Epigenetic Functional Nutrition

Epigenetic Functional Nutrition:

When Science Meets the Table

By

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This book is dedicated to all individuals who suffer from chronic diseases including mental disorders and cancer, with the hope that embracing a healthy lifestyle and nutrition becomes part of the cure.

DISCLAIMER

This book contains the author's research results, opinions, ideas, and personal experiences.

This book is not intended in any way to substitute conventional pharmacological treatments, someone's doctor or a healthcare professional's advice, suggestions, and treatment options, or to interfere with the doctor-patient relationship based on what is discussed in this book. The author disclaims all responsibilities for harm, misinterpretations, misunderstanding, damage, or loss that the reader may relate, directly or indirectly, to the content described in this book.

TABLE OF CONTENTS

PREFACE

We live in the era of *individualized medicine* and *functional recovery*, and it would be inconvenient to *dissect* morbidity from *environment*: the *microenvironment* of specific pathological conditions, such as the microenvironment of cancer cells or neuronal cells in the brain, and the *macroenvironment* we all live in. Epigenetics represents both the *science* and the *technique* that studies and links, via experimental procedures, the environment to gene regulation, either gene expression or suppression, leading to phenotype manifestation. Keeping this in mind, the main concept behind the creation of this book comes from the need to provide easy access to fast-growing scientific evidence and research studies on the latest developments on the epigenetic effects of specific bioactive ingredients found in *functional* foods, and their effects on regulating gene expression, and ultimately affecting our overall health. This book aims to provide some scientific evidence on the role of functional nutrition rich in bioactive compounds in brain development and major mental disorders and certain types of cancer, and its epigenetic role.

The amount of scientific evidence about the beneficial effects of functional food and its potential clinical applications has tremendously increased in the past few years, so it is practically impossible to discuss or report all of it in this book. Thus, *Epigenetic Functional Nutrition* focuses primarily on inflammatory-based chronic diseases of psychiatric interest such as autism, depression, and schizophrenia, and two specific cancers, breast and colorectal cancer, because of the role of inflammatory processes underlying their pathogenesis and their close relation to dietary risk factors.

The book combines the benefits of a macrobiotic diet, considered as a naturally grown, nutrient-dense, chemical-free, and mainly plant-based diet, with *epigenetics*, which consists of a complex molecular machinery finely orchestrated in response to environmental factors including foods that regulates gene expression. Food is considered by most people only as a calorie-dense source of the so-called macronutrients (carbohydrates, proteins, and fats) that provides energy for our cells in order to function properly. But food is also a source of *bioactive compounds*, such as the micronutrients vitamins, minerals, phytonutrients, polyphenols, and

bioflavonoids, amongst others, capable of exerting biological effects: antioxidant, neuroprotective, anticancer, and anti-inflammatory effects. Epigenetic modifications can help fight the dangerous effects caused by adverse environmental factors, including unhealthy diets and lifestyles (such as physical inactivity) that potentially lead to chronic systemic low-grade inflammation and brain inflammation (neuroinflammation), DNA damage, cell damage, cell transformation, cell death, and eventually disease manifestation.

Thus, the primary goal of this book is to report the beneficial role of certain bioactive compounds as demonstrated by scientific studies and evidence for our overall health and their potential beneficial properties in altering disease course.

The study focuses on the role of *inflammation*, which has been recently considered in playing a major role in the development of chronic neuropathologies including autism and schizophrenia spectrum disorders, and mood disorders – in particular major depression and postpartum depression, in which neuroinflammatory mechanisms play an important role in the regulation of emotional behaviors and cognition.

Epigenetic Functional Nutrition also discusses the role of functional foods in two different types of cancer: breast cancer (BC) and colorectal cancer (CRC), which, amongst other pathological determinants, seem to have a close relation to dietary quality.

The book highlights the importance of choices that we make every day about food, and its impact on our body. By strongly supporting the concept that a simple gesture such as picking the right food in any circumstances, including at social events or at school, we can indeed make a difference to our body, even at the epigenetic level.

This book intends to inform and encourage those individuals who are fighting against chronic diseases to embrace a better lifestyle that includes functional nutrition to achieve a better quality of life and health promotion.

Moreover, this book shows how certain bioactive compounds affect gene expression by modulating epigenetic mechanisms including DNA methylation and histone acetylation.

Finally, *Epigenetic Functional Nutrition* aims to instruct communities and specific subpopulations of a disadvantageous socioeconomic status that have limited access to knowledge and educational programs, as well as

those struggle with chronic diseases and poor quality of life, about the relevance of nutrition for their overall health. The book is an easy and accessible source to improve the quality of their food choices.

1. INTRODUCTION

This book provides the latest scientific-based information and research evidence on the epigenetic role of a macrobiotic epigenetic functional diet for chronic mental disorders, including autism spectrum disorder (ASD), schizophrenia and major depression, and certain types of cancer, in particular breast and colorectal cancers – which represent the two most common type of cancers (after lung cancer) and are strongly associated with epigenetic alterations.

Chronic debilitating mental disorders such as schizophrenia and certain types of cancer share the fact that besides the individual genetic vulnerability, the environment plays a relevant role in the disease manifestation and outcomes.

Environmental *modifiable risk factors* including unhealthy diets correlate with a higher risk of inflammatory-based systemic alterations associated with different pathologies such as metabolic syndrome, cardiovascular diseases, diabetes, obesity, and cancer (Hariharan et al., 2022; Fahed et al., 2022; Zhang et al., 2021; Macedo and Calder, 2018).

It has been reported that vulnerable individuals with mental disorders, for example, are more prone to adopt a high-risk lifestyle such as poor nutrition and lack of exercise, and they are more susceptible to developing cardiovascular disease, obesity, altered metabolic conditions, and even certain types of cancer or obesity-related cancers (van Zonneveld et al., 2022; Nordentoft et al., 2021).

Addressing this matter is crucial to help people and their healthcare providers make significant changes regarding food choices and to create the conditions for a better quality of life, increasing the life expectancy of these populations.

Understanding cell biology and how every single cell in our body works constantly and cooperates with the surrounding environment would create in all of us a state of appreciation and fascination. In fact, inside every single cell there are so many different biochemicals and hundreds of metabolic processes occur every single moment in a highly orchestrated and finely regulated manner. Each compartment of a cell perfectly communicates with

one another, with all the complex events under the control of specific molecular signals. All the molecular and biochemical processes are well determined too. These highly complex molecular processes are also influenced by factors, positive or negative, arriving from the environment, receiving millions of signals constantly, and it is up to our organism to protect us from potential damage through a constant state of surveillance. If our defense system (the immune system) functions efficiently, we then stay healthy.

Under stress conditions, environmental factors negatively change the proper function of our cells creating the basis for the development of a pathological process. In the case of a cancer cell, for example, we know that abnormal signals (genetically or epigenetically determined) instruct a single cell to proliferate continuously losing the physiological inhibition or the capacity to arrest the cell cycle. This step of uncontrolled proliferation follows other steps that lead to tumor initiation (or exiting the quiescence state), a reversible cell cycle arrest that is finely and highly regulated, cell transformation, and invasion. Eventually cancer cells gain the capability to metastasize (the spread of cancer cells to a different organ).

Moreover, under severe and prolonged stress conditions, immune cells lose their ability to neutralize a cell that is changing its phenotype and becoming cancerous. The tumor then grows uncontrolled and eventually leaves the original site causing metastasis.

In the brain, stress affects epigenetic mechanisms that have significant effects on gene expression, for example the expression of schizophrenia-related genes including reelin, glutamate decarboxylase (GAD), and brain-derived neurotrophic factor (BDNF). It alters epigenetic dynamics including DNA methylation/demethylation and histone acetylation/deacetylation processes (Matrisciano 2016).

Our immune system represents the defense mechanism that constantly protects our organism against offenders such as external pathogens (physical or chemical), germs, and microorganisms (including bacteria, viruses and parasites). It is also active against abnormal signals underlying cell transformation, attacking them and neutralizing them. Prolonged abnormal pathological signals may promote dysregulations of the immune system with consequent activation of pro-inflammatory mechanisms. Systemic inflammation, if not properly modulated, can create a status of chronic low-grade inflammation that has been linked to several pathological processes. Therefore, it’s important to keep the immune system efficient

and the systemic inflammation under control avoiding negative triggers as much as possible.

Under certain adverse situations, a dysfunctional immune system releases pro-inflammatory cytokines inducing systemic inflammation or, if in the brain, neuroinflammation. Both systemic low-grade inflammation and neuroinflammation are considered the underlying cause of several pathological conditions such as obesity, cancers, and neuropsychiatric disorders (Diakos et al., 2014; Kolb et al., 2016; Nogo et al., 2021).

Thus, preventing or regulating inflammation by adopting an anti-inflammatory lifestyle including functional diets and physical activity, helps lower the risk of developing chronic diseases.

Bioactive compounds are commonly found in several functional foods (identified as *functional* because of their physiological role) and they have been investigated and scientifically proven to improve overall health. By targeting specific molecular factors and intracellular pathways underlying pathological conditions such as systemic inflammation and neuroinflammation, functional foods may become a suitable *tool* and lifestyle strategy to enhance quality of life in terms of the prevention and management of chronic diseases.

This book intends to underly the importance of the impact of environmental adverse factors including unhealthy diets on genome and gene expression (epigenetic mechanisms) by regulating epigenetic events, and how these factors lead to disease manifestation.

It is important to consider that epigenetic alterations are, by definition, *heritable* and *reversible* (Zhang et al., 2020), which means that they can be potentially passed to the second generation. On the other hand, they can be *treatable* by eliminating harmful external factors including unhealthy diets or adopting healthier diets rich in functional foods with epigenetic properties able to reverse or correct the epigenetic alterations. Certain types of food rich in bioactive ingredients, called functional foods, exert epigenetic effects by modulating gene expression.

Altered epigenetic mechanisms, which include DNA methylation, histone modification, and microRNA (miRNA), constitute the underlying pathological factors of several conditions including neuropsychiatric disorders and cancer (Matrisciano et al., 2018; Ilango et al., 2020).

Recently the role of specific bioactive compounds found in functional foods on epigenetic mechanisms and their effects on regulating gene expression has been reported in several scientific publications (Hardy and Tollefsbol, 2011; Rasmussen et al., 2021).

Thus, a functional epigenetic diet may be considered a potential synergistic strategy to adopt for improving these pathological conditions and improve overall health.

Epigenetics is a relatively young science that has been deeply studied in oncology. It is well-established that several cancers have a strong epigenetic component in their pathogenesis in absence of mutations in the DNA sequence, suggesting that epigenetic alterations reflect the abnormal interaction between genes and the environment.

Epigenetic studies have been progressively extended to other pathological conditions such as neuropsychiatric disorders including autism spectrum disorders, schizophrenia, mood disorders, and dementia, including Alzheimer's disease (Dawson and Kouzarides, 2012; Peedicayil and Grayson, 2018).

Epigenetic markers such as DNA methyltransferases (DNMTs), and histone deacetylases (HDACs), amongst others, are open also to novel therapeutic strategies. Different *epigenetic drugs* are under clinical trials for cancer treatment and some of them have already been approved (as briefly described in the editorial by Esteller (2017).

Obesity and unhealthy dietary patterns are well-known risk factors for several conditions, ranging from cancer and dementia to metabolic syndromes associated with schizophrenia and autism populations (Avila et al., 2015; Stevens et al., 2018; Dhaliwal et al., 2019). We know, for example, that individuals with schizophrenia are at a higher risk of developing dementia at a relatively young age, or medical pathological conditions including cardiovascular diseases, diabetes, and metabolic syndrome, not necessarily or solely related to antipsychotic medications (Penninx and Lange, 2018). Nowadays, the Centers for Disease Control and Prevention (CDC) states that "the US obesity prevalence was 41.9% in 2017 – March 2020" (National Health and Nutrition Examination Survey [NHANES]). We are thus in a health crisis. But obesity represents only the tip of the iceberg. It is not just a problem for itself: obesity leads also to a higher risk of developing other diseases including cardiovascular diseases, dementia, and cancers (Avgerinos et al., 2019).

Although the Mediterranean diet remains one of the most recommended diets against inflammatory-based chronic diseases, including Alzheimer's disease, the information and the attention given to the role of micronutrients contained in functional foods for health promotion remain poor. The western world's diet is becoming more homogeneous, despite regional differences, with more consumption of prepacked, processed and ultra-processed foods rich in sugar, salt, and preservatives that trigger inflammatory processes. Consumption of ultra-processed foods is associated with a higher risk of all-cause mortality (Kim et al., 2019).

Functional food can be defined as natural or processed food that has a potentially positive effect on health beyond basic nutrition. It contains biologically active compounds which provide clinically proven and documented health benefits for the prevention, management, or treatment of chronic diseases. This definition of functional foods represents a breakthrough in highlighting the role of foods, usually whole plant-based foods, as components of a normal diet for optimized nutrition, and the importance of "bioactive compounds" which represent the *effectors* of food effectiveness. Examples of *functional foods* are grapes rich in resveratrol, blueberries rich in anthocyanins (flavonoids that have proven to be a powerful group of antioxidants), and walnuts rich in minerals and omega-3 fatty acids which exert anti-inflammatory activity. Adopting a functional food diet may also help the overall health of children and adults with ASD by reducing the risk of comorbidities such as obesity and inflammatory-based diseases including certain types of cancer.

Switching from a disease *treatment* view to an individual *cure* and functional recovery approach may improve disease course, clinical severity, and outcomes. Providing information about the latest research evidence on the potential epigenetic benefits of functional nutrition and bioactive ingredients in chronic mental disorders and cancer may help individuals to adopt a functional diet as a part of their plan to stay healthy or manage their disorders. An integrated and synergistic approach used for inflammatory-based chronic diseases and disabilities is urgently needed.

Individuals affected by severe mental disorders or cancer face challenging times in terms of the higher risk of comorbidities, medications' side effects, and poor quality of life. Thus, this book aims also to provide a comprehensive source of scientific knowledge for clinicians, researchers, residents, fellows and students who are devoted to improving the quality of life of people who are suffering with inflammatory-based chronic diseases

by adopting a preventive and holistic approach which includes a functional nutrition program.

So, dietary interventions, including community-based, family-based, and individual-based nutritional education, are urgently needed to prevent the risk of associated medical conditions and to improve the course of underlying pathologies for these populations (Matrisciano, 2023).

Individuals with chronic psychosis and mood disorders such as bipolar disorders adopt an unhealthy lifestyle and poor dietary habits by eating mostly unhealthy foods like highly processed foods, and neglecting fruits, vegetables, and fibers. Psychoeducational programs are needed to guide patients toward the right path in terms of food and nutrition.

In a community mental health care setting instead, with a controlled functional nutrition program mostly based on fibers, fruits, vegetables, legumes, nuts and seeds rich in phytonutrients and bioflavonoids, and which avoids highly processed foods, processed meats, sugary beverages and foods such as cakes and candies, patients seem to reduce their risk of developing metabolic disorders including obesity, diabetes or insulin resistance. Patients can keep their weight under control by losing the excessive weight caused by gut dysmicrobiosis and inflammation, and alterations in blood sugar and blood pressure may also be corrected.

Overall, in the long run, these individuals experience a better outcome in terms of reduced associated medical conditions and in general a better quality of life.

Chapter 1 discusses some molecular aspects of epigenetics mechanisms.

Chapter 2 discusses autism spectrum disorder (ASD): its pathogenesis, clinical manifestation, medical comorbidities including the risk of obesity, and the role of functional foods.

Chapter 3 shows the clinical and pathogenetic characteristics of schizophrenia and the role of unhealthy lifestyles and poor nutrition in those individuals, and the consequent risk of premature death.

Chapter 4 illustrates the role of diet in breast cancer and colorectal cancer, which represent two very common cancers strongly related to unhealthy dietary habits. It shows us how adopting a functional epigenetic nutrition may improve outcomes and quality of life.

Chapter 5 discusses the role of functional foods in mood disorders including major depression and postpartum depression.

Chapter 6 reports on functional foods with anti-inflammatory and anti-cancer properties.

Chapter 7 discusses the epigenetic activity of some of the bioactive compounds found in functional foods.

Chapter 8 discusses the role of an anti-inflammatory epigenetic diet.

Chapter 9 focuses on health promotion with regard to the role of herbs and spices.

For more information, the major scientific sources used for this book are *the National Center for Biotechnology Information (NCBI)-PubMed.gov*, the *American Cancer Association (ACS)*, *Centers for Disease Control and Prevention (CDC)*, the *US Department of Agriculture (USDA)*, and *clinicaltrials.gov.*

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2. EPIGENETICS: BASIC PRINCIPLES

The term *epigenetic*, which has a Greek origin and means literally above (epi) the genome, commonly refers to stable, environment-depending changes in gene expression that occur without altering the underlying DNA sequence (Haig, 2004).

According to the National Human Genome Research Institute, the epigenome consists of a set of chemical modifications to the DNA and DNA-associated proteins in the cell, which modifies gene expression, and are heritable (via meiosis and mitosis). These modifications occur as a natural process of development and tissue differentiation but can also be altered in response to environmental factors or disease. Chromatin represents the form of how the DNA is packed and refers to the complex of DNA and the proteins that package DNA into the nucleus of cells. Chromatin can undergo structural modifications as well by dynamic changes in targeted proteins called histones with the consequence of allowing or obstructing transcription factors to access DNA for gene expression. Epigenomes through biochemical modifications in the DNA and/or protein level alters gene expression in specific cell types, under specific conditions and developmental phases. Every cell in our body has the same DNA sequence no matter if that cell belongs to the heart, brain, or bones. What makes a cell specialized for each organ and tissue is the ability of the epigenome to turn "on" or "off" certain genes to produce the proteins needed for that particular organ and tissue. Haemoglobin is the protein that carries iron in the blood while the dopamine transporter is a protein that allows dopamine to enter neuronal cells. Thus, epigenetic mechanisms are fundamental for the normal development and maintenance of tissue-specific gene expression. Under pathological conditions, abnormalities in epigenetic processes induced by lifestyle or environmental factors including smoking, diet, infectious diseases, severe prolonged stress, and trauma, amongst others, can lead to alterations in gene expression and disease development including neuropsychiatric disorders and cancer (Moos et al., 2016; Furtado et al., 2019; McEwen BS, 2017).

Evidence suggests that several diseases and behavioral disorders result from defects in gene function. Cancer and other diseases such as autoimmune disease, asthma, type 2 diabetes, metabolic disorders, neuropsychiatric disorders, and autism display aberrant gene expression. Several compounds targeting enzymes involved in histone acetylation and deacetylation, histone methylation, and DNA methylation and demethylation have been developed as epigenetic drugs for haematological malignancies and solid tumors (Pechalrieu et al., 2017). Recently researchers are focusing on finding additional epigenetic targets for the development of new molecules for the treatment of different CNS disorders such as autism and schizophrenia, targeting specific enzymes that play a significant role in gene expression and function. The same concepts apply to those cancers with known epigenetic modifications as underlying pathogenetic mechanisms including breast cancer and colorectal cancer (Garcia-Martinez et al., 2021; Okugawa et al., 2015).

The basic biology of epigenetics

It is well-established that our genetic constitution affects our overall health and risk of disease development, but also our lifestyle and behaviors affect our health and the risk of getting sick from several pathological conditions including neuropsychiatric disorders and cancer. The influence of gene expression induced by environmental factors including physical exercise and diet is studied by *epigenetics*. Epigenetics represents a relatively young science that studies the modifications of the genome (or gene expression), both heritable (in fact, epigenetic changes such as DNA methylation pass to daughter cells during cell division) and reversible, without causing changes in DNA sequence. Conrad Waddington in 1946 was the first to use the term "*epigenetic*" to describe the effect of gene-environment interactions on the expression of phenotypes created by the regulation of gene expression. Nowadays, the term epigenetic commonly refers to stable, environment-depending changes in gene expression that occur without altering the underlying DNA sequence (Haig, 2004). Epigenetic changes are known to be stable enough to be inherited through generations of mitotic cell divisions and may result in stable phenotypic alterations in the organisms (Campos et al., 2014). The different mechanisms and the molecular pathways underlying the regulation of the epigenome have been extensively studied; all of them appear to be able to regulate gene expression by modifying chromatin structure.

Until recently, chromatin was defined as the form that DNA takes in eukaryotic cells; it is composed of several units (nucleosomes), each consisting of 147 base pairs of DNA wrapped around a complex of 8 "core" histone proteins. This octamer is composed of two copies of core histones H2A, H2B, H3, and H4; an additional histone protein, H1, acts as a linker that further compacts the nucleosomes into higher-order structures. Chromatin may exist in different structural states, reflecting its accessibility to the transcriptional machinery and therefore its functional state. When the chromatin exists in a more open state, where the DNA is broadly accessible to the transcription factors, it is called *euchromatin*. Since this is the state in which transcription is allowed, euchromatin is often referred to as the "active" state. On the other hand, the "inactive" and "silent" state of chromatin is called *heterochromatin*, and refers to a highly condensed combination of DNA and structural proteins which protects the genome from damages and prevents it from being accessible to the transcriptional machinery. Between these two extremes, chromatin may exist in a continuum of structural and functional conformations, i.e., active, permissive, repressed, and inactive; modifications altering the structure of the histones at a particular allele of a gene represent the main way through which the transcription of the genome is differentially modulated.

More recently, it became well-recognized that chromatin plays an important role in the regulation of gene activity (Even-Faitelson et al., 2016; Reyes et al., 2021). This may occur because histones' covalent modifications alter the affinity of these proteins for the DNA and/or for other structural proteins, making chromatin accessible to the transcriptional machinery. Alternatively, these modifications may regulate gene expression by attracting or repelling different transcriptional activators or repressors (Fyodorov et al., 2018). Several epigenetic mechanisms that control the chromatin remodelling have been described and most involve strong, covalent modification of histone proteins, such as acetylation, methylation, ubiquitylation, SUMOylation, ADP-ribosylation and phosphorylation. Amongst them, the most characterized mechanisms, and the most used in experimental procedures, are *acetylation* and *methylation of histones*. Hyperacetylation of histones is associated with chromatin decondensation, and consequently with an increased gene transcription, whereas hypoacetylation leads to the opposite state, more compact, with decreased gene transcription (Shen et al., 2015; Carrera et al., 2021). The catalyzing acetylation enzymes are called histone acetyltransferases (HATs), whereas histone deacetylases (HDACs) produce deacetylation; these modifications generally occur on the amino-acid lysine residues of the N-terminal tail and are perfectly balanced to accurately regulate chromatin activity and gene

expression (Narlikar et al., 2002). Methylation of the histones may occur on lysine or arginine residues and is mediated by enzymes called histone methyltransferase (HMTs) (Lachner and Jenuwein, 2002). In general, methylation of lysine residues is considered an extremely stable and almost irreversible modification, leading to a robust repression of gene expression.

Nevertheless, the subsequent discovery of histone-demethylating enzymes (HDMs) has proved that methylation is not a permanent mark (Hyun et al., 2017). Histone methylation may correlate with either gene activation or repression, depending on the residue involved (i.e., methylation on lysine 9 residue of H3 histone is associated with gene repression, whereas methylation on lysine 4 of the same histone relates to actively transcribed genes) (Martin and Zhang, 2005). Methylation may occur even directly on the DNA, usually by transferring the methyl group S-Adenosyl-methionine (Sathe M) to C5 position of the cytosine at the dinucleotide sequence CpG to form 5-methylcytosine; this modification is catalyzed by a family of enzymes called *DNA methyltransferases* (DNMTs) (Moore et al., 2013). When methylated at CpG clusters (known also CpG islands) within the gene promoter or body, the DNA is strongly inaccessible to transcription factors and generally gene expression is repressed. This repression is further strengthened by the binding with protein complexes containing methyl binding domain proteins (MBDs), histone deacetylases (HDACs) and histone methyltransferases (HMTs) (Du et al., 2015). The amount of methylation, especially at promoter regions of the genes but also within the gene body, directly correlates with the level of inactivation of gene transcription (Neri et al., 2017).

Evidence is emerging that several diseases and behavioral pathologies result from defects in gene function. It is well-known in the case of cancer, but other diseases such as autoimmune diseases, asthma, type 2 diabetes, metabolic disorders, and neuropsychiatric disorders including autism and schizophrenia display aberrant gene expression. Gene function may be altered by either a change in the sequence of the DNA or a change in the epigenetic programming of a gene in the absence of DNA sequence changes. With epigenetic drugs, it is potentially possible to reverse aberrant gene expression profiles associated with different disease states. Several epigenetic compounds targeting DNA methylation and histone deacetylation enzymes (DNMTs and HDACs) have been tested in clinical trials (Karsli-Ceppioglu, 2016). Understanding the epigenetic machinery and the differential roles of its components in specific disease states is essential for developing targeted epigenetic therapy (Szyf, 2009).

DNA methylation

DNA methylation is the most common epigenetic tool used by cells to express or silence a gene. DNA methylation occurs at CpG dinucleotides on the 5th carbon of the cytosine base, forming 5-methylcytosine (5-mC). Clusters of methylated CpG, called CpG islands, are usually associated with gene inactivation.

DNA methylation may be studied using different methods. For example, DNA methylation can be detected using *bisulfite conversion*, *methylation sensitive restriction enzymes*, *methyl binding proteins* and *anti-methylcytosine (anti-mC) antibodies via immunoprecipitation (IP) techniques*. At present, the most common and accurate method for the analysis of DNA methylation is via sodium bisulfite modification, or bisulfite conversion. Treatment of DNA with sodium bisulfite leads to a deamination of unmethylated cytosine bases, and their subsequent conversion into the residue uracil, whereas methylated cytosines remain unchanged. Bisulfite-modified DNA can then be amplified and sequenced by methylation-specific PCR methods (MSP), which provide very accurate, sensitive, and specific information about the methylation status of different blocks of CpG sites in a CpG island. Nevertheless, since this approach can recognize only small (<40bps) strands of methylated DNA within each CpG island, it is insufficient for use in clinical analysis (Hayatsu et al., 2008). In the preclinical setting, our lab measured DNA methylation using an immunoprecipitation technique called *MeDIP* (Methylated DNA Immunoprecipitation) analysis in low amounts of DNA samples (Matrisciano et al., 2016). By MeDIP analysis we were able to measure the amount of methylated cytosines in brain regions of the mouse model for schizophrenia induced by prenatal stress. We demonstrated that in prenatally stressed mice epigenetic abnormalities occurred in genes related to schizophrenia (Matrisciano et al., 2013). More recently, in oncology, DNA methylation has been measured by using cell-free DNA samples which represents a non-invasive dynamic assessment of disease status in patients with cancer (Galardi et al., 2020).

Histone modification

Histone modification patterns are dynamically regulated by enzymes that add and remove covalent modifications to histone proteins. Histone acetyltransferases (HATs) and histone methyltransferases (HMTs) add acetyl and methyl groups, respectively, whereas HDACs and histone demethylases (HDMs) remove acetyl and methyl groups, respectively.

Several histone-modifying enzymes including various HATs, HMTs, HDACs and HDMs have been identified (Kouzarides, 2007). These histone-modifying enzymes interact with each other as well as other DNA regulatory mechanisms to tightly link chromatin state and transcription (Sharma et al., 2010).

Histone acetylation neutralizes the positive charge on the histone tail, opening chromatin and increasing the access of transcription factors to their DNA binding sites. Acetylation commonly occurs at lysine residues, such as the H3K9, and is catalyzed by histone acetyltransferases and reversed by HDACs. HDACs remove acetyl groups from histone tails and prevent subsequent acetylation (Shahbazian and Grunstein, 2007; Konsoula and Barile, 2012). Cytosine methylation attracts repressor complexes comprised of HDACs such that DNA methylation and histone acetylation are usually inversely related. Histone acetylation directly modifies chromatin structure through effects on the local physicochemical environment that define the chromatin state (Turner, 2000; Taverna et al, 2007). Additional histone modifications, notably histone methylation, influence transcription through indirect pathways that involve a complex array of transcriptional mediators.

Multiple lysine and arginine residues on the histone tails are subject to methylation, which is catalyzed by distinct histone methyltransferases and reversed by histone demethylases. This process provides a signaling pathway that begins with the activation of the intracellular signals that activate the individual methylating or demethylating enzymes producing a specific epigenetic profile on the histone tails. This process links specific intracellular signals to specific histone methylation marks. The methylation profile of the histone tails is highly variable. Methylation can occur at multiple sites along the histone tails and vary in the level of methylation (mono-, di-, or tri-methylation). The resulting profile acts as a ‘code’ for various protein complexes that remodel chromatin and alter transcriptional activity, thus indicating an indirect influence of histone methylation on transcription (Hake and Allis, 2006; Zhang et al., 2013). More recently, it has been reported that histone acetylation modifications, under a controlled activity between HAT and HDAC, play an important role in cancer development including breast cancer. Specific HDAC inhibitors have been approved by the FDA for certain cancer treatments, in addition to HAT inhibitors or activators (Guo et al., 2018).

MicroRNAs (miRNAs)

MicroRNAs (miRNAs) are small (about 21–22) noncoding RNAs, and are a known regulator of essential biological processes in animals and plants. Each miRNA can regulate different messenger RNAs (mRNAs).

Dysregulated miRNAs play critical roles in the progression of various diseases, such as aging, cardiovascular disease, neuropsychiatric disorders, and cancer. In children with autism spectrum disorders (ASDs), aberrant expression of several miRNAs has been found and they could play an important role as link factor between genetics and the environment in ASD pathogenesis (Vasu et al., 2019). Similarly, studies have shown that miRNAs are implicated in the pathogenesis of schizophrenia for their role in neurodevelopment, synaptic plasticity, and neuronal activity and are susceptible to environmental factors including maternal immune activation and cannabis exposure during adolescence, supporting the hypothesis that early life adverse events alter the brain miRNA profile and contribute to disease development (Thomas and Zakharenko, 2021). Several studies showed the role or miRNA in cancer development (Mishra et al., 2016).

Epigenetics and neurodevelopment

Here, a few pathological conditions determined by epigenetic alterations in their pathogenesis or with potential epigenetic interventions for therapy will be described.

Epigenetic mechanisms mediate gene-environment interplay during the entire lifespan. The implication of epigenetic regulation in neurodevelopment, embryonic, post-natal, and adult neurogenesis has been demonstrated in several studies (Fagiolini et al., 2009; Gonzales-Roybal and Lim, 2013; Ma et al., 2010; Roth and Sweatt, 2011). Altered epigenetic regulation has been hypothesized to underlie several neuropsychiatric diseases with well-established neurodevelopmental impairments in their pathogenesis, such as autism spectrum disorders and schizophrenia (Rangasamy et al., 2013; Zhubi et al., 2014). We all know that ASDs and schizophrenia comprise a complex group of behaviourally related disorders that are primarily genetic in origin. Involvement of epigenetic regulatory mechanisms in the pathogenesis of ASDs has been suggested by the occurrence of these disorders in patients with diseases arising from epigenetic abnormalities (Fragile X syndrome) or that involve key epigenetic regulatory factors (Rett syndrome) (Fetit et al., 2021; Imamura et al., 2020).

Rett Syndrome, an X-linked, neurodevelopmental disorder which first begins to be symptomatic between 6 and 18 months of age, is considered to belong to the spectrum of autism disorders because of its main manifestations (stereotyped movements, seizures, severe cognitive impairment, loss of acquired language, and autistic-like behaviour, such as indifference to other people, absence of speech, avoidance of eye-contact, and social isolation). It appears to be caused by a loss-of-function mutation in the gene encoding the transcriptional repressor MeCP2 (Zoghbi, 2005; Sandweiss et al., 2020), which leads to a pathological overexpression of target genes, eventually resulting in an increased synaptic inhibition in cortical neurons (Calfa et al., 2015). This phenomenon is thought to occur because impairments in MeCP2 function seem to lead to a misinterpretation of the DNA methylation pattern (Horike et al., 2005). Interestingly, experimental procedures which reverse the MeCP2 deficiency showed efficacy in rescuing a Rett-like phenotype, both *in vivo* and *in vitro*, and even in adult, fully differentiated neurons (Giacometti et al., 2007; Nelson et al., 2006).

Angelman Syndrome (AS) includes features like impaired speech, mental retardation, puppet-like motor symptoms, paroxysms of laughter, and seizures; it represents another example of genetic/epigenetic dysfunctional disorder. It is caused by a loss-of-function of the maternal copy of the Ubiquitin E3 Ligase gene (UBE3A), which encodes for a protein involved in the ubiquitin-dependent protein degradation process (Mabb et al., 2011). Since in neurons the paternal UBE3A allele is epigenetically silenced (Lee and Bartolomei, 2013), AS is determined by mutations or deletions in the maternal copy of the gene, and epigenetic interventions are hypothesized to activate the paternal copy (Zylka, 2020).

Prader-Willi Syndrome (PWS) is another disease sharing symptoms with disorders of the autistic spectrum, other than manifestations like hypotonia, hypogonadism and hyperphagia that may result in obesity. It is caused by loss of function of several genes on the paternal copy of 15q11-q13 that may occur via different mechanisms, including imprinting errors silencing the paternal allele. Epigenetic mechanisms leading to the aberrant silencing of the paternal allele include DNA methylation, covalent histone modifications and antisense RNA (Bittel and Butler, 2005; Duker et al., 2010).

Fragile X Syndrome (FXS) is the most common heritable form of cognitive impairment and the second most common cause of comorbid autism (Kraan et al., 2019). FXS patients show mental retardation, repetitive speech, stereotyped behavior, autistic features, anxiety, and ADHD-like symptoms, as well as somatic manifestation like facial abnormalities (elongated face,

large ears), microcephaly, hypotonia and macroorchidism (Terracciano et al., 2005). The mutation underlying the pathogenesis of the disease has been identified in the expansion on a cytosine-guanine-guanine (CGG) repeat in the 5'UTR of the *fmr1* gene (Gallagher and Hallahan, 2012); normally, the alleles contain between 5 and 50 repeats; unstable, pre-mutation, alleles contain up to 200 repeats; whereas overtly mutant *fmr1* alleles contain more than 200 CGG repeats. Via mechanisms still to clarify, the CGG repeats lead to a repression of the *fmr1* gene via hypermethylation of CpG islands in its promoter region, blocking the synthesis of its product, the Fragile X Mental Retardation Protein (FMRP) (Godler et al., 2010), which in turn produces abnormal dendrite growth and impaired synaptic connections (He and Portera-Cailliau, 2013). More epigenetic mechanisms other than DNA hypermethylation have been described in the pathogenesis of FXS, such as demethylation at histone H3 at lysine 4, hypermethylation of histone H3 at lysine 9 (Eiges et al., 2007) and RNA interference (Zhou et al., 2019).

Recently, altered behavioral tests associated with biochemistry analysis in a mouse model of idiopathic ASD adult forms supported the hypothesis of a pivotal role of mGlu5 metabotropic glutamate receptors as candidate drug targets (Matrisciano et al., 2022). In this study, we found changes in the expression and function of mGlu5 receptors in the striatum, which represents the brain region that encodes habit memory, and, therefore, plays a key role in the programming of repetitive stereotyped behaviors. associated with altered ASD-like phenotype of Btbr mice including stereotypic and repetitive behaviors. The pharmacological blockade of the overactive mGlu5 receptors reversed all the behavioral deficits and represented a valuable treatment option for high mGlu5-expressing non-syndromic ASD.

Epigenetics and psychiatric disorders

Major psychiatric disorders such as schizophrenia and bipolar disorder with psychosis express a complex symptomatology characterized by positive symptoms, negative symptoms, and cognitive impairment for schizophrenia and mood swings (mania and depression) for bipolar disorder (Grayson and Guidotti, 2013). There is a growing body of evidence suggesting that the understanding of the genetic etiology of psychiatric illnesses, including schizophrenia, will be more successful with integrative approaches considering both genetic and *epigenetic* factors (Abdolmaleky et al., 2008). For example, several genes including those encoding dopamine receptors (*DRD2, DRD3*, and *DRD4*), serotonin receptor 2A (*HTR2A*) and catechol-

O-methyltransferase (*COMT*) have been implicated in the etiology of schizophrenia and related disorders through meta-analyses and large, multicenter studies. There is also growing evidence for the role of *DRD1*, NMDA receptor genes (*GRIN1, GRIN2A, GRIN2B*), brain-derived neurotrophic factor (*BDNF*), and the dopamine transporter (*SLC6A3*) in both schizophrenia and bipolar disorder (Kaalund et al., 2014; Uno and Coyle, 2019). Recent studies have indicated that epigenetic modification of reelin (*RELN*), *BDNF*, and the *DRD2* promoters confer susceptibility to clinical psychiatric conditions (Guidotti et al., 2016). Recent advances in schizophrenia research indicate that a deficit of brain γ-aminobutyric acid GABAergic function detected in these patients is related to the downregulation of several GABAergic genes, including glutamic acid decarboxylase 67 (GAD) and reelin (Tao et al., 2018). This downregulation could be a pathogenetic mechanism underlying the complex symptomatology of schizophrenia.

In the prefrontal cortex and basal ganglia GABAergic neurons of schizophrenia patients, an increase of DNA methyltransferase 1 (the enzyme that transfers a methyl group from S-adenosylmethionine to carbon 5 of the cytosine pyrimidine ring embedded in cytosine-phospho-guanine [CpG] islands containing promoters) increases methylation of selected GABAergic promoters. This increase is probably among the leading causes of GAD, reelin and other gene-expression downregulation (Guidotti et al., 2009). The complex etiology of schizophrenia, and other psychiatric disorders, warrants the consideration of both genetic and epigenetic systems and the careful design of experiments to unveil the genetic mechanisms conferring liability for these disorders and the benefit of existing and new therapies. In our lab, we have shown that systemic injection of the brain-permeant mGlu2/3 receptor agonist (-)-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylic acid (LY379268) increased the mRNA and protein levels of growth arrest and DNA damage 45-β (Gadd45-β), a molecular player of DNA demethylation, in the frontal cortex and hippocampus of mice. Treatment with LY379268 also increased the amount of Gadd45-β bound to specific promoter regions of reelin, brain-derived neurotrophic factor (BDNF), and glutamate decarboxylase-67 (GAD67). The action of LY379268 on Gadd45-β was mimicked by valproate and clozapine but not haloperidol. These findings show that pharmacological activation of mGlu2/3 receptors has a strong impact on the epigenetic regulation of genes that have been linked to the pathophysiology of schizophrenia (Matrisciano et al., 2011).

More recently, a substantial amount of evidence from Dr. Guidotti's group (Matrisciano et al., 2012; 2013) and other researchers (McGowan and Szyf, 2010) suggests that epigenetic modifications of DNA and chromatin induced by environmental factors, including stress, may contribute to the complex phenotypes of neuropsychiatric disorders.

Patients with psychosis express an increase in brain DNA methyltransferases (DNMT1 and 3a), and an increase in ten-eleven translocation hydroxylase (TET1). DNMT and TET are important components of the DNA-methylation/demethylation dynamic regulating the expression of key molecules involved in brain function (Dong et al., 2014).

In addition, recently published papers show that epigenetic mechanisms like histone modifications and DNA methylation affect diverse pathways leading to depression-like behaviors in animal models. Major depressive disorder is a chronic, remitting syndrome involving widely distributed circuits in the brain. Stable alterations in gene expression that contribute to structural and functional changes in multiple brain regions are implicated in the heterogeneity and pathogenesis of the illness. Epigenetic events that alter chromatin structure to regulate programs of gene expression have been associated with depression-related behavior, antidepressant action, and resistance to depression or 'resilience' in animal models, with increasing evidence for similar mechanisms occurring in postmortem brains of depressed humans (Sun et al., 2013). Adverse alterations of gene expression profiles, including glucocorticoid receptor or brain-derived neurotrophic factor, were shown to be inducible by early life stress and reversible by epigenetic drugs. Postmortem studies revealed epigenetic changes in the frontal cortex of depressed suicide victims. There exists profound evidence for histone deacetylase inhibitors to be a novel line of effective antidepressants via counteracting previously acquired adverse epigenetic marks (Schroeder et al., 2010). The epigenetic regulation of the promoter of the glucocorticoid receptor gene has been suggested as a molecular basis of such stress vulnerability. It has also been suggested that antidepressant treatment, including antidepressant medications and electroconvulsive therapy, may be mediated by histone modification on the promoter of the brain-derived neurotrophic factor gene (McGowan and Kato, 2008). Recently, a significant epigenetic component in the pathology of suicide has been realized. Murphy et al. showed that psychiatric patients with a history of suicide attempt had significantly higher levels of global DNA methylation compared with controls (Murphy et al., 2013).