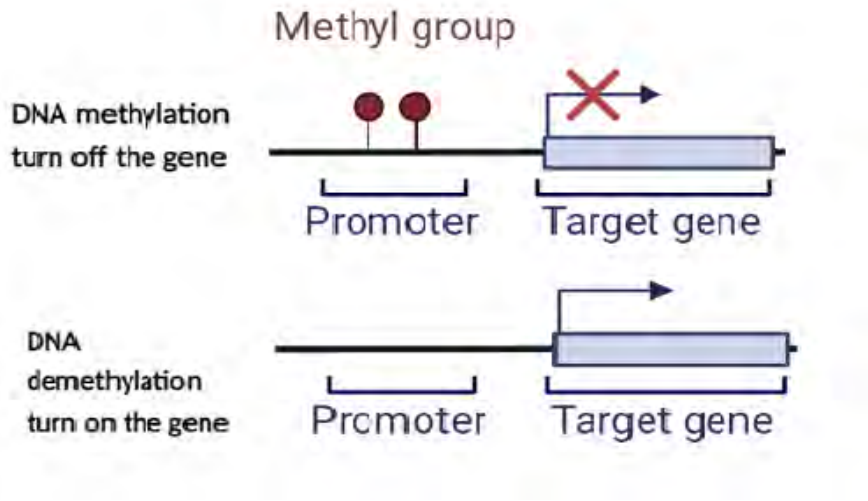

Figure 2: Gene expression regulation by DNA methylation and DNA demethylation (Adapted from Liaw et al., 2022)

3.2.2 Histone Modifications

Histone proteins are surrounded by chromatin. If the DNA is tightly coiled around histone, the DNA is transcriptionally inactive as the transcription factors and other protein complexes initiating the transcription process cannot access the gene to be transcribed. This highly condensed form of DNA is known as heterochromatin which is transcriptionally silent and the less condensed form of DNA is known as euchromatin which is transcriptionally active.

Acetylation, methylation, phosphorylation, and ubiquitination are just a few of the posttranslational modifications which histones go through (Figure 3). These modifications alter the structure of the chromatin, which has an impact on gene expression. According to the "histone code" theory, various alterations can significantly change the chromatin structure and transcriptional ability. Even though the factors that determine whether a gene is expressed (repressed or promoted) are complicated and depending on the location of the lysine and the degree of its methylation, they are still poorly understood, histone lysine methylation indicates additional significant group of histone modifications which affects gene expression (Loscalzo & Handy, 2014).

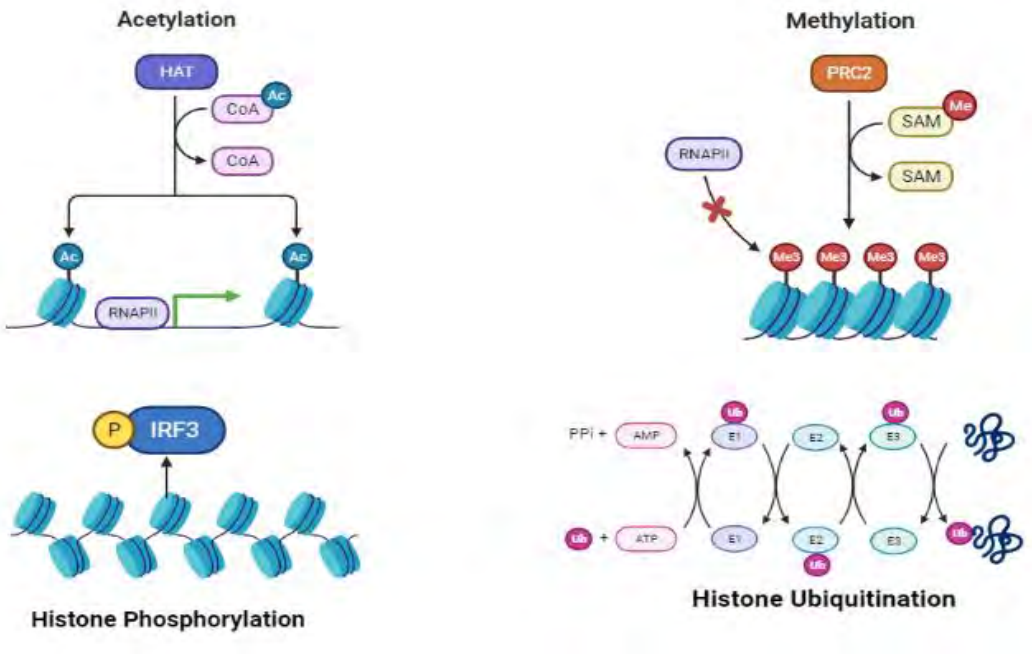

Figure 3: Histone modifications (Adapted from Suchitra Ajjarapu, 2022)

Histone acetylation

Histone acetyltransferases (HATs) and histone deacetylases are two categories of enzymes that maintain the level of histone acetylation (HDACs). HATs catalyze the elimination of a positive charge from the histones, decreasing the connection between histones and DNA's which is charged negatively by transferring an acetyl group from acetyl-CoA to an amino acid group of the target lysine residues in the histone tails. As a result, the chromatin normally becomes less compact and more available to the transcription factor. HDACs function as epigenetic regulation of expression of genes by removing acetyl groups from histone tail lysine residues (Alaskhar Alhamwe et al., 2018).

Histone methylation

Histone methyltransferases (HMTs), which include lysine methyltransferases (KMTs) and arginine methyltransferases (PRMTs), and histone demethylases mediate histone methylation and histone demethylation, respectively (HDMs). The electrical charge of histones is changed by acetylation of histone lysine, which has an impact on how well they interact with DNA. So far, this electrostatic connection is not directly affected by the methylation of histone lysine or arginine. Instead, it has an indirect impact on the new recruits and attachment of several transcription factors to chromatin. The enzymes known as histone methyltransferases (HMTs) can add up to three methyl groups from the cofactor S-adenosyl-L-methionine (SAM) to the histones' lysine or arginine residues (Alaskhar Alhamwe et al., 2018).

Histone phosphorylation

Histone phosphorylation state is regulated by two different types of enzymes that work in opposite ways. Phosphatases take away phosphate groups, whereas kinases give them. There are at least three recognized uses for phosphorylated histones, including the repair of DNA damage, management of chromatin compaction throughout mitosis and meiosis, and regulating of transcriptional activity. Histone phosphorylation, in contrast to histone acetylation and methylation, works in concert with some other histone modifications to lay the groundwork for their interplay. As a result of this interaction, the regulation of the chromatin condition down and its effects are complicated (Alaskhar Alhamwe et al., 2018).

Histone ubiquitination

Through a variety of cell signaling pathways, protein ubiquitination is an essential post-translational alteration that controls almost every element of cellular activity in eukaryotes. The goal proteins' stability and activity are controlled by the ubiquitin-proteasome mechanism, which links the 8.5 kD protein ubiquitin to substrate proteins. Histone ubiquitin ligases carry out histone ubiquitination, and deubiquitinating enzymes (DUBs), commonly referred to as ubiquitin-specific peptidases, can undo it (Alaskhar Alhamwe et al., 2018).

3.2.3 Non-coding RNA

Although RNA which fails to encode a protein is often referred to as a non-coding RNA (ncRNA), this isn't meant to imply that certain RNAs lack information or activity. Since it has long been believed that now the large amount of genetic information is carried by proteins, current research indicates that the majority of the genes of humans and some other biological creatures are actually translated to ncRNAs, which are frequently then converted to fewer items through regulating the expression (Mattick & Makunin, 2006). Non-coding RNAs are two categories for non-coding RNAs (non-coding RNAs) that are not translated into proteins. Short chain non-coding RNAs, which include siRNAs, miRNAs, and piRNAs, and long non-coding RNAs (lncRNAs), are the two primary groups of RNA that play a key role in the regulation. Non-coding RNAs have been implicated in epigenetic alteration in recent decades, and numerous research has found that they can affect gene and chromosomal expression to regulate cell development (Jian-Wei Wei et al., 2016).

siRNAs

Small interfering RNA (SiRNAs), a particular class of RNA molecules, are essential for controlling how genes are expressed. RNA interference is carried out by a double-stranded RNA molecule, which is typically 21 to 23 nucleotides long (RNAi). The biological process called RNA interference (RNAi) allows cells to mute or lower the expression of particular genes. The enzyme Dicer breaks down larger double-stranded RNA molecules to create siRNAs. As siRNAs are created, they join forces with a protein known as the RNA-induced silencing complex (RISC), that has the capacity to target and attach to corresponding messenger RNA (mRNA) molecules. The

siRNA-RISC binds specifically to mRNA molecules and causes their destruction or blockage of translation, which then in turn causes the relevant gene to be silenced or expressed at a lower level (Jian-Wei Wei et al., 2016).

miRNAs

MiRNAs are single-stranded RNA molecules that range in length from 19 to 24, and 50% of them are found in chromosomal areas that are susceptible to structural alterations. The siRNA and miRNA categories of control RNAs were once considered to differ from one another in two key ways. One is because siRNA is exogenous and originates by viral infection, the area of the transfer of genes, or the gene target, however miRNA is endogenous and the expression outcome of the actual gene. Another distinction is that siRNA is made from fully complementary, lengthy double-stranded RNA, while miRNA is made up of insufficient, hairpin-shaped double-stranded RNA that has been analyzed through Drosha and Dicer. Despite these variations, it is hypothesized that miRNA and siRNA share a common mode of activity in inducing silence of transcriptional genes due to their close link, for example, the similar sized of the two components. Nearly 1,800 potential miRNAs have recently been discovered in the human genome, and the number of miRNAs is still growing quickly as a result of the advancement of high-throughput sequencing methods (Jian-Wei Wei et al., 2016).

piRNAs

Piwi-interacting RNAs (piRNAs) are a subclass of RNA molecules that range in size from 26 to 31 nt. Piwi-interacting RNA, or piRNA, gets its name from its ability that it binds to Piwi proteins in a physiologic context. By attaching to chromosomal PcG react and PcGs, the Piwi protein reduces the homeobox gene, demonstrating its function as an epigenetic control component. Therefore, it has been hypothesized that the piRNAs linked to the Piwi protein might play important functions in the control of epigenetic processes. According to pertinent studies, piRNAs can be separated into two sub-clusters. One of them is the pachytene piRNA cluster, which predominately develops throughout meiosis and is still produced throughout the haploid spermatid stage (Jian-Wei Wei et al., 2016).

Long non-coding RNAs (lncRNAs)

Among non-coding regulation RNAs, LncRNAs are one more group. LncRNAs are typically >200 nt long, found in the cytoplasm or nucleus, and infrequently encode proteins. Sense, Antisense, Bidirectional, Intronic, and Intergenic lncRNAs are the most common divisions of LncRNAs. Nevertheless, only four classic lncRNA methods for controlling gene expression are primarily involved in each of these groups: Scaffolds, decoys, signals, and guides. The first investigations to uncover the function for lncRNAs in epigenetic control, finding functions for two lncRNAs, H19 RNA and Xist RNA, accordingly, focused on investigations into genomic imprinting and X chromosome inactivity. H19 is a genomic imprinting lncRNA which may go to the cytoplasm, has been spliced and polyadenylated, and reaches a high cytoplasmic concentration. Despite the fact that H19 remains the initial gene known to be strongly related with genomic imprinting, its function is still unknown (Jian-Wei Wei et al., 2016).

3.2.4 Other Modifications

Chromatin remodeling

Chromatin is a common name for the protein and DNA complex. Gene silencing results from the tight folding of DNA surrounding histones, which stops it from accessing numerous chromosomal regulation proteins. Chromatin remodeling is a key method for controlling the expression of genes because it allows numerous regulatory proteins, including transcription factors and DNA replication components, to gain entry to tightly enclosed DNA. The three dynamic nucleosome features of rebuilding, enzyme-induced covalent modification, and repositioning determine the fundamental process of chromatin remodeling. Nucleosome composition can be changed during reconstruction utilizing either standard histones or unique variations. Chromatin remodeling can also result from the covalent alteration of histones by ATP-dependent protein complexes, histone acetyltransferases, deacetylases, and methyltransferases. Additionally, remodeler complexes have the ability to influence nucleosome repositioning. Each of these mechanisms eventually end up in DNA activation and interaction with transcription protein regulators (Dr. Sanchari Sinha Dutta, 2018).

DNA hydroxymethylation

DNA hydroxymethylation, a newly identified kind of DNA modification that occurs when in cytosine at the C5 position the hydrogen atom gets replaced via a hydroxymethyl group, has only lately come to be understood for its significance to biological and its function as an epigenetic marker. Several differences in animal genomes have been found to include 5-hydroxymethylcytosine (5-hmC), and in humans' tissues have the highest concentrations of this substance. The TET family of methyl cytosine dioxygenases oxidizes 5mC to produce 5-hmC, which is thought to be a potential intermediary stage in a replication-independent DNA demethylation process (Figure 4). Understanding towards the DNA methylation mechanism responsible for numerous biological processes, which are relevant to the functioning of the nervous system, is currently provided by the recent finding of a hydroxylated form of 5mC (5-hmC) and the TET family of enzymes needed for its transformation (Richa & Sinha, 2014).

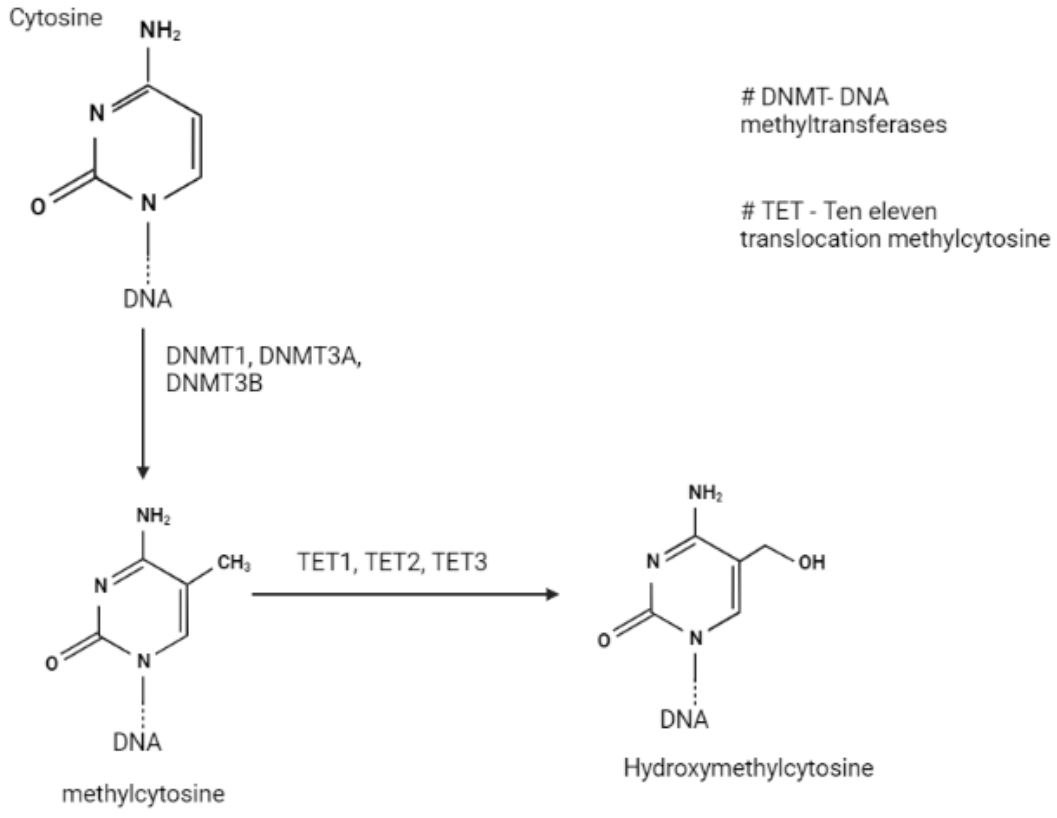

Figure 4: Hydroxymethylation of the DNA (Pfeifer et al., 2013)

3.3 Importance of Epigenetics

Epigenetics is largely acknowledged as one of current biology's most significant and important topics. From conception until maturity, epigenetics plays a crucial role in the growth of organisms. Throughout embryonic development, it aids in controlling gene expression to make sure the proper genes are expressed at the appropriate time and location. Cancer, diabetes, and neurological illnesses are just a few of the diseases that epigenetic modifications have been linked to contributing to their onset. For instance, epigenetic modifications can alter how some genes are expressed, which can result in the unchecked cell growth that is a hallmark of cancer. Without affecting the underlying DNA sequence, epigenetic alterations can be transmitted from one generation to another. Hence, epigenetics can contribute to the understanding of how traits and diseases are handed down through generations.

Epigenetic indicators are capable of helping forecast how a person will react to specific medications or therapies. It can enhance patient outcomes and customize treatments. All things considered, the science of epigenetics is revolutionizing how we comprehend how genes and the environment affect human well-being and growth. In reaction to environmental stressors, epigenetic alterations can happen quite rapidly and have an impact on an organism's health and lifespan. It implies that epigenetics can contribute to the understanding of how organisms can undergo mutations and modify their surroundings.

The study of epigenetics can help us comprehend and protect threatened animals. Scientists may discover more about the genetic variety and health of communities by examining epigenetic sequences, and they can create plans for preservation as well as restoration.

3.4 Factors Affecting Epigenetics

A few things can have an impact on epigenetics:

- Environmental elements, such as poisons, pollutants, and anxiety
- Aging
- Genetic variation
- Lifestyle choices like exercise and sleep (Alegría-Torres et al., 2011).

Chapter 4

Metabolic Disorders

4.1 History of Metabolic Disorders

Metabolic disorders have been recognized for centuries, with some of the earliest descriptions dating back to ancient times. However, the concept of metabolism as a fundamental process in the body did not emerge until the 19th century.

In the early 20th century, the British physician Archibald Garrod made significant contributions to the understanding of inborn errors of metabolism. He proposed that these disorders were caused by defects in specific metabolic pathways, which led to the accumulation of toxic substances in the body (Arnold, 2018). It was proposed that inherited metabolic illnesses result from gene mutations that result in faulty enzymes with reduced or nonexistent function. The first hereditary condition in humans to be linked to an enzymatic deficiency was methemoglobinuria, which was discovered in 1948 (Gregory Enns, 2019). Herman Haller, who was researching the risks of atherosclerosis, introduced the phrase "metabolic syndrome" in 1977. He developed the word to discuss the links between fatty liver disease, obesity, diabetes mellitus, high blood lipids, a high uric acid level, in addition to the way having a combination of all of these conditions together increases the chance of atherosclerosis developing (Dr. Ananya Mandal, 2019).

So, the definition is when abnormal chemical processes interfere with the body's metabolism, a metabolic disease develops. This may impair the body's capacity to break down big molecules for energy, reduce the effectiveness of cell energy production, or interfere with the body's ability to regulate its energy levels.

4.2 Etiologies of Metabolic Disorder

The illnesses which are known as metabolic disorders have an impact on the body's metabolic functions. Many factors, such as genetics, way of life, and environmental influences, are responsible for these illnesses. A number of the most prevalent etiologies of metabolic diseases

exist. Inherited genetic mutations are the root cause of many metabolic diseases. These mutations can interfere with several metabolic pathways and result in diseases. Therefore significant lifestyle variables including poor diet, inactivity, and obesity can influence the development of metabolic diseases like type 2 diabetes, hypertension, and high cholesterol. Then, contact with pollution and poisons might result in metabolic problems. Nevertheless, hormonal abnormalities, including those caused by thyroid conditions or polycystic ovarian syndrome (PCOS), might impact how well the metabolism works. Moreover, certain drugs have the potential to disrupt metabolism and result in metabolic diseases. Then, some viruses, including Hepatitis B and C, can disrupt metabolic processes and result in abnormalities of metabolism. DNA methylation and histone acetylation are examples of epigenetic alterations that can impact metabolic pathways and cause metabolic diseases.

It is significant to highlight that numerous metabolic illnesses may have various etiologies and complicated etiological origins. The etiology of metabolic disorders is assisted by oxidative stress, a state when there is an unbalance between the synthesis of reactive oxygen species and the antioxidative defense mechanism. Oxidative stress can harm cells as well as tissues.

4.3 Classification of Metabolic Disorders

These disorders can affect various aspects of metabolism, including energy production, the breakdown of proteins, carbohydrates, and fats, and the removal of waste products from the body. Here is a classification of metabolic disorders based on their underlying causes:

1. Inborn Errors of Metabolism
2. Mitochondrial Disorders
3. Hormonal Disorders
4. Lysosomal Storage Disorders
5. Disorders of Lipid Metabolism
6. Disorders of Carbohydrate Metabolism
7. Organic Acidemias
8. Peroxisomal Disorders
9. Glycosylation Disorders
10. Metal Metabolism Disorders

11. Urea Cycle Disorders
12. Mitochondrial DNA Depletion Syndromes
13. Disorders of Purine and Pyrimidine Metabolism
14. Disorders of Amino Acid Transport
15. Disorders of Heme Metabolism
16. Neurotransmitter Disorders
17. Congenital Disorders of Autophagy
18. Endocrine metabolic disorders.
19. Disorders of vitamin and cofactor metabolism Vitamins and cofactors relevant for various metabolic pathways.
20. Disorders of organelle biogenesis, dynamics and interactions disorders.

4.4 Common Metabolic Disorders and Syndromes

Common metabolic disorders are given below:

1. Diabetes
2. Wilson's Disease
3. Obesity
4. Cardiovascular Disorders
5. Hyperthyroidism
6. Hypothyroidism
7. Cancer
8. Phenylketonuria (PKU)
9. Galactosemia
10. Maple Syrup Urine Disease
11. Lactose Intolerance
12. Hunter Syndrome
13. Krabbe Disease

The common symptoms of metabolic disorder is given below:

- Tiredness
- Weakness of muscles
- Overweight or loss the weight
- Changes in skin like rashes, dryness.
- Feeling pain in stomach
- nausea or vomiting
- Reduced appetite
- Growth issues in babies and infants
- Changes in mental state

4.5 Diagnosis of Metabolic Disorders

The diagnosis of metabolic disorders usually involves a series of tests and examinations to identify the underlying cause of symptoms. Here is an overview of the typical process of diagnosing metabolic disorders:

1. Medical history and physical exam: A healthcare provider will take a detailed medical history and conduct a physical exam to look for signs and symptoms of a metabolic disorder. They may ask about family history of similar disorders, growth and developmental milestones, and other medical conditions.

2. Blood tests: Blood tests are often the first step in diagnosing metabolic disorders. These tests can measure levels of various substances in the blood, including glucose, amino acids, enzymes, and hormones.

3. Newborn screening: Many metabolic disorders can be detected through newborn screening, which involves testing a newborn's blood for certain substances that may indicate a metabolic disorder.

4. Differential diagnosis: Metabolic disorders can have overlapping symptoms with other medical conditions, so healthcare providers will often perform a differential diagnosis to rule out other possible causes.

5. Collaborative care: The diagnosis of metabolic disorders often involves collaboration between different healthcare providers, including specialists in genetics, metabolism, endocrinology, and other related fields.

6. Monitoring and follow-up: Once a metabolic disorder is diagnosed, ongoing monitoring and follow-up are essential to ensure that treatment is working effectively and to catch any complications or changes in the condition

7. Urine tests: Urine tests can also provide important information about metabolic disorders. These tests can measure levels of substances such as glucose, ketones, and organic acids, which can indicate problems with metabolism.

8. Imaging tests: Imaging tests such as X-rays, CT scans, and MRIs can be used to look for abnormalities in organs or tissues that may be related to a metabolic disorder.

9. Genetic testing: Some metabolic disorders are caused by genetic mutations, so genetic testing may be recommended to confirm a diagnosis.

10. Biopsy: In some cases, a tissue biopsy may be needed to confirm a diagnosis.

4.6 Treatment of Metabolic Disorders

The treatment of metabolic disorders varies depending on the specific disorder and its underlying cause. Here are some general approaches to treating metabolic disorders:

1. Dietary changes: Many metabolic disorders can be managed through dietary changes. This may involve avoiding certain foods or increasing the intake of others.

2. Medications: Medications may be used to manage symptoms or to address the underlying cause of a metabolic disorder.

3. Supplements: Some metabolic disorders may require supplementation with vitamins, minerals, or other nutrients.

4. Organ transplantation: In some cases, a metabolic disorder may lead to organ damage that requires transplantation.

5. Gene therapy: In recent years, gene therapy has emerged as a potential treatment option for some genetic metabolic disorders. This approach involves delivering a healthy copy of a gene to replace a defective one, with the goal of restoring normal function to affected cells.

Also, some medicine can be used for the treatment of the metabolic disorder. Such as-

1. Metformin
2. Statins
3. Levothyroxine
4. Insulin
5. Glucagon-like peptide-1 (GLP-1) agonists
6. Thyroid hormone replacement therapy
7. Allopurinol
8. Bisphosphonates
9. Fibrates
10. Corticosteroids

Chapter 5

Role of Epigenetics in the Development of Metabolic Disorders

5.1 Epigenetics in the Development of Obesity

5.1.1 Obesity

Obesity is a medical condition characterized by an excess amount of body fat that can negatively affect a person's health. It is typically defined as having a body mass index (BMI) of 30 or higher, which is calculated by dividing a person's weight in kilograms by their height in meters squared. By 2030, there will be over 2.16 billion overweight people and 1.12 billion obese people worldwide, increasing the prevalence of both conditions (T Kelly et al., 2008). This evinces a clear global medical and social health impact together with societal and individual criticism. Additionally, it is linked to a higher risk of death, cardiovascular disease, cancer, and type 2 diabetes (T2D) (Flegal et al., 2007). Although extensive study, contemporary initiatives to combat the growing epidemic of obesity with nutrition, physical education, having surgery, and drugs have not succeeded to address the problem permanently (Kerstin Rohde et al., 2019).

Classification of Obesity and diagnosis of Obesity

Table 1: World Health Organization adult body mass index classification (Ruban et al., 2019)

Classification	**Body mass index (kg/m^2)**
Underweight	<18.5
Normal weight	18.5–24.9
Overweight	25.0–29.9

Obese class I	30.0–34.9
Obese class II	35.0–39.9
Obese class III	≥40

By measuring the body weight, a healthcare professional can diagnose a patient whether he or she is suffering from obesity or not.

Causes

An imbalance in the number of calories consumed compared to those burnt is the primary root cause of obesity. However, there are other risk factors that contribute to the illness. Even while poor eating habits and inadequate physical activity are the primary causes of being overweight, some people are genetically predisposed to being obese or experiencing a variety of medical conditions. Diet imbalance, lack of sleep, insufficient exercise, stress, genetic variation or mutation are the causes of obesity (Yasmine S. Ali, 2022).

Signaling Pathway

i. Obesity and the JAK/STAT pathway

Specifically linked to the development of obesity is the JAK)/STAT pathway. As a result of cytokine and growth factor release, the entire STAT protein family may be triggered by tyrosine phosphorylation. It turns on JAKs when they attach to the specific receptors, allowing the enzymes to transphosphorylate both the cytoplasmic tail and one another. The STAT proteins receive a place to dock with the receptor parts and are phosphorylated. The cell's nucleus is where the phosphorylated STAT proteins move, attach to particular DNA sequences, and control the gene expression of the desired genes. In the gene expression if any negative mechanism occurs then the dysfunction of this pathway may take place. The obesity is directly influenced by the JAK/STAT signaling pathway's dysregulation (Wen et al., 2022).

ii. Obesity and the AMPK pathway

By controlling eating, sensitivity to insulin, brown adipose tissue (BAT) thermogenesis and white adipose tissue (WAT) browning, AMPK contributes an essential part in the formation of obesity. Gaining weight happens when AMPK is activated in the CNS. The hypothalamic AMPK controls consuming behavior. In vivo application of ghrelin which is a hunger hormone stimulated hypothalamic AMPK production and raised consumption of food (Wen et al., 2022).

5.1.2 Role of Epigenetics in Obesity

Obesity is a metabolic disorder. The modification of the epigenetic marks also causes obesity. The modifications are given below:

DNA Modification

From the list of genes associated with obesity, leptin and adiponectin come out on the very top of the list. These hormones control energy production and metabolism and are mostly generated through adipose tissue. According to a study leptin (LEP) gene promoter is negatively associated with body weight and DNA methylation. Additionally connected to changed lipid profiles, reduced sensitivity to insulin, and damaged metabolism of glucose results from LEP promoter hypomethylation. In addition to this, beneath the skin adipose tissues, the total body weight index of obese adults showed a positive correlation with adiponectin gene (ADIPOQ) methylation and an opposite relationship with LEP methylation. There are many investigations claiming that the connection between the LEP and ADIPOQ genes develop obesity because of the insulin resistance and disturbance of metabolism (Mahmoud, 2022).

Histone Modifications

Changes in the histone have been observed in obesity and connected to the stimulation of expression of several genes which are involved in adiposity and metabolic functions. The appetite-regulating genes POMC and NPY have their gene expression controlled by histone acetylation. High-fat diet-related weight gain has been linked to decreased H3K9 acetylation at the PMOC and improved acetylation of the identical remains at the NPY gene. The gastrointestinal tract improved acetylation of H3K9 and H3K18 at the TNF (tumor necrosis factor) and CCL2 (monocyte

chemotactic protein 1) genes, which is probable what causes the inflammation. This modification also develops obesity (Mahmoud, 2022).

Non-Coding RNA

It was discovered that white adipose tissues had a caused expression of miRNA, which was associated with decreased blood vessel function. Already in DNA methylation it explained that the leptin is responsible of the obesity. MiRNA, which targets leptin, was overexpressed in obese individuals which enhancements in body weight and metabolic activity. It was found that the expression of an extensive list of miRNAs related to adipogenesis, metabolism of fat, signaling of insulin, hypoxia, inflammation, and cell growth which offer a thorough list of miRNAs associated with metabolic disorders and obesity (Mahmoud, 2022).

5.1.3 Treatment

A variety of epigenetic medications are being studied for the prevention and treatment of obesity. For example, it has been shown that histone deacetylase inhibitors (HDACi) may assist in the weight loss of obese individuals by improving their blood sugar metabolism. The enhanced acetylation of histones by HDACi leads to the activation of genes that control blood sugar and calories metabolism. (Arguelles et al., 2016).

Inhibitors of DNA methyltransferase (DNMTi), another kind of epigenetic drugs, have shown promise in the treatment of obesity. DNMTi reduces DNA methylation, that may result in amplification of genes associated with appetite and the decomposition of energy (Arguelles et al., 2016). Though these medications have not yet received approval for the treatment of obesity, it is crucial to remember that they remain in the very beginning stages of progress.

5.2 Epigenetics in the Development Diabetes

5.2.1 Diabetes

Blood glucose levels that increase in people with diabetes are long term metabolic disorders that eventually cause substantial harm to the circulatory system, blood vessels, eyes, kidneys, and nerves. Diabetes can be 2 type which is Type 1 Diabetes T1D and Type 2 Diabetes T2D.

Type 1 diabetes mellitus (T1DM), also known as diabetes caused by autoimmune disease, is a chronic condition that results in hyperglycemia when the pancreatic beta cells in the pancreas don't succeed to produce enough insulin. While indications typically begin in childhood or adolescence, they may show up much later in life. Although the root cause of T1DM is unknown, it recently was reported that T cell-mediated death of -cells may have an effect on how it develops (Katsarou et al., 2017).

Prolonged type 2 diabetes interferes with the body's ability to use insulin as intended. Type 2 diabetics are referred regarded as having insulin resistance. Middle-aged or older persons are the age group at most risk for this kind of diabetes. Adult-onset diabetes used to be the name for it. But type 2 diabetes also affects kids and teenagers, frequently as a result of childhood obesity. Type 2 diabetes affects around 29 million Americans. Another 84 million people have prediabetes, which is elevated blood sugar (or blood glucose) but not yet diabetes (Michael Dansinger, 2023).

Causes

Following are most probable causes of the diabetes:

- Viral infection: Researchers believe a virus, such as the flu or a cold, could be the root cause of type 1 diabetes. Following a viral infection like Epstein-Barr, measles, rubella, CMV, measles, influenza, or encephalitis, type 1 diabetes frequently develops just a few days later.
- Destroying or eliminating the pancreas: Rarely, type 1 diabetes can result from a pancreatic injury or trauma. The ability of the body to produce insulin is also lost as a consequence of a surgical elimination of the pancreas, which causes type 1 diabetes.

- Family history: If someone possesses a relative or sibling with type 1 diabetes, their risk of developing the condition is slightly higher. Genetics. Type 1 diabetes is more likely to occur in those who have specific genes.
- Age: Type 1 diabetes can appear at any point in life, but it usually does so over two stages. Little ones experience their initial period between the years of 4 and 7. The next stage impacts children between the years of 10 and 14 (Sarah Watts, 2022).
- Genetic factors: Specific DNA sequences affect the body's ability to produce insulin.
- Weight gain: Being overweight, especially if accompanied by belly fat, might cause insulin resistance.
- Metabolic syndrome: Excessive blood sugar levels, obesity of the abdomen, hypertension, high cholesterol, and high triglycerides are disorders that are frequently accompanied by insulin resistance.
- Liver overproduction of glucose: Some livers remain to manufacture glucose even though the blood sugar levels are appropriate.
- Communication problems between cells: Improper signaling or poor reception of messages among cells might interfere with the processes of converting glucose into insulin, possibly resulting in diabetes.
- Malfunctioning beta cells: Beta cells that aren't working properly can cause blood sugar levels to fluctuate, and excessive blood sugar can make them even worse (Michael Dansinger, 2023).

Signaling Pathway

For Type 1 Diabetes:

T cells of the immune response kill pancreatic beta cells and causing type 1 diabetes (T1D). Due to the T cell, the beta cell in the pancreas may die or suffer damage. T cells leave the thymus and travel to the pancreas lymph node. Autoreactive CD4 T cells may transform into T helper 1 (TH1), Follicular helper T (TFH), peripherally derived Regulatory T cell (pTreg), or anergic cells in the pancreas lymph node by connecting with dendritic cells transmitting islet antigen. Th1 cells two type of pro-inflammatory cytokines interferon gamma (IFNγ) and tumor necrosis factor alpha (TNFα) produce when the Th1 cell passing the pancreas. It the IFNγ and TNFα derived from the

Th1 cell which will stimulate the M1 macrophages. This M1 macrophages then produce reactive oxygen species (ROS), TNFα, and interleukin 1 beta (IL-1β) which may increase the Beta cell death or damage. In this situation the Beta cell in pancreas cannot produce enough insulin in the body to control the level of glucose which causes the type 1 diabetes (Figure 5) (Burrack et al., 2017).

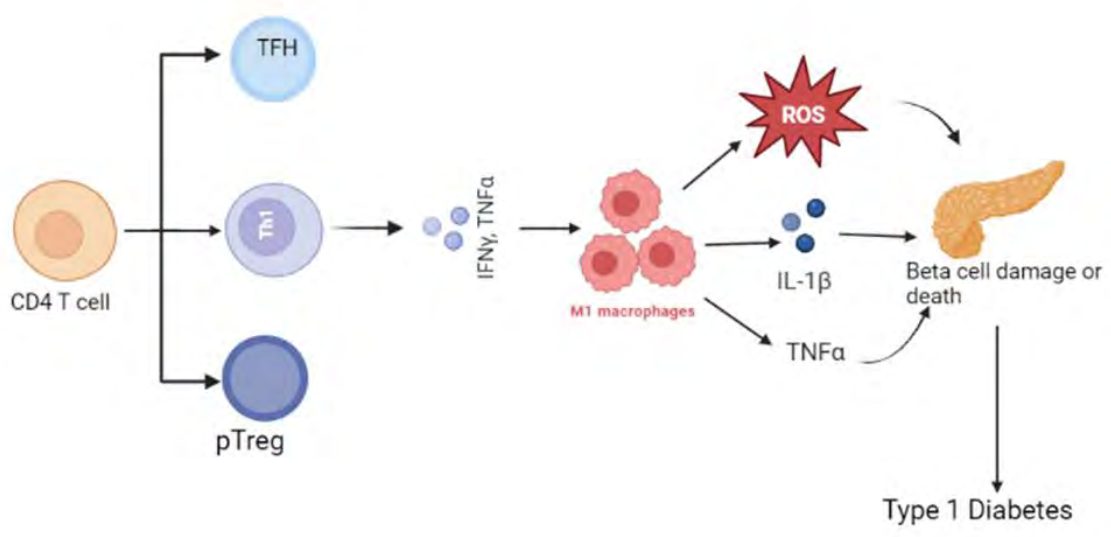

Figure 5: T cell induced Type 1 diabetes (Adapted from Guo et al., 2019)

For Type 2 Diabetes:

The tyrosine-protein kinase activity of insulin is activated when the insulin receptor binds to it. In order to create PIP3, the insulin receptor is able to directly activate PI3K or it may indirectly via phosphorylating and activating AKT. AKT suppresses the action of GSK-3 by phosphorylating it at the Ser9 location. The PI3K/AKT/GSK-3 signaling pathway that is connected to the regulation of glycogen formation is implicated in the insulin signalling pathway and GSK-3 is subject to control and regulation by insulin within the context of this signaling pathway. Hyperglycemia happens when the action of GS, which encourages glycogen synthesis and lowers blood glucose levels, isn't inhibited. Because the insulin signal isdusfuncction, the body boosts GSK-3 activity

in order to raise blood sugar levels. This is done by phosphorylating GSK-3 at its Tyr 216 site, which also stops PI3K/AKT from being active that reduce blood glucose (Arneth et al., 2019).

5.2.2 Role of Epigenetics in Diabetes

Diabetes develops and progresses in large part due to epigenetics. The term "epigenetics" describes modifications to gene expression which have no effect in changes to the base DNA sequence. External factors might have an impact with these modifications, which may have long-lasting consequences on how genes are regulated. The epigenetic processes of DNA methylation, histone changes, and non-coding RNA molecules have all been linked to diabetes.

DNA methylation

T1D condition-association research use monozygotic (MZ) twins discordant with the illness. DNA methylation patterns of 15 T1DM-discordant MZ twin pairs' isolated CD14+ monocytes. Immune beneficial cells called monocytes can develop into tissue macrophages, which have been linked to islet cell apoptosis and insulin shortage. Researchers found 58 hypermethylated and 74 hypomethylated sites, which are T1DM-related methylation changes. Additionally, they discovered hypomethylation in essential genes like GAD2, that produces GAD65, a significant T1DM autoantigen involved in the cause of the disease, HLA-DQB1, that confers a significant single risk factor for T1DM in addition to HLA-DRB1, RFXAP, an enzyme that regulates of HLA class II, and NFKB1A (Cerna, 2020).

The earliest research on islets in humans focused on specific candidates and looked into genes having a recognized role in the functioning of cells or metabolism of cells. DNA methylation is influenced by someone's environment, age, genetics, and gender. Therefore it might change how genes are expressed. The results of these investigations showed that islets had hypermethylated promoters for the genes encoding the proteins insulin (INS), a transcription factor and the activity of mature beta cells (PDX1), the mitochondrial regulator PGC1A (PPARGC1A), and the GLP1 receptor (GLP1R) that induces insulin secretion. These DNA hypermethylations disrupt beta cell lifespan and insulin output, that may result in the development of T2D (Davegårdh et al., 2018).

Histone modifications

Variations in multiple histone modifications (H3K9Ac, H4K16Ac, H3K4me3, H3K9me2,3, and H3K27me3) of genes associated with diabetes systems comparing T1DM and control subjects. Histone-modifying enzymes perform an essential part in controlling immune cell responses, that influence the development of T1D. The locations of different chromatin modifications was likewise adjacent to the DQB1 and DRB1 genes: H3K9Ac levels in monocytes from T1DM patients was greater in the region 4 kb above of HLA-DQB1 and reduced in the region 4 kb upstream of HLA-DRB1 respectively. The enhanced transcription in the monocyte cell line is correlated with the higher acetylation at these locations. Nevertheless, it is impossible to determine either the variations are the consequence of the disease's associated hyperglycemia or its etiology (Cerna, 2020). There is a significant hereditary component to T1D, and specific genetic variations can be linked to a higher chance of acquiring the condition. The existence of certain variations in the human leukocyte antigen (HLA) genes, specifically the HLA class II genes, is a particularly noteworthy cause for T1D. These variations in genetics may have an impact on immune system performance as well as the instability of histone modifications linked to the onset of T1D.

When transiently high glucose levels, multiple histone lysine changes have been observed as well. These modifications are possibly responsible for the constant transcriptional activation of the RELA gene, which codes for the p65 subunit of NF-kB, regardless of whether endothelial cells were subsequently incubated with regular glucose levels. Overall, this action has the effect of repressing some target genes linked to endothelium dysfunction while activating others through transcription. HMOX1, MMP10, SLC7A11, MMP1, MCP-1, and ICAM gene activity may be enhanced as a result of acetylation or hyperacetylation. Which is the reason for higher glucose which is hyperglycemia in the blood and occurs T2D (De Rosa et al., 2018).

Non-coding RNA

Genetic connection provided the first convincing proof of lncRNA contributing to cell functioning. The molecular impact of variations in non-protein-coding sequences can be more difficult to ascertain than that of changes in protein-coding cities, which frequently cause an amino acid substitution or protein terminating. Numerous T2D-associated SNPs, such as CDKN2B-AS1 (ANRIL) and KCNQ1OT1, have been found at loci encoding lncRNAs. In fact, the CDKN2B-

AS1 gene has been implicated in so numerous investigations diabetes risk. Each of the three thoroughly researched cyclin-dependent inhibitors of kinase p16INK4A, p15INK4B, and p14ARF are encoded by two protein-coding genes at the CDKN2A/B locus, together with the lncRNA gene CDKN2B-AS1. The significance of this locus for controlling the growth of cells emphasizes the possibility that dysregulation of beta-cell growth may be to blame for the higher T2D risk (Wilson & Pullen, 2021).

Genome-wide miRNA expression profiles of Tregs in T1DM patients showed significantly higher levels of miR-510 compared to healthy controls and lower levels of miR-342 and miR-191. Additionally, an analysis of miRNA levels between T cells and Tregs revealed that Tregs had significantly lower levels of eight particular miRNAs and a significantly higher level of miR-146a, suggesting that they play a role in T1DM.

5.2.3 Treatment

Epigenetic treatments include medications that suppress epigenetic enzymes; examples include DNA methyltransferase (DNMT) inhibitors and histone deacetylase (HDAC) inhibitors. Furthermore, numerous studies have demonstrated that silencing and overexpressing histone demethylases and HDACs affect beta cell activity and insulin release (Ling, 2022).

Some of the most common treatments for diabetes include:

Metformin, Sulfonylureas, glipizide, glyburide, DPP-4 inhibitors, SGLT2 inhibitors, GIP and GLP-1 receptor agonist, Insulin.

5.3 Epigenetics in the Development of Cardiovascular Diseases

5.3.1 Cardiovascular Diseases

According to estimates, 17.9 million deaths worldwide in 2019 were attributable to CVDs, or 32% of all fatalities. Heart attack and stroke deaths accounted for 85% of these fatalities. The majority of CVD fatalities occur in nations with low or middle incomes. In 2019, noncommunicable

diseases induced 17 million premature deaths (before the age of 70), and 38% of those fatalities were attributable to CVDs ((CVDs), 2021)

A set of illnesses which impact the blood vessels and heart are referred to as cardiovascular diseases. A few parts of the heart and/or circulatory system may be impacted by these disorders. A person can have symptoms (physical manifestations of the disease) or they may not have any symptoms at all. Heart or circulation problems are included in cardiovascular disease (*Cardiovascular Disease: Types, Causes & Symptoms*, 2022).

Types of Cardiovascular Diseases

There are four main types of CVD- coronary heart disease, stroke, peripheral arterial disease, aortic disease (Cardiovascular Disease - Illnesses & Conditions | NHS Inform, 2022). Other types are arrhythmia, valve disease, heart failure, congenital heart disease, pericardial disease, cerebrovascular disease, deep vein thrombosis (DVT) (*Cardiovascular Disease: Types, Causes & Symptoms*, 2022).

Causes of Cardiovascular Diseases

The most common causes of CVS are family history of CVD, age, gender, alcohol, being overweight or obese, sedentary lifestyle, high blood pressure (Cardiovascular Disease- NHS Inform, 2022).

Signaling Pathway

There are several signaling pathways present for the CVD, one of which is explained in this review paper:

In the cardiovascular system the guanine nucleotide regulatory protein (G protein) plays a vital regulatory function in a number of transmission of signals pathways. Adenylate cyclase/cAMP and phospholipase C (PLC)/phosphatidylinositol turnover (PI) are examples of these signalling pathways. These are linked to the control of a number of physiological processes, include the function of platelets, which includes aggregation, release, and formation of blood clots, and cardiovascular function, which includes arterial relaxation and responsiveness. Research studies have shown that abnormal changes in adenylate cyclase activity, cAMP levels, G protein, and

PLC/PKC will alter cardiac and vascular function, which can be detected by changes in the levels of inhibitory G protein (Gi-2 and Gi-3), but not activation G protein (Gs). At the onset of high blood pressure, several G protein modifications take place ("Cardiovascular and Signal Transduction," n.d.; Krupnick & Benovic, 1998).

5.3.2 Role of Epigenetics in Cardiovascular Diseases

DNA methylation

Multiple investigations have revealed that DNA methylation is crucial in the development of cardiovascular disorders. Over the past few years, it is being discovered that DNA methylation is connected to the activity of genes that are linked to cardiac disease, heart failure, hypertension, and other cardiovascular disorders.

Human cardiomyocyte regulation connected DNA methylation of DNA methyltransferase DNMT3a. In addition to altering the expression of the contractile protein gene in cardiomyocytes, DNMT3a knockout additionally led to damage to mitochondria and decreased glucose metabolism in these cells.

When compared with parents with no previous experiences of hypertension, those having a family background exhibited increased average arterial pressure and decreased total placental DNA methylation in placental specimens. The process of inflammation, oxidative damage, and the renin-angiotensin system (RAS) are all directly associated to the fusion of mitochondria 2, which can decrease the growth of VSMCs. Hypomethylation associated with the gene MF2 could decrease this gene's activity. It thus caused endothelial cell injury and VSMC expansion, which in turn aided in the formation of hypertension (Shi et al., 2022).

Histone modification

Histone methylation is another way to affect heart disease. The research discovered that the catalytic subunit EZH2 of PRC2 and histone methyltransferase G9a worked together to silence genes. With dimethylation of lysine 9 on histone H3 and association with EZH2, G9a reduced the activity of various types of genes in cardiomyocytes (Shi et al., 2022).

The onset and progression of hypertension are both highly correlated with histone acetylation. HDAC6 mRNA and protein expression were discovered to be elevated by Ang II in a research project to look into the function of HDAC6 in hypertension. Cystathionine -lyase (CSE) was deacetylated by HDAC6, which caused CSE to be degraded by the ubiquitin-albumin route. The primary enzyme responsible for creating the physiological vasodilator hydrogen sulfide is CSE. The breakdown of CSE decreased hydrogen sulfide generation, which exacerbated endothelial dysfunction and hastened hypertension (Shi et al., 2022).

Non-Coding RNA

Currently, miRNA has been linked to the emergence of the condition known as acute coronary syndrome and coronary artery disease. According to one study, the miRNA-SNP rs41291957 enhanced the production of miRNA-143 and miRNA-145 within human coronary smooth muscle cell(s) and controlled how VSMCs changed phenotypically. According to the study, the miRNA-SNP rs41291957 can be considered a key gene for determining the possibility and outcome of coronary heart disease (Shi et al., 2022).

Vascular calcification's onset and progression are closely related to miRNA. Researchers discovered that miRNA-30b had a preventative effect on calcification and may be an endogenous regulating component of vascular calcification. By blocking the mTOR signaling pathway, miRNA-30b enhances MMPs and degradation in VSMCs, preserves mitochondrial equilibrium. Additionally, miRNA-30b inhibited SOX9, decreased the synthesis of the protein bone morphogenetic protein 2 (BMP2), and prevented bone development in VSMCs that had received treatment with -glycerophosphorate. These actions decreased the incidence of vascular calcification (Shi et al., 2022).

5.3.3 Treatment

5 types of drugs used for the cardiovascular diseases:

There are lots of different medicines used to treat diseases of the heart, but they all belong to a few main groups, including:

- ACE inhibitors like ramipril.
- Angiotensin-II antagonists like losartan.
- Anti-arrhythmic medicines like amiodarone.
- Anticoagulant medicines like warfarin.
- Anti-platelet medicines like aspirin (Heart Medicines - Heart Treatments | NHS Inform, 2023).

Some drugs that are used for a CVD in heart failure are- (Gorica et al., 2022):

- Zolinza (Vorinostat) - HDAC inhibitor
- Givinostat - HDAC inhibitor
- Apicidin - HDAC inhibitor

5.4 Epigenetics in the Development of Cancer

5.4.1 Cancer

Due to the considerable changes in cell metabolism associated with cancer, the disease is frequently referred to as a metabolic condition. In order to accommodate their energy requirements and preserve cellular processes, regular cells closely regulate their metabolisms. Nevertheless, significant modifications lead to changes in metabolic processes in cancer cells The Warburg effect is a well-known metabolic change seen in cancer cells. Cancer is a condition when a few of the body's cells grow out of control and propagate across bodily regions. In the millions of cells that make up the human body, cancer can develop practically anywhere. Occasionally, this systematic process fails, causing damaged or aberrant cells to proliferate. Tumors, referred to as tissue masses, can develop from these cells. Tumors may or may not be cancerous (Hanahan & Weinberg, 2000).

Causes of Cancer

Approximately 33 percent of cancer mortality might be related to tobacco use, alcohol usage, having an elevated body mass index (BMI), eating few fruits and vegetables, and not exercising enough, according to the WHO Trusted Source (Faith Selchick, 2022). Mutations, or alterations to

the DNA in cells are the primary cause of cancer. Mutations in the DNA can be inherited. In addition, they may develop later in life because of environmental factors.

Signaling Pathway

Cancer can be many types and different type of cancer have different cell signaling pathways. In this review all the signaling pathway is not explained but the basic understanding how cancer develop in our body is explained below:

Genetic factors include mutant or inactive genes that suppress tumors and cancer-causing genes. These defective genes cause the affected cell to split wildly as well as to live longer. A lump forms at the spot as an increasing number of the cells with aberrant genes separate, and it has no purpose other than to grow and quickly attack other tissues. A few cells separate from the tumor and disperse to different parts of the body. This indicates metastasis. Tumors that have spread are the primary cause of cancer-related deaths. New tumors successfully take hold in a variety of sites. In order to get to the vitamins and minerals in the bloodstream, they develop new blood vessels. No-specialized-function tumors might hinder the regular operation of the organ in which they are present (Hanahan & Weinberg, 2000).

5.4.2 Role of Epigenetics in Cancer

DNA methylation

In addition to alterations in genetics, epigenetic changes including DNA methylation function as a supplementary pathway for the deactivation of tumor suppressor genes (TSGs). Numerous recognized tumor suppressor genes are silenced as an outcome of the hypermethylation of CGI promoters, which is inversely linked with gene expression in cancer cells (Figure 6). DNA methylation is known to silence genes involved in repairing DNA and regulation of cell cycles in a variety of cancer types; this process is frequently associated with hereditary mutation of the gene. Because of promoter hypermethylation, spontaneous breast and ovarian tumors exhibit a decrease of BRCA1 expression. According to this, promoter methylation causes to a number of cancers, which includes renal cell carcinoma, by epigenetically silencing the tumor suppressor VHL (Lakshminarasimhan & Liang, 2016).

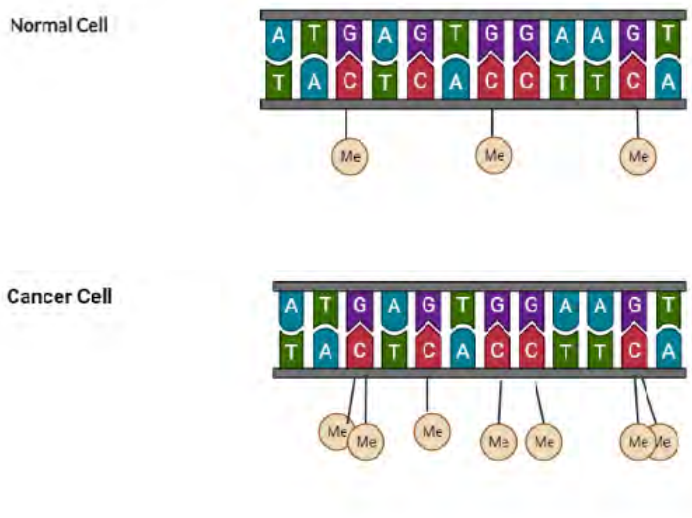

Figure 6: Hypermethylation of CpGs leads to cancer ("DNA Methylation and Hydroxymethylation," n.d.)

Histone Modifications

On the other hand, increased HAT action has a general cancer-causing effect and typically results from chromosomal mutations that involve multiple fusion collaborators, including combined lineage leukemia (MLL)-CBP, MLL-EP300, MOZ-EP300, or MOZ-CBP in hematological malignancies. Whenever the mutations result in chimeric oncoproteins, the carcinogenic impact happens. The genetic domains of the fusion partners could then be inappropriately acetylated by HATs as a result (Audia & Campbell, 2016).

Like HATs, it's possible that HDACs play an essential part in cancer that isn't restricted to histones. Alpha-tubulin, HSP90 and cortactin (HDAC6), p53 (HDAC5), and ERR (HDAC8) are among the several known HDAC substrates that are deacetylated. HDACs can modify proteins that participate in tumor migration, metastasis, and growth (Audia & Campbell, 2016).

Non coding RNA

MiRNAs may operate as tumor suppressors and oncogenes. A well-known example of an oncogenic miRNA is miR-21. Most cancers, such as breast cancer, glioblastoma, colorectal cancer, lung cancer, pancreatic cancer, as well as leukemia, overexpress miR-21. Elements of the miR-34

family (miR-34s) are referred to as tumor suppressive miRNAs. In reaction to DNA damage, the tumor suppressor TP53 through transcription activates the miR-34 family. Unlike miR-34b and miR-34c, which have been synthesized from a single the primary transcripts from chromosome 11, miR-34a is formed by the sequence on chromosome 11. Prostate cancer spread and recurrence were linked to miR-34a deletion (Huang et al., 2013).

Recent research using the latest generation of sequencing showed that stem cells and cells with differentiation produce ncRNA differently. For instance, differentiated cells and embryonic stem cells change considerably in terms of small non-polyadenylated (NPA)-conserved RNA (snacRNA). SnacRNAs might operate in CSCs or cancer cells, however this is uncertain (Huang et al., 2013).

5.4.3 Treatment

The most common types of treatment are:

- Surgery
- Chemotherapy
- Radiation therapy
- Stem cell (bone marrow) transplant
- Immunotherapy (biological therapy)
- Hormone therapy
- Targeted drug therapy (Faith Selchick, 2022).

DNA methyltransferase inhibitors (DNMTi), 5-Azacytidine is currently FDA approved to treat high-risk myelodysplastic syndromes (MDS) patients and has resulted in successful clinical outcomes (Lakshminarasimhan & Liang, 2016).

There are more FDA approved epidrugs for cancer. The list of FDA approved epidrugs with its mechanism and treatment is given in Table 2.

Table 2: List of FDA approved epigenetic drugs for cancer (Miranda Furtado et al., 2019)

FDA approved Drug (commercial name)	Year	Mechanism of action	Treatment
Azacitidine+ decitabine or low-dose cytarabine (Venclexta)	2018	DNA methyltransferase inhibitors (DNTMi)	Acute myeloid leukemia
Panobinostat + Bortezomib + Dexamethasone (Farydak)	2015	Histone Deacetylase Inhibitor (HDACi)	Multiple myeloma
Belinostat (Belodaq)	2014	Histone Deacetylase Inhibitor (HDACi)	Peripheral T Cells Lymphoma
Romidepsin (Ixodax)	2009	Histone Deacetylase Inhibitor (HDACi)	Cutaneous T-cell lymphoma
5 Azacitidine (Vidaza)	2009	DNA methyltransferase inhibitors (DNTMi)	Myelodysplastic syndrome
Vorinostat (Zolinza)	2006	Histone Deacetylase Inhibitor (HDACi)	Cutaneous T-cell lymphoma

Chapter 6

Conclusion

The role of epigenetics in the development of metabolic disorders is being widely studied for the past few decades. Epigenetic modifications encompassing DNA methylation, histone modifications, and non-coding RNA expression, play a critical role in gene expression regulation and is involved in various metabolic processes. The aberrant epigenetic modifications observed in individuals with metabolic disorders have been associated with dysregulation of glucose metabolism, lipid metabolism, adipogenesis, and inflammation. The interplay between genetics, epigenetics and other environments factors contribute to the development of metabolic disorders. Investigating the role of epigenetics in the development and progression of metabolic syndromes will unravel the underlying mechanisms of the diseases and aid researchers to find out potential therapeutic interventions targeting theabnormal epigenetic marks.

References

Alaskhar Alhamwe, B., Khalaila, R., Wolf, J., von Bülow, V., Harb, H., Alhamdan, F., Hii, C. S., Prescott, S. L., Ferrante, A., Renz, H., Garn, H., & Potaczek, D. P. (2018). Histone modifications and their role in epigenetics of atopy and allergic diseases. *Allergy, Asthma & Clinical Immunology*, *14*(1), 39. https://doi.org/10.1186/s13223-018-0259-4

Alegría-Torres, J. A., Baccarelli, A., & Bollati, V. (2011). Epigenetics and lifestyle. *Epigenomics*, *3*(3), 267–277. https://doi.org/10.2217/epi.11.22

Arguelles, A. O., Meruvu, S., Bowman, J. D., & Choudhury, M. (2016). Are epigenetic drugs for diabetes and obesity at our door step? *Drug Discovery Today*, *21*(3), 499–509. https://doi.org/https://doi.org/10.1016/j.drudis.2015.12.001

Arneth, B., Arneth, R., & Shams, M. (2019). Metabolomics of Type 1 and Type 2 Diabetes. *International Journal of Molecular Sciences*, *20*(10). https://doi.org/10.3390/ijms20102467

Arnold, G. L. (2018). Inborn errors of metabolism in the 21(st) century: past to present. *Annals of Translational Medicine*, *6*(24), 467. https://doi.org/10.21037/atm.2018.11.36

Audia, J. E., & Campbell, R. M. (2016). Histone Modifications and Cancer. *Cold Spring Harbor Perspectives in Biology*, *8*(4), a019521–a019521. https://doi.org/10.1101/cshperspect.a019521

Burrack, A. L., Martinov, T., & Fife, B. T. (2017). T Cell-Mediated Beta Cell Destruction: Autoimmunity and Alloimmunity in the Context of Type 1 Diabetes. *Frontiers in Endocrinology*, *8*. https://doi.org/10.3389/fendo.2017.00343

Cardiovascular and Signal Transduction. (n.d.). *CD Creative-Diagnostics*. Retrieved June 6, 2023, from https://www.creative-diagnostics.com/cardiovascular-and-signal-transduction.htm

Cardiovascular disease - Illnesses & conditions | NHS inform. (2022, November 18). NHS Inform. https://www.nhsinform.scot/illnesses-and-conditions/heart-and-blood-vessels/conditions/cardiovascular-disease

Cardiovascular Disease: Types, Causes & Symptoms. (2022, January 9). Cleveland Clinic. https://my.clevelandclinic.org/health/diseases/21493-cardiovascular-disease

Cardiovascular diseases (CVDs). (2021, June 11). World Health Organization. https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)

Cerna, M. (2020). Epigenetic Regulation in Etiology of Type 1 Diabetes Mellitus. *International Journal of Molecular Sciences*, *21*(1). https://doi.org/10.3390/ijms21010036

Davegårdh, C., García-Calzón, S., Bacos, K., & Ling, C. (2018). DNA methylation in the pathogenesis of type 2 diabetes in humans. *Molecular Metabolism*, *14*, 12–25. https://doi.org/10.1016/J.MOLMET.2018.01.022

De Rosa, S., Arcidiacono, B., Chiefari, E., Brunetti, A., Indolfi, C., & Foti, D. P. (2018). Type 2 diabetes mellitus and cardiovascular disease: Genetic and epigenetic links. *Frontiers in Endocrinology*, *9*(JAN), 2. https://doi.org/10.3389/FENDO.2018.00002/BIBTEX

DNA methylation and hydroxymethylation. (n.d.). *Theoretical and Computational Biophysics Group*. Retrieved June 6, 2023, from https://www.ks.uiuc.edu/Research/methylation/

Dr. Ananya Mandal. (2019). History of Metabolic Syndrome. *News-Medical*. https://www.news-medical.net/health/History-of-Metabolic-Syndrome.aspx. MLA

Dr. Sanchari Sinha Dutta. (2018). Chromatin Remodeling: Mechanism and Importance. *News-Medical*. https://www.news-medical.net/life-sciences/Chromatin-Remodeling-Mechanisms-and-Importance.aspx.

Faith Selchick, D. A. (2022, February 17). *Cancer: Types, Causes, Treatment, and Prevention*. Healthline. https://www.healthline.com/health/cancer

Flegal, K. M., Graubard, B. I., Williamson, D. F., & Gail, M. H. (2007). Cause-Specific Excess Deaths Associated With Underweight, Overweight, and Obesity. *JAMA*, *298*(17), 2028–2037. https://doi.org/10.1001/jama.298.17.2028

Gorica, E., Mohammed, S. A., Ambrosini, S., Calderone, V., Costantino, S., & Paneni, F. (2022). Epi-Drugs in Heart Failure. *Frontiers in Cardiovascular Medicine*, *9*, 923014. https://doi.org/10.3389/fcvm.2022.923014

Gregory Enns. (2019). metabolic disease. *Britannica*. https://www.britannica.com/science/metabolic-disease

Guo, Z., Lou, Y., Kong, M., Luo, Q., Liu, Z., & Wu, J. (2019). A systematic review of phytochemistry, pharmacology and pharmacokinetics on astragali radix: Implications for astragali radix as a personalized medicine. *International Journal of Molecular Sciences*, *20*(6). https://doi.org/10.3390/IJMS20061463

Hanahan, D., & Weinberg, R. A. (2000). The hallmarks of cancer. *Cell*, *100*(1), 57–70. https://doi.org/10.1016/S0092-8674(00)81683-9

Heart medicines - Heart treatments | NHS inform. (2023, March 1). NHS Inform. https://www.nhsinform.scot/tests-and-treatments/medicines-and-medical-aids/types-of-medicine/heart-medicines

Huang, T., Alvarez, A., Hu, B., & Cheng, S.-Y. (2013). Noncoding RNAs in cancer and cancer stem cells. *Chinese Journal of Cancer*, *32*(11), 582–593. https://doi.org/10.5732/cjc.013.10170

Jian-Wei Wei, Kai Huang, Chao Yang, & Chun-Sheng Kang. (2016). Non-coding RNAs regulates in epigenetics (Review). *Oncology Reports*, 3–9. https://doi.org/10.3892/or.2016.5236

Jin, B., Li, Y., & Robertson, K. D. (2011). DNA methylation: superior or subordinate in the epigenetic hierarchy? *Genes & Cancer*, *2*(6), 607–617. https://doi.org/10.1177/1947601910393957

Katsarou, A., Gudbjörnsdottir, S., Rawshani, A., Dabelea, D., Bonifacio, E., Anderson, B. J., Jacobsen, L. M., Schatz, D. A., & Lernmark, Å. (2017). Type 1 diabetes mellitus. *Nature Reviews Disease Primers*, *3*(1), 17016. https://doi.org/10.1038/nrdp.2017.16

Kerstin Rohde, Maria Keller, Lars la Cour Poulsen, Matthias Blüher, Peter Kovacs, & Yvonne Böttcher. (2019). Genetics and epigenetics in obesity. *National Library of Medicine*, *93*, 37–50. https://pubmed.ncbi.nlm.nih.gov/30399374/

Krupnick, J. G., & Benovic, J. L. (1998). The role of receptor kinases and arrestins in G protein-coupled receptor regulation. *Annual Review of Pharmacology and Toxicology*, *38*, 289–319. https://doi.org/10.1146/ANNUREV.PHARMTOX.38.1.289

Kuneš, J., Vaněčková, I., Mikulášková, B., Behuliak, M., Malínská, L., & Zicha, J. (2015). Epigenetics and a New Look on Metabolic Syndrome. *Physiological Research / Academia Scientiarum Bohemoslovaca*, *64*, 611–620. https://doi.org/10.33549/physiolres.933174

Lakshminarasimhan, R., & Liang, G. (2016). The Role of DNA Methylation in Cancer. *Advances in Experimental Medicine and Biology*, *945*, 151–172. https://doi.org/10.1007/978-3-319-43624-1_7

Leppert, S., & Matarazzo, M. (2014). De Novo DNMTs and DNA Methylation: Novel Insights into Disease Pathogenesis and Therapy from Epigenomics. *Current Pharmaceutical Design*, *20*(11), 1812–1818. https://doi.org/10.2174/13816128113199990534

Liaw, A., Liu, C., Ivanovski, S., & Han, P. (2022). The Relevance of DNA Methylation and Histone Modification in Periodontitis: A Scoping Review. *Cells 2022, Vol. 11, Page 3211*, *11*(20), 3211. https://doi.org/10.3390/CELLS11203211

Ling, C. (2022). Pharmacoepigenetics in type 2 diabetes: is it clinically relevant? *Diabetologia*, *65*(11), 1849–1853. https://doi.org/10.1007/s00125-022-05681-x

Loscalzo, J., & Handy, D. E. (2014). Epigenetic modifications: basic mechanisms and role in cardiovascular disease (2013 Grover Conference series). *Pulmonary Circulation*, *4*(2), 169–174. https://doi.org/10.1086/675979

Mahmoud, A. M. (2022). An Overview of Epigenetics in Obesity: The Role of Lifestyle and Therapeutic Interventions. *International Journal of Molecular Sciences*, *23*(3). https://doi.org/10.3390/ijms23031341

Michael Dansinger, M. (2023, March 16). *Type 2 Diabetes: Symptoms, Causes, Diagnosis, and Treatment*. WebMD. https://www.webmd.com/diabetes/type-2-diabetes

Miranda Furtado, C. L., Dos Santos Luciano, M. C., Silva Santos, R. Da, Furtado, G. P., Moraes, M. O., & Pessoa, C. (2019). Epidrugs: targeting epigenetic marks in cancer treatment. *Epigenetics*, *14*(12), 1164–1176. https://doi.org/10.1080/15592294.2019.1640546

Olinski, R., Slupphaug, G., Foksinski, M., & Krokan, H. E. (2021). Genomic Uracil and Aberrant Profile of Demethylation Intermediates in Epigenetics and Hematologic Malignancies.

International Journal of Molecular Sciences, *22*(8), 4212. https://doi.org/10.3390/ijms22084212

Ospelt, C. (2022). A brief history of epigenetics. *Immunology Letters*, *249*, 1–4. https://doi.org/10.1016/J.IMLET.2022.08.001

Pfeifer, G. P., Kadam, S., & Jin, S. G. (2013). 5-hydroxymethylcytosine and its potential roles in development and cancer. *Epigenetics and Chromatin*, *6*(1). https://doi.org/10.1186/1756-8935-6-10

Richa, R., & Sinha, R. P. (2014). Hydroxymethylation of DNA: an epigenetic marker. *EXCLI Journal*, *13*, 592–610. https://pubmed.ncbi.nlm.nih.gov/26417286

Rotondo, J. C., Mazziotta, C., Lanzillotti, C., Tognon, M., & Martini, F. (2021). Epigenetic Dysregulations in Merkel Cell Polyomavirus-Driven Merkel Cell Carcinoma. *International Journal of Molecular Sciences 2021, Vol. 22, Page 11464*, *22*(21), 11464. https://doi.org/10.3390/IJMS222111464

Ruban, A., Stoenchev, K., Ashrafian, H., & Teare, J. (2019). Current treatments for obesity. *Clinical Medicine (London, England)*, *19*(3), 205–212. https://doi.org/10.7861/clinmedicine.19-3-205

Sarah Watts. (2022, November 18). *Type 1 Diabetes Symptoms, Causes, Diagnosis and Treatments*. Endocrineweb. https://www.endocrineweb.com/conditions/type-1-diabetes

Shi, Y., Zhang, H., Huang, S., Yin, L., Wang, F., Luo, P., & Huang, H. (2022). Epigenetic regulation in cardiovascular disease: mechanisms and advances in clinical trials. *Signal Transduction and Targeted Therapy*, *7*(1), 200. https://doi.org/10.1038/s41392-022-01055-2

Suchitra Ajjarapu. (2022, June 25). *Covalent Histone Modification- Definition, Types, Functions*. The Biology Notes. https://thebiologynotes.com/covalent-histone-modification/

T Kelly, W Yang, C-S Chen, K Reynolds, & J He. (2008). Global burden of obesity in 2005 and projections to 2030. *International Journal of Obesity (2005)*. https://pubmed.ncbi.nlm.nih.gov/18607383/

Wen, X., Zhang, B., Wu, B., Xiao, H., Li, Z., Li, R., Xu, X., & Li, T. (2022). Signaling pathways in obesity: mechanisms and therapeutic interventions. *Signal Transduction and Targeted Therapy*, *7*(1), 298. https://doi.org/10.1038/s41392-022-01149-x

What is epigenetics? (2021, June 11). https://medlineplus.gov/genetics/understanding/howgeneswork/epigenome/

Wilson, M. E., & Pullen, T. J. (2021). The role of long non-coding RNAs in the regulation of pancreatic beta cell identity. *Biochemical Society Transactions*, *49*(5), 2153–2161. https://doi.org/10.1042/BST20210207

Yasmine S. Ali. (2022). Causes and Risk Factors of Obesity. *Verywell Health*. https://www.verywellhealth.com/obesity-causes-4014562

Zakhari, S. (2013). Alcohol Metabolism and Epigenetics Changes. *Alcohol Research: Current Reviews*, *35*, 6–16.