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Depressive disorder and antidepressants from an epigenetic point of view

Depressive disorder and epigenetics

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Abstract

Depressive disorder is a complex, heterogeneous disease that affects approximately 280 million people worldwide. Environmental, genetic, and neurobiological factors contribute to the depressive state. Since the nervous system is susceptible to shifts in activity of epigenetic modifiers, these allow for significant plasticity and response to rapid changes in the environment. Among the most studied epigenetic modifications in depressive disorder is DNA methylation, with findings centered on the brain-derived neurotrophic factor gene, the glucocorticoid receptor gene, and the serotonin transporter gene. In order to identify biomarkers that would be useful in clinical settings, for diagnosis and for treatment response, further research on antidepressants and alterations they cause in the epigenetic landscape throughout the genome is needed. Studies on corner stone antidepressants, such as selective serotonin reuptake inhibitors, selective serotonin and norepinephrine reuptake inhibitors, norepinephrine, and dopamine reuptake inhibitors and their effects on depressive disorder are available, but systematic conclusions on their effects are still hard to draw due to the highly heterogeneous nature of the studies. In addition, two novel drugs, ketamine and esketamine, are being investigated particularly in association with treatment of resistant depression, which is one of the hot topics of contemporary research and the field of precision psychiatry.

Key Words: Epigenetics; Depression; DNA Methylation; Histone Tail Modification; miRNA; Antidepressants

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Core Tip: Deeper knowledge on the biological background of depressive disorder could be achieved through understanding of epigenetic mechanisms that alter the response of cells to environmental stimuli. Among the later are also antidepressants, since it has

been shown that they affect DNA methylation, histone modifications, and microRNA expression. As not all patients respond to prescribed antidepressants, it is of interest to discover specific biomarkers that could be used in a clinical setting.

INTRODUCTION

DEPRESSIVE DISORDER

Depressive disorder is a complex heterogeneous disease that affects more than 280 million people [1]. The principal form of depressive disorder is major depressive disorder (MDD). Symptoms of depressive disorder are persistent depressive mood, diminished ability to feel pleasure and rejoice, weight changing, disturbed sleep, loss of energy, lowered self-esteem, trouble with concentration, elevated emotional psychomotor activity in children and teenagers, psychomotor agitation or motor retardation, and self-injuring or suicidal ideation [2]. The suicidality phenotype includes ideation, suicide attempt, and death by suicide. MDD is, along with bipolar disorder, schizophrenia, and substance use disorder, one of the most common mental disorders in people who die by suicide [3]. Depression contributes to suicidality and it increases mortality risk by 60–80% [4]. According to the Diagnostic and Statistical Manual of Mental Disorder Diagnosis, MDD must exhibit five (or more) out of ten symptoms [2]. The prevalence of depression is higher for women (4.1%) than for men (2.7%) [5]. Sex differences are exhibited in multiple cells of the central nervous system (CNS), neurons, astrocytes, and microglia [6]. Emerging data is showing that besides hormones, epigenetic differences have considerable sexual dimorphism [7]. However, steroid hormone levels influence levels of DNA methyltransferases (DNMTs). For example, female rats had higher levels of DNMT3a and methyl CpG binding protein 2 (MeCP2) in the amygdala (an important center for modulating juvenile social play, aggression, and anxiety) [6] and the preoptic area [7]. As a result of a difference in DNMT3a, there is also a difference in DNA methylation level [6]. Moreover, people aged 50 years and more have a 1.5 times higher risk for developing depression than younger people [5]. Modern lifestyle promotes independence of the environmental light/dark cycle, which

leads to shifting in sleep-wake patterns. Circadian rhythm disruption is affected by the increase in nocturnal activity, decrease of sleep, and extended exposure to artificial light during night time [8]. Limbic brain regions, monoamine neurotransmitters, and the hypothalamic-pituitary-adrenal (HPA) axis are under circadian regulation. It is thought that the perturbation of circadian rhythms contributes to the prevalence of depression and other mood disorders [9].

Depressive disorder is a result of the interplay of many different factors: environmental, genetic, neurobiological, and cultural [10]. Known environmental risk factors for developing depressive disorder are poverty, negative experiences in the family (bad relationship, violence, divorce, child maltreatment), or other stressful life events. In the time after a stressful life event, the risk for depressive disorder is elevated but the effects of adversity can persist over time [4]. In depressive symptoms that persist over time, stable molecular adaptations in the brain, especially at the level of epigenetics, might be involved [11].

Genetic heritability for depressive disorder, estimated from twin studies, is around 35–40% [10, 12]. Genome-wide association studies have discovered multiple loci with small effects that contribute to MDD [13]. Pandya *et al.* (2012) collected results from neuroimaging, neuropsychiatric, and brain stimulations studies and showed similar results [14]. In recent years, more and more studies are oriented towards epigenetics, to understand new mechanisms and the way epigenetics is linked to a depressive state.

The nervous system is susceptible to shifts in the activity of epigenetic modifiers, which allow for significant plasticity and response to rapid changes in the environment [15]. Epigenetic mechanisms are dynamic. They are very important for early development of the organism and as well as later in life, as a response to external factors [16].

From a biological perspective, there are four theories of depressive disorder: monoamine theory, stress induced theory, neurotrophic theory, and cytokine theory.

<figure 1 about here>

Figure 1 Depressive disorder risk factors. Depressive disorders are influenced by various —and often overlapping—risk factors, that form theories of depressive disorders.

1.2. *THEORIES OF DEPRESSIVE DISORDER*

1.2.1. THE MONOAMINE THEORY OF DEPRESSIVE DISORDER

Monoamine neurotransmitters (serotonin, norepinephrine, and dopamine) are chemical messengers involved in the regulation of emotion, arousal, and certain types of memory. The monoamine hypothesis of depressive disorder proposes development of depressive disorder by signal dysfunction between neurons – that a decreased level of neurotransmitters leads to the depressive state [2, 17].

1.2.2. THE STRESS INDUCED THEORY OF DEPRESSIVE DISORDER

Prenatal stress, early-life adversities, chronic stress, and stressful life events are all strong predictors of the onset of depressive disorder. The HPA axis, a neuroendocrine system, is responsible for adaptation to changing environments. Response to stress begins in the hypothalamus, with the secretion of corticotropin-releasing hormone, which affects the pituitary gland to release adrenocorticotropic hormone. Adrenocorticotropic hormone circulates in the blood and stimulates the release of glucocorticoid hormones (cortisol) in the adrenal cortex. Cortisol binds to glucocorticoid receptors in the brain, which are key regulators of the stress response. Cortisol with a negative loop inhibits the HPA axis. Dysregulation of the negative loop is associated with depressive disorder [2, 17].

1.2.3. NEUROTROPHIC THEORY OF DEPRESSIVE DISORDER

Neurotrophic factors are peptides or small proteins that support the growth, survival, and differentiation of developing and mature neurons. Decreased neurotrophic support affects the development of depressive symptoms. Brain-derived neurotrophic factor (BDNF) is a very well examined neurotrophic factor. Many studies made on brain and

blood showed decreased expression of *BDNF* in patients with depressive disorder. Also, decreased *BDNF* expression has been associated with epigenetic modifications of the *BDNF* gene [17].

1.2.4. CYTOKINE THEORY OF DEPRESSIVE DISORDER

Cytokines are small secreting proteins important in cell signaling. Cytokines include chemokines, interferons, interleukins (IL), lymphokines, and tumor necrosis factors (TNF) [18]. The cytokine (or inflammation) theory of depressive disorder suggests that inflammation has a significant role in its pathophysiology. Patients with depressive disorder have increased inflammatory markers – IL-1β, IL-6, TNF-α, and C-reactive protein [19]. Depressive disorder is not a typical autoimmune disease, so the elevation of cytokines in patients with depressive disorder is lower than in autoimmune or infectious diseases [2].

There are several proposed theories by which the immune system (cytokines and immune cells) could affect depressive-like behavior [20]. For example, inflammation in peripheral tissue can signal the brain *via* the vagus nerve, cytokine transport systems, and a leaky blood-brain barrier caused by rising of TNF-α, which leads to brain accessibility for other peripheral signals [19].

Cytokines in the brain elevate during chronic stress and depressive disorder, but besides peripheral cytokines, they can arise also from the CNS. Cytokines IL-6 and TNF-α activate indoleamine-2,3-dioxygenase (IDO) which decreases tryptophan (a serotonin precursor) and consequently reduces serotonin. Moreover, IDO is included also in the kynurenine pathway. Metabolites from this pathway activate monoamine oxidase (MAO) which degrades serotonin, dopamine, and norepinephrine. Cytokines might also act directly on neurons, changing excitability, synaptic strength, and synaptic scaling. Furthermore, cytokine IL-1β can contribute to heightened activation of the HPA axis and lowering inflammatory response to stress. During chronic stress microglia (neural immune cells) enhance phagocytic activity and synaptic remodeling [20]

Microglia represent 10% of all brain cells [21]. During the development of the organism, microglia are extremely active. They significantly contribute to shaping and refining the developing neural circuits by regulating neurogenesis, synaptogenesis, synaptic pruning, and behavior. Early life stress, which is strongly associated with depressive disorder and other mental disorders, can trigger microglia perturbations and affect development through changed morphological and functional changes of microglia. For example, microglial phagocytic activity and neuronal-microglial signaling can disrupt neural circuits and alter the formation of behavior. Furthermore, aberrant functionality of maturing microglial cells can alter their developmental programs and have long-lasting consequences for their reactivity [22]. It is thought that innate immune memory is mediated through epigenetic reprogramming and can last *in vivo* for several months [23].

1.3. *EPIGENETICS*

In the 1940s, Waddington named the environmental influence of the genome epigenetics. Epigenetic modifications alter gene expression without changing the DNA sequence. The three key types of epigenetic change that occur in cells are DNA methylation, histone posttranslational modifications, and non-coding RNAs. The first two regulate gene transcription through altered chromatin structure and DNA accessibility, while the latter one regulates already transcribed messenger RNA (mRNA) [10]. Studies of epigenetics have escalated in the last 20 years and are gaining importance also in the field of psychiatry. Through epigenetic studies further understanding of depressive disorder is being achieved, but there are still many questions left to answer.

<figure 2 about here>

Figure 2 Epigenetic mechanisms. Epigenetic mechanisms include DNA methylation, noncoding RNA activity (such as micro RNA), and posttranslational histone tail modifications. Ac: histone acetylation, Me: histone methylation, miRNA: micro RNA.

1.3.1. DNA METHYLATION

DNA methylation is a process in which a single methyl group is added on the 5C of the cytosine DNA base. Methyl groups are transferred from S-adenosyl-L-methionine to cytosine by DNMTs [17]. In mammals, there are three groups of DNMTs; DNMT1, DNMT2, and DNMT3. DNMT1 maintains DNA methylation, DNMT3a and DNMT3b carry out *de novo* DNA methylation, and DNMT3L modulates DNMT3a and DNMT3b. DNMT2 has no DNA methylation activity. Instead it catalyzes RNA methylation, specifically on transfer RNAs [24]. DNA methylation mainly occurs at cytosine-phosphate-guanine (CpG) dinucleotides. When those dinucleotides are repeated many times in DNA sequence, they are called CpG islands. CpG islands have an average length of 1000 bp and they contain more than 50% of guanines and cytosines. Approximately 40% of genes contain CpG islands in promoter regions. Methylation of a promoter results in the inability of transcription factors to bind properly to regulatory elements and repression of gene transcription [17]. However, in mammals DNA methylation also occurs at CpA, CpT, and CpC. Those non-CpG methylation sites are common in brain tissue and several other tissue types [25], but at a three times lower rate than CpG methylation [26]. Besides methylation in promoter regions, it can also occur in the gene body and in intergenic regions and affect gene transcription [27]. DNA methylation is a stable cell state, but it can be reversed. Demethylation occurs when 5-methylcytosines are oxidized back to cytosines *via* three cytosine derivate forms: 5-hydoxymethylcytosine, 5-formylcytosine and 5-carboxylcytosine [28].

1.3.2. HISTONE TAIL MODIFICATION

The basic unit of chromatin is the nucleosome, which consists of negatively charged DNA and positively charged histone proteins. The nucleosome is an octamer, containing two copies of H2A, H2B, H3, and H4 proteins. Typically, a 147 bp long segment of DNA is wrapped around each nucleosome. H1 protein serves as a linker protein between the other histones that helps to condense nucleosome even more [29]. Histone proteins have a long amino acid tail on their N-terminal. In contrast with the core part of the histone protein, this extended part is very dynamic and is prone to

chemical modifications [30]. To describe histone modifications we follow a standard nomenclature: first we write the name of the histone protein (H2A, H2B, H3, H4, or H1), then the modified amino acid residue (the name of amino acid and its site; for example, K4 – lysine at site 4), and finally the type of modification (for example trimethylation – me3). An example a final structure is H3K4me3. Specific proteins chemically modify histones and change chromatin conformation. Changes in conformation lead to the opening or closing of the chromatin, which allows or prevents transcription. There are many different types of histone posttranslational modification, such as acetylation, methylation, phosphorylation, ubiquitination, *etc.*, that can be modified differently and by different proteins called "writers" and "erasers". Furthermore, "readers" are proteins important for cross-talk between different epigenetic modifications, for example, DNA methylation, and histone modifications mutually influence each other. There are many different reader domains that recognize histone modifications [31]. The most studied histone modifications are acetylation and methylation [29].

Histone acetyltransferases (HATs) are proteins that transfer acetyl groups to lysine residues on the amino acid tail of histone proteins, while histone deacetylases (HDACs) are proteins that remove acetyl groups from the histone tails. Addition of a negative acetyl group loosens the tight bond between the negatively charged DNA and positively charged histones. This enables access of transcriptional machinery to the regulatory parts of DNA and consequently gene transcription [10].

Histone methylation is the adding of methyl group to lysine and arginine residues on the histone tail. Histone methyltransferases (HMTs) add methyl groups to the histone tail, and histone demethylases (HDMs) remove methyl groups. Methylation of the histone tail can work in two ways. It can open chromatin or condense it. This depends on the position of the lysine/arginine residue in the histone tail and the number of methyl groups added to the amino acid [10].

1.3.3. MICRO RNAS

Non-coding RNAs include many different RNAs: PIWI-interacting RNAs, small nucleolar RNAs, long non-coding RNAs and the most studied, micro RNAs (miRNAs). MiRNAs are noncoding, 19–24 nt long RNAs that bind to mRNAs. A mature miRNA goes through biogenesis before it achieves its final form. Briefly, it is transcribed as a 1 kb long primary RNA (pri-miRNA) with a stem and loop structure. Pri-miRNA is cleaved by Drosha ribonuclease III (DROSHA) into a 60–100 bp long precursor miRNA (pre-miRNA). Pre-miRNA is then translocated from the nucleus into the cytoplasm where the endonuclease Dicer converts it into an unstable, double stranded small RNA. One strand of the duplex is degraded and other, the mature miRNA, incorporates into the RNA-induced silencing complex (RISC) along with Argonaut protein. Mature miRNA is complementary to one or more mRNAs. It binds to the 3' untranslated region (UTR) of the target mRNA and silences targeted mRNA or sends mRNA to degradation when binding is highly complementary [32].

2. EPIGENETICS AND DEPRESSIVE DISORDER

Among biomarkers that could be associated with MDD are: BDNF, the cortisol response, cytokines, and neuroimaging. However, due to complex nature of depressive disorder a single biomarker is not sufficient for use in diagnosis or monitoring of the disorder. Therefore, it has been proposed to examine multiple biomarkers and use them for patient examination [33]. In genetic studies several polymorphisms associated with depressive state in genes of the monoaminergic system (the gene that encodes for serotonin transporter, receptor genes for dopamine and serotonin, genes involved in signaling of noradrenaline and dopamine…), and genes involved in the functioning and regulation of the HPA axis have been implicated [2], but did not reveal the role of the DNA sequence itself in the etiology of depressive disorder. In future epigenetics may present new findings, which could be included as possible biomarkers for MDD [33].

Epigenetic modifications are studied on saliva and blood of the depressed patients, *post mortem* brain tissue of depressed patients who died by suicide, and rodent animal models (rats and mice). There are several ways to induce stress and depressed state in

animal models [34]. Chronic stress is induced with "bullying" by a bigger more aggressive mouse or witnessing another mouse being physically aggressed for several days [10]. Early life stress from humans can be evoked also on animal models by maternal separation of offspring during early postnatal periods. Such induced stress in animals results in mimicking certain behavioral features of human depressive disorder. It has been shown that these methods evoke epigenetic changes, similar to those seen in humans [34].

Tables 1–4 show selected studies of epigenetic changes detected in samples of depressed patients and animal models. The most studied epigenetic modification is DNA methylation and it has been rather extensively investigated in the *BDNF* gene, specifically exon I. In studies of depressive disorder induced by stress in the prenatal and early stages of life, methylation of glucocorticoid receptor gene (*NR3C1*) was the most analyzed. Lately, more studies are also considering histone 3 modifications among which are methylations of the lysine 27, 9, 4, and acetylation of the lysine 14. Studies of miRNAs are diverse and are showing that more standardized approach is needed.

3. POSSIBLE TREATMENTS OF DEPRESSIVE DISORDER

There are pharmacological and nonpharmacological (psychotherapy, lifestyle interventions, and neuromodulatory treatment) ways of treating depressive disorder. For pharmacological treatment, there are many different antidepressants available and they are a cornerstone for treating depressive disorder [81]. The main drug classes of antidepressants are selective serotonin reuptake inhibitors (SSRIs), selective serotonin and norepinephrine reuptake inhibitors (SNRIs), norepinephrine and dopamine reuptake inhibitors (NDRI), noradrenergic and specific serotonergic agents (NaSSAs), tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAO-I) and melatonin modulators (agomelatine) [82]. However, there is no universally effective treatment for all depressed patients [81]. People suffering from depressive disorder can recover in a year, or not recover in more than 20 years. Furthermore, depressive episodes recur in almost half of recovered patients [5]. Even though there are many different

antidepressants available and many different treatment options, 34–46% of MDD patients still do not respond effectively to one or more antidepressant treatments (i.e. fail to achieve remission). That is why there is still a great need for new antidepressants for curing treatment resistant depression [83]. Among novel drugs ketamine and eskatemine are being extensively used. Also, the histone deacetylase inhibitors (HDACis) are being tested on animal models as one possibility of treatment.

3.1. *SELECTIVE SEROTONIN INHIBITORS*

SSRIs are the most commonly prescribed antidepressants and are used as the first treatment step for depressive disorder. Serotonin or 5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter that modulates mood, reward, learning, and memory. Deficiency in serotonin release is not associated with serotonin biosynthesis. The serotonin deficit is more likely due to less serotonin neuron firing and less serotonin release. However, SSRIs block the reabsorption of serotonin into presynaptic neuron cell and with that, improve message transmission between cells [82].

Fluoxetine was the first SSRI to be developed and is the most used antidepressant for children and adolescents. Many different SSRIs have now been developed that vary in binding affinity - some are more specific to serotonin than others. It became clear that using the available antidepressants targeting specific monoamines also have side effects. Those side effects come from neurotransmitters binding to different receptors. For example, when serotonin binds to the 5HT1A receptor there is an antidepressant and anxiolytic effect; when it binds to 5HT2A/C receptor there is an effect on sexual dysfunction. Multimodal antidepressants directly target specific serotonin receptors and inhibit reuptake of serotonin. Vilazodone is an example of a multimodal antidepressant, which targets a specific receptor (5HT1A). Still, vilazodone is not as superior as it was expected to be, compared to other antidepressants [82, 84]. Vortioxetine is more promising since it shows superior efficacy compared to the other antidepressants in trials. Vortioxetine is an agonist of 5HT1A, (partial) antagonist of

other receptors, and a potent serotonin reuptake inhibitor. Besides the antidepressant effect, it also improves cognitive function [82, 84].

3.2. *KETAMINE*

Novel treatments that target outside of the monoaminergic system are ketamine (targeting the glutamate system through N-methyl-aspartate (NMDA) receptor antagonism), and agomelatin (a melatonin receptor agonist) [82]. Agomelatin is a melatonin agonist and a selective serotonin antagonist. For antidepressant effect, both actions are necessary. Agomelatin showed good antidepressant effect for people with seasonal affective disorder [85].

Ketamine is used in many clinical studies for treatment-resistant patients who fail to respond to SSRIs. Ketamine showed good results, with a response rate between 40 and 90% [85]. Intravenous infusion of ketamine produces a rapid and prolonged effect within a few hours of administration. It is accompanied by psychotomimetic effects, which subside within 2 h. The effect of a single intravenous insertion lasts 2–14 days and it has an anti-suicide effect [83]. Ketamine is restricted for routine clinical use due to its side effects – dissociative effects, changes in sensory perception, intravenous administration, and risk of abuse [86]. Ketamine is a mixture of two enantiomers, S-ketamine and R-ketamine. In the past few years, esketamine (S-ketamine) has been studied as a better option than ketamine because of its easier administration. Esketamine can be inserted intranasally and is therefore easier for at home administration. Recently, researchers investigated also R-ketamine. Preclinical and clinical studies on intravenously infused R-ketamine elicit a fast and sustained antidepressant state, without psychotic symptoms [87].

3.2.1. KETAMINE'S ACTION

Ketamine affects the glutamate system. Glutamate is an excitatory neurotransmitter and is involved in neurodevelopment, neurocognitive (memory learning) function, and neuroplasticity (neurogenesis, neuronal growth and remodeling, maintenance, and

synaptic plasticity). Dysregulation of neuroplasticity can contribute to MDD and other neuropsychiatric conditions. The majority of neurons use glutamate as a neurotransmitter. Two types of glutamate receptors (ionotropic or metabotropic glutamate receptors) are categorized into four major classes: α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors (AMPAR), NMDA receptors (NMDAR), kainate receptors and metabotropic glutamate receptors [88]. NMDARs are located at the postsynaptic and presynaptic side of glutamatergic synapses in the central nervous system [89]. In *post mortem* brains of MDD patients, many studies have revealed alteration in NMDAR. Several changes were discovered, such as NMDAR dysfunction (reduced glutamate recognition and allosteric regulation) and altered expression of NMDAR subunits. The latter might be manifested by altered glutamatergic input and abnormal glutamate neurotransmission [88].

There are several mechanisms of ketamine action, which may act complementary. Ketamine can bind to NMDAR on presynaptic or postsynaptic glutamatergic neuron, and on GABAergic interneurons. Binding leads to blockade and inhibition of NMDAR. For the antidepressant effects of ketamine, cascade of actions happen – Ɣ-aminobutyric acid ($GABA_A$) decrease, glutamate release, AMPAR activation, BDNF release, tropomyosin receptor kinase B (TrkB) activation, and mammalian target of rapamycin complex 1 (mTORC1) activation. The result is an acute change in synaptic plasticity and sustained strengthening of excitatory synapses [86]. The process of synaptogenesis is activated and further probably affects cognition, mood, and thought patterns [90].

3.3. *HISTONE DEACETYLASE INHIBITORS*

Decreased acetylation is associated with depressive state and because of that, histone deacetylases (HDACs) (as erasers of acetylation) might become a novel treatment target [10]. HDACs, "erasers" of histone acetylation, are classified into two categories - the zinc-dependent, and nicotinamide-adenine-dinucleotide (NAD)–dependent sirtuins (Table 5) [91].

HDACs I, II, and IV are expressed in brain, primarily in neurons. Class I and II regulate histone deacetylation at most genes, class III deacetylate nuclear and cytoplasmic substrates beside histones [92]. The balance between HAT and HDAC activity determines the (de)condensation status of the chromatin and gene transcription [10].

Histone deacetylase inhibitors (HDACis) are potent to specific classes of HDACs. The US Food and Drug Administration has approved a few HDACis (vorinostat (SAHA), belinostat, panobinostat, and romidepsin) for treatment of some types of cancers. Many preclinical studies on mice showed an antidepressant effect of HDACis by reversing acetylated state. Moreover, HDACis also promote neuronal rewiring and recovery of motor functions after traumatic brain injury. Use in clinical practice is limited due to severe side effects including thrombocytopenia and neutropenia [93].

4. DEPRESSIVE DISORDER ASSOCIATED GENES AND CLASSICAL ANTIDEPRESSANT DRUGS

How different antidepressants affect depressive symptoms can be measured by a subject's phenotype (behavior for animals and psychiatric evaluation for humans). Epigenetic alterations might become one of the tools to check how well specific subjects respond to the antidepressant [94].

4.1. *BDNF AND DEPRESSIVE DISORDER*

One of the most studied genes of depressive disorder is *BDNF*. BDNF is one of the most important neutrophins. The human *BDNF* gene contains nine exons (I–IX), each regulated by its own promoter. All the different transcripts are translated into an identical BDNF protein [52]. It is highly expressed in the central nervous system [95] and plays an important role in proper brain development and functioning, including neuronal proliferation, migration, differentiation, and survival [52]. BDNF binds to p75 neurotrophin receptor (p75NTR) and TrkB [95]. In many studies, exon I and IV showed alteration in expression levels in depressed subjects. Splice variant TrkB.T1 is an astrocytic variant and has gained a lot of interest in the study of the depressive state [10].

Two single nucleotide polymorphisms: Val66Met and *BE5.2* of *BDNF* reduce BDNF release. In addition, studies show also significant effects of epigenetic changes on the depressive state [52]. Treatment with SSRIs and HDACis antidepressants increases levels of BDNF in peripheral tissues. If BDNF does not increase early after administration, this predicts non-response to antidepressants [96].

4.1.1. BDNF AND ANTIDEPRESSANTS

4.1.1.1. HUMAN STUDIES

The studies on DNA methylation and antidepressant effect in general include a rather low number of subjects, but several different antidepressants.

Two studies analyzed H3K27me3 modification and both reported decreased H3K27me3 in patients with MDD. Chen *et al.* (2011) performed a study on Caucasians (French Canadian origin), 9 control subjects, 11 MDD subjects without a history of antidepressant use, and 7 MDD subjects who used antidepressants. All MDD subjects died due to suicide. Several different antidepressants were administered: fluoxetine (n = 1), venlafaxine (n = 2), clomipramine (n = 1), amitriptyline (n = 1), citalopram (n = 1), and doxepin (n = 1). Analysis of the epigenetic modification H3K27me3 in brain tissue from Brodmann area 10 between the control group and the non-medicated MDD group showed no differences. Subjects with a history of antidepressant use showed an increase in *BDNF* IV expression, but not *BDNF* I, II, and III expression and a decreased level of H3K27me3 at the *BDNF* IV promoter [97]. Lopez *et al.* (2013) investigated 25 MDD patients (13 females and 12 males) whose blood levels of total BDNF and H3K27me3 were measured before antidepressant treatment and after 8 wk of citalopram administration. After treatment, there was an elevation of peripheral *BDNF* mRNA in patients responsive to antidepressant treatment and a decrease in H3K27me3 Level at promoter IV of the *BDNF* gene [98].

Increase of *BDNF* DNA methylation level after antidepressant administration was shown in three studies. Carlberg *et al.* (2014) studied *BDNF* methylation on PBMC of 207 MDD patients, and 278 control subjects from Vienna (Austria). From 207 MDD

patients, 140 subjects were treated with antidepressant medication and 25 subjects were not. There was alteration in DNA methylation at the *BDNF* exon I promoter. After antidepressant administration, there was an increase in methylation in MDD patients, compared with patients without antidepressants medication and healthy controls [43]. D'Addario *et al.* (2013) report that there was an increase in DNA methylation at *BDNF* promoter, in 41 MDD patients with stable pharmacological treatment, in comparison to 44 healthy control subjects. In addition, there was a significant reduction in expressed *BDNF* from PBMCs in MDD patients than in control group. Patients who took only SSRI or SNRI antidepressant had a higher methylation level of *BDNF* promoter than patients who received antidepressants and mood stabilizers [99]. In a study of Wang *et al.* (2018), 85 Chinese Han patients with MDD (females and males) were treated with escitalopram. Blood samples were tested for DNA methylation in the *BDNF* region. DNA methylation before treatment was significantly lower than after 8 wk of treatment. A difference was seen also between remitted and non-remitted patients. Patients with remission had higher DNA methylation than non-remitters [16].

Two studies included analysis of patients who responded and those who did not. In both, higher methylation level was an important contributor to treatment response. Hsieh *et al.* (2019) included 39 patients with MDD (females and males) and 62 healthy controls (females and males). Higher methylation level was detected at CpG site 217, and lower methylation level at CpG sites 327 and 362 in the *BDNF* exon IX promoter in MDD patients compared to controls. After drug administration (SSRIs; fluoxetine, paroxetine, and escitalopram), 25 patients who responded to SSRIs had a higher methylation level at CpG sites 24 and 324 than patients who did not respond (n = 11). Methylation analysis results also show consistent results of BDNF protein level and mRNA level in peripheral blood [46]. The study of Tadić *et al.* (2014) included 46 MDD patients (females and males) with different monoaminergic antidepressants prescribed: escitalopram (n = 5), fluoxetine (n = 2), sertraline (n = 6), venlafaxine (n = 19), duloxetine (n = 2), mirtazapine (n = 6), amitriptyline (n = 1), clomipramine (n = 3), trimipramine (n = 1), or tranylcypromine (n = 1). Although different antidepressants were used, the

main observation of the study was the response or non-response to the antidepressant treatment. From 13 CpG sites checked for methylation status on blood samples within the *BDNF* IV promoter, one stood out: antidepressant non-responders had lower methylation at CpG position –87 (relative to the first nucleotide of exon IV). There were no other DNA methylation changes after treatment [94].

4.1.1.2. ANIMAL STUDIES

In animal models, it has been shown that histone tail modifications significantly affect gene expression and that they are also changed after antidepressant administration.

In the study by Park *et al.*, (2018) male Sprague-Dawley rat pups were separated from mothers during early life. Maternal separation evoked a decrease of exon I mRNA *Bdnf*, H3 acetylation (ac) levels, and an increase in *Dnmt1* and *Dnmt3a* mRNA level in the hippocampus. After 3 wk of escitalopram administration in adult rats subjected to maternal separation, the result was an increase in BDNF protein, exon I mRNA, levels of H3ac, and a decrease in *Mecp2*, *Dnmt1*, and *Dnmt3a* mRNA levels [34]. Xu *et al.* (2018), showed that mice stressed in the adolescent period show epigenetic changes also in adult life. Stress in tested male C57BL/6J mice were induced by confrontation of aggressor mice CD1. The expression level of total *Bdnf* and *Bdnf* IV mRNA were decreased in the medial prefrontal cortex (the same results were observed also in the hippocampus). *Bdnf* I and VI mRNA levels changed over time in medial prefrontal cortex. Adult mice had upregulated H3K9me2 in a region downstream of the promoter of the gene *Bdnf* IV but there were no differences in H3K4me3, H3K9ac, and H3K4ac. Tranylcypromine administration reversed this change and increased levels of H3K4me3. Tranylcypromine is a non-selective MAOI [100].

Tsankova *et al.* (2006) showed decreased expression of *Bdnf* III and IV, which manifested also in the total level of *Bdnf* mRNA in the hippocampus in chronically defeated BL6/C57 mice. Changes in *Bdnf* III and IV expression persist a month after cessation of the chronic defeat stress. On the promoter of *Bdnf* III and *Bdnf* IV there was an increase of H3K27me2, but not H3K9me2. Chronic imipramine (a TCA)

administration reversed changes of *Bdnf* expression but did not reverse H3K27me2 to the base level. After chronic social defeat stress and imipramine administration, H3 was hyperacetylated (H3K9/14ac) at the promoter *Bdnf* III and IV, which affects mRNA expression. Furthermore, H3K4me2 was similarly enriched in the *Bdnf* III promoter and correlated with transcriptional activation. There were no changes in H4ac. There was a decrease also in *Hdac5* mRNA level, but only on chronically stressed mice treated with chronic imipramine. Acute imipramine did not influence *Hdac* level [71].

4.2. *SLC6A4 AND DEPRESSIVE DISORDER*

Solute carrier family 6 member 4 (*SLC6A4*) is a gene that codes for serotonin transporter (5-HTT). The protein's name comes from the name of the monoamine neurotransmitter serotonin (5-HT) that binds to it. The gene *SLC6A4* was associated with the protein later. 5-HTT is an integral membrane protein that transports serotonin from synapse to presynaptic neuron. Besides involvement in regulation of the serotonergic system, *SLC6A4* also acts as an important element of stress susceptibility. Serotonin transporter linked promoter region (5-HTTLPR) polymorphism at gene *SLC6A4* has 2 variants, a short allele (S) and a long allele (L). The S allele results in lower gene transcription and is therefore associated with a depressive state [101]. In addition, there are also several epigenetic studies explaining its dysfunction. Some studies have shown how treatment with classical antidepressants affects epigenetic changes of the *SLC6A4* gene. Therefore, *SLC6A4* is a key target for antidepressant treatment research.

4.2.1. *SLC6A4* AND ANTIDEPRESSANTS

4.2.1.1. HUMAN STUDIES

There is a difference in the response to antidepressants seen when analyzing DNA methylation in *SLC6A4* gene. Two studies reported higher methylation status after antidepressant administration and one lower methylation status.

Booij *et al.* (2015) included in their study 33 MDD patients (females and males). MDD patients who were taking SSRIs had higher methylation levels at CpG 11 and 12 within

the regulatory region upstream of the promoter of the *SLC6A4* than patients who did not use antidepressants (n = 36). Research was done on whole blood samples. There was no association between mRNA expression and DNA methylation [102]. In the study of Okada *et al.*, (2014) peripheral blood was taken from 50 Japanese MDD patients (females and males) before and after antidepressant treatment. Different antidepressants (paroxetine, fluvoxamine, milnacipran) were used in this study. There were no differences in DNA methylation of *SLC6A4* exon I promoter between the healthy control group (n = 50) and patients without antidepressant administration. There was a significant increase in methylation at the CpG 3 site after 6 wk of antidepressant treatment [103].

Domschke *et al.* (2014) included 61 Caucasian MDD patients who were tested for changes in DNA methylation from blood cells. Administration of escitalopram was evaluated 6 wk after treatment. There was lower average methylation in the transcriptional control region upstream of exon 1A of *SLC6A4* gene. The CpG 2 site specifically stands out from these results [104].

CONCLUSION

Depressive disorder is affected by dysregulation of many different genes, each contributing a small effect. All hypotheses of depressive disorder involve a variety of changes that can occur in a depressive state. These are a consequence of gene variations or epigenetic changes that affect DNA transcription and/or mRNA translation resulting in imbalanced protein levels regulating the processes in the CNS. With the development of technologies and new knowledge, epigenetic research has become accessible for investigation in the field of psychiatry. Among candidate genes particular interest was placed on *BDNF, NR3C1,* and *SLC6A4*, as their roles in CNS regulation has been identified in association with response to external stress stimuli and mood regulation. Although the research has been fairly extensive, we still cannot identify a reliable biomarker or a set of them, either proteomic or (epi)genetic, to be used in a clinical setting.

However, in many studies scientists discuss the importance of epigenetic factors (DNA methylation and histone modifications) as playing a key role in predicting antidepressant response. The aggregation of subthreshold levels of the epigenetic changes in several different genes might show alterations caused by depressive state. It appears that to date we have uncovered a few pieces of the jigsaw but that more studies are needed for understanding this complex disorder. For example, it has been determined that classical antidepressants change the epigenome and it has been proposed that this effect might be an important contributor to treatment. These results have triggered further investigation of drugs targeting epigenetic modifiers (HDACs, HMTs). HDACis seem to be promising drugs but until now, there are no HDACis used for depression treatment.

Further research in clinical settings will be important to determine which epigenetic markers are informative for treatment response prediction, and which markers actually change as a response to treatment. Although the field of pharmacoepigenetics is only starting to develop, we can see some potential genes, which we can expect to become biomarkers with clinical value. With rapid technological advancement, enabling determination of markers from multi-omic data with the use of artificial intelligence, and carefully designed studies in the growing field of psychiatry, we could expect to obtain relevant biomarkers that could be used by clinicians as meaningful guidance in addition to clinical interview in the future. With the development of the field of pharmacoepigenetics, it will be possible to move towards personalized treatments, where combinations of genetic and environmental factors will need to be incorporated in treatment selection.

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