Novel approaches in schizophrenia-from risk factors and hypotheses to novel drug targets

Matej Ľupták, Danica Michaličková, Zdeněk Fišar, Eva Kitzlerová, Jana Hroudová

ORCID number: Matej Ľupták 0000-0001-8002-2477; Danica Michaličková 0000-0002-9690-9846; Zdeněk Fišar 0000-0002-3389-0287; Eva Kitzlerová 0000-0002-2989-0030; Jana Hroudová 0000-0001-5448-1717.

Matej Ľupták, Danica Michaličková, Jana Hroudová, Institute of Pharmacology, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague 12800, Czech Republic

Zdeněk Fišar, Eva Kitzlerová, Jana Hroudová, Department of Psychiatry, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague 12000, Czech Republic

Corresponding author: Jana Hroudová, MD, PharmD, PhD, Associate Professor, Department of Psychiatry, First Faculty of Medicine, Charles University and General University Hospital in Prague, Albertov 4, Prague 12000, Czech Republic. hroudova.jana@gmail.com

Abstract

Schizophrenia is a severe psychiatric disorder characterized by emotional, behavioral and cognitive disturbances, and the treatment of schizophrenia is often complicated by noncompliance and pharmacoresistance. The search for the pathophysiological mechanisms underlying schizophrenia has resulted in the proposal of several hypotheses to explain the impacts of environmental, genetic, neurodevelopmental, immune and inflammatory factors on disease onset and progression. This review discusses the newest insights into the pathophysiology of and risk factors for schizophrenia and notes novel approaches in antipsychotic treatment and potential diagnostic and theranostic biomarkers. The current hypotheses focusing on neuromediators (dopamine, glutamate, and serotonin), neuroinflammation, the cannabinoid hypothesis, the gut-brain axis model, and oxidative stress are summarized. Key genetic features, including small nucleotide polymorphisms, copy number variations, microdeletions, mutations and epigenetic changes, are highlighted. Current pharmacotherapy of schizophrenia relies mostly on dopaminergic and serotonergic antagonists/partial agonists, but new findings in the pathophysiology of schizophrenia have allowed the expansion of novel approaches in pharmacotherapy and the establishment of more reliable biomarkers. Substances with promising results in preclinical and clinical studies include lumateperone, pimavanserin, xanomeline, roluperidone, agonists of trace amine-associated receptor 1, inhibitors of glycine transporters, AMPA allosteric modulators, $mGLUR_{2-3}$ agonists, D-amino acid oxidase inhibitors and cannabidiol. The use of anti-inflammatory agents as an add-on therapy is mentioned.

Key Words: Schizophrenia; Immune system; Inflammation; Genetics; Novel antipsy-

chotics; Add-on therapy

Core Tip: This review discusses the newest insights in the pathophysiology and risk factors for schizophrenia and points out the novel approaches of antipsychotic treatment, potential diagnostic and theranostic biomarkers. The hypotheses focusing on neuromediators (dopamine, glutamate, serotonin), neuroinflammation, cannabinoid hypothesis, gut brain axis model, and other currently discussed hypotheses are summarized. Key genetic features and new findings in the pathophysiology of schizophrenia support the expansion of novel approaches in pharmacotherapy and development of non-dopaminergic antipsychotics.

INTRODUCTION

Schizophrenia is a serious mental disorder with a lifelong prevalence of approximately 1% and a peak age of onset of 23-34 years in women and the early twenties in men. It is a very complex syndrome that involves widespread brain multi-dysconnectivity. It is characterized by cognitive, behavioral and emotional dysfunctions. To fulfil the diagnostic criteria for schizophrenia, patients must exhibit two or more negative, disorganized or positive symptoms that persist for a minimum of six months, and at least one symptom must be disorganized speech or a positive symptom[1]. Positive symptoms include hallucinations and delusions; negative symptoms are characterized by deficits in normal behavior, including asociality, alogia, anhedonia, blunted affect, and avolition[2]. There is a wide range of treatment possibilities; however, the effectiveness and/or adverse effects of antipsychotics with different pharmacological profiles vary. Successful treatment of schizophrenia is complicated by noncompliance and pharmacoresistance. The prevalence of pharmacoresistant schizophrenia is estimated to range from 12.9% to 48%[3]. It has been estimated that approximately 20% of patients with schizophrenia receive combination treatment and/or antipsychotic polypharmacy[4]. Augmentation strategies used in clinical practice include the addition of another antipsychotic, concurrent administration of benzo-diazepines or mood stabilizers, repetitive transcranial magnetic stimulation or electroconvulsive therapy.

The pathophysiological mechanism of the onset and progression of schizophrenia, the diagnostic neuropathology, and sensitive and specific biomarkers have not yet been identified. Several different hypotheses have been proposed to explain the neuropathology of schizophrenia that focus on environmental, genetic, neurodevelopmental, and neurochemical effects. Research and development in imaging methods and in preclinical studies have led to the improvement of these theories. Positron emission tomography (PET) and single photon emission computer tomography enable *in vivo* quantification of dopaminergic functions in the brain and dopamine synthesis, release, and availability in postsynaptic dopaminergic neurons and transporters.

The targeting of existing and new drugs is based primarily on the dopamine and glutamate hypotheses of schizophrenia. All current antipsychotics modulate the function of the dopamine D_2 receptor. A nonlinear relationship between D_2 receptor occupancy, clinical response, and adverse effects of current antipsychotics was found. A small response to antipsychotic treatment appears at 50% dopamine receptor occupancy; as receptor occupancy increases, the response increases as well as the risk of extrapyramidal adverse effects[5]. These findings were proven in a double-blind study in patients with first episode schizophrenia; 65% occupancy of D_2 receptors was the borderline between responders and nonresponders[6]. Recently, research has focused on the prodromal phase of schizophrenia. Dopamine synthesis increases

during the acute phase of the disease. Stress and other risk factors affect the dopamine systems, leading to their dysregulation and consequently to the development of psychotic disorder[7].

Excitatory glutamate neurotransmission occurs though ionotropic and metabotropic glutamate receptors. The glutamate hypothesis of schizophrenia is based on the dysfunction of the N-methyl-D-aspartate (NMDA) receptor. Currently, the effects of ketamine on brain function in healthy volunteers are being examined; studies are focused on glutamate concentrations in the brains of patients with prodromal symptoms during the first episode and other episodes of schizophrenia. Dysfunction of both NMDA receptors and presynaptic synthesis of dopamine has been implicated in the clinical symptoms of schizophrenia. Relationships between presynaptic dopamine dysfunction and positive symptoms and between glutamate dysfunction and negative and cognitive symptoms are expected[7].

To improve the diagnosis of schizophrenia, predict the therapeutic response to antipsychotics, develop new drugs, and personalize treatment, it is necessary to identify new specific and sensitive biomarkers of the disease[8]. Blood-based biomarkers are regarded as a feasible option because the dysregulation of gene expression, epigenetic patterns, protein quantities, and metabolic and inflammatory molecules in peripheral blood have been shown to have distinct patterns in patients with schizophrenia[8]. The aim of this review is to provide the newest insights into the pathophysiology and risk factors of schizophrenia and novel approaches to antipsychotic treatment.

GENETICS AND SCHIZOPHRENIA

Schizophrenia is closely linked to genetic factors, including small nucleotide polymorphisms (SNPs), copy number variations and changes in gene expression. Combinations of different pathogenic mechanisms, including aberrant DNA methylation, altered histone code, dysregulated long noncoding RNA (lncRNA)-dependent tethering of epigenetic complexes to DNA, aberrant polyadenylation of pre-mRNAs, and mis-splicing, have been reported to play a role in schizophrenia development[9]. The hereditary burden of schizophrenia is estimated to be approximately 80%. Genome-wide association studies (GWAS) have identified more than 100 loci, many of which contain multiple genes that are significantly associated with schizophrenia. The assessment of polygenic scores allows us to determine the risk of schizophrenia based on the number of risk alleles weighted by the odds ratio of each allele.

DNA methylation, an epigenetic process that produces 5-methylcytosine, is mediated by DNA methyltransferases and has a key role in several processes, such as imprinting, inactivation of the X-chromosome, silencing of transposons or regulation of genomic stability and chromatin structure. Schizophrenia is linked to pathophysiological DNA methylation of several genes, including those encoding reelin, catechol-O-methyltransferase (COMT), monoamine oxidase A, serotonin receptor 2A, the transcription factor SOX-10, and others. Unfortunately, no schizophrenia-specific "methylation panel" has been proposed, and it has not yet been clarified whether these changes represent causes or consequences of schizophrenia development[9].

Approximately 70%-80% of the genome is transcribed into noncoding transcripts, and the majority of schizophrenia-associated risk variants have been found in noncoding regions. LncRNAs can interact with DNA, RNA, and proteins, influencing transcription and posttranscriptional processes such as splicing, polyadenylation and/or regulation of transcript stability. MicroRNAs (miRNAs) are small noncoding RNAs that regulate more than 50% of protein-coding genes by acting as promoter or enhancer elements; miRNAs might participate in histone, DNA, or chromatin methylation and modification. Both lncRNAs and miRNAs can be affected by different genetic variants, especially SNPs, which could increase the risk of schizophrenia onset [9,10].

Microdeletions in chromosomal region 22q11.2 are one of the well-established genetic risk factors for schizophrenia and increase the risk of schizophrenia development to 30%-40%[11,12]. COMT is a major dopamine catabolic enzyme, and its gene is located in this microdeletion region. In addition, a functional COMT polymorphism [valine/methionine (VAL/MET) substitution at codon 108] causes differences in its catabolic activity, dopamine baselines and stress-induced cortical dopamine release[13]. The MET version of the allele is not as stable as the VAL version, causing decreased COMT activity and an increase in dopamine levels,

especially in the prefrontal cortex[14].

The major histocompatibility complex (MHC) locus located on chromosome 6, which contains genes encoding proteins essential for adaptive immunity, has one of the strongest links to schizophrenia. Specifically, there was increased expression of complement component 4A (C4A). Sex differences in the *C4* gene could explain the higher male susceptibility to schizophrenia. Schizophrenia patients with higher C4 Levels were characterized as low responders or nonresponders to antipsychotic medication. The expression of the genes encoding CSMD1 and CSMD2, which are important regulators of C4, has been found to be decreased in schizophrenia and connected with reduced cognition and executive function[15,16]. Other immune receptors, including toll-like receptors (TLRs), which take part in microbe-derived molecular signaling, early brain development, synaptic plasticity, and neurogenesis, have been identified as schizophrenia susceptibility genes by GWAS. Both TLR2 and TLR4 were altered in the blood and brain tissue of schizophrenic patients[15].

The genes encoding for neuregulin 1 and neuregulin 3 are candidate schizophrenia genes and produce several possible proteins that influence neuronal differentiation and migration. The role of neuregulin 1 in schizophrenia is not well known, but increased neuregulin 1 signaling led to NMDA receptor hypofunction (in accordance with the glutamate hypofunction hypothesis of schizophrenia). There is no evidence of hyperexpression of neuregulin 1 itself; however, the possibility of mutations causing the production of proteins with enhanced function is still present[14]. Neuregulin 3 is a ligand for receptor tyrosine-protein kinase erbB-4 (ErbB4), and different genetic variants of the neuregulin 3 gene, especially the rs10748842 allele, are connected with higher schizophrenia risk and cognitive impairment[17]. Mutant mice with ErbB4 deletion from fast-spiking interneurons exhibited increased cortical excitability and oscillatory activity and desynchronized neurons in the cortical region, probably caused by the disruption of the proper function of inhibitory GABA circuits in interneurons. These functional changes manifested in increased locomotion, impaired social and emotional behavior and cognitive dysfunction, which are common symptoms of schizophrenia[18,19].

The gene encoding dystrobrevin-binding protein 1 (also referred to as dysbindin or DTNBP1) has been identified as a gene associated with schizophrenia; however, no specific protein coding mutations increasing the risk of schizophrenia have been identified. Decreased dysbindin expression has been found in the brains of schizophrenia patients, and dysbindin risk haplotypes have been associated with increased negative symptomatology in schizophrenia[14].

The gene most closely linked to schizophrenia is probably the gene encoding the protein disrupted in schizophrenia 1 (DISC1), which has been associated with schizophrenia mainly due to a mutation causing a translocation between exons 8 and 9. The molecular mechanism of this mutation is not known, but the shortened mutant DISC1 protein is incapable of dimerization, and it may interact with other proteins. DISC1 expression is especially high during neurodevelopment in the late fetal and early postnatal phases, during which it participates in hippocampal development; however, DISC1 expression continues into adulthood. In schizophrenia pathophysiology, not only DISC1 itself but also its binding and interaction partners, such as microtubule-associated protein 1A, glycogen synthase kinase 3β, phosphodiesterase 4 and fasciculation and elongation protein zeta-1, might play a crucial role[14,20-22].

The synaptosomal-associated protein SNAP25 is involved in synaptic vesicle docking and fusion during neurotransmitter release. The promoter variant rs6039769 with the C risk allele caused an increase in SNAP25 expression, probably causing a larger amygdala and greater functional connectivity between the amygdala and ventromedial prefrontal cortex in male schizophrenic patients. This modulation in the plasticity of the prefrontal cortex-limbic connection caused higher schizophrenia risk[23].

The gene encoding transcription factor 4 (TCF4) is another GWAS-confirmed gene associated with schizophrenia. It encodes class I basic helix-loop-helix transcription factors and plays a role in neurodevelopment. Altered expression of TCF4 in the forebrain of a transgenic mouse caused altered cognition and long-term depression increased the density of immature spines[24]. Many other genes have been associated with schizophrenia diagnosis and have been reported in the literature[25-27]; description of all schizophrenia-linked genes is beyond the scope of this review.

TRIGGERS AND RISK FACTORS

Environmental model of schizophrenia

The onset and severity of schizophrenia are always modulated by an interplay between genetic and environmental risk factors[28]. Many epidemiological studies have investigated putative environmental risk factors for schizophrenia and peripheral biomarkers of the disease[29,30]. According to an umbrella review of meta-analyses on risk factors and peripheral biomarkers for schizophrenia[31], history of obstetric complications, exposure to stressful events in adulthood or to childhood adversity, cannabis use, and serum folate level showed robust evidence of association with schizophrenia.

The prenatal and perinatal periods are characterized by great neural vulnerability to environmental insults. A recent systematic review and meta-analysis of 152 studies revealed numerous prenatal and perinatal risk factors, calculated with odds ratios (ORs), that were statistically linked to schizophrenia onset[32]. The biggest risk factors for schizophrenia onset are any familial psychopathology, especially maternal psychosis (OR: 7.61). Maternal infections (herpes simplex 2, OR: 1.35; unspecified infections, OR: 1.27), a suboptimal number of antenatal care visits (OR: 1.83), or maternal stress (OR: 2.4) can lead to a higher prevalence of obstetric events (OR: 1.52), which are the longest-studied and best replicated environmental risk factors for schizophrenia. Significantly relevant obstetric events include maternal hypertension (OR: 1.4), hypoxia (OR: 1.63), premature rupture of membranes (OR: 2.29) and polyhydramniosis (OR: 3.05). There is experimental and clinical evidence showing significant risks of prenatal infection and inflammation for the later development of schizophrenia. According to the viral model of schizophrenia, prenatal viral and bacterial infections and inflammation play an important role in the development of schizophrenia[33].

Nutritional deficits or famine in pregnancy (OR: 1.4) or more than two pregnancies (OR: 1.3) can be associated with reduced allocation or lower socioeconomic status. Another risk factor is congenital malformations (OR: 2.35)[32]. The most relevant postnatal environmental risk factors are childhood trauma (OR: 2.87), urban living (OR: 2.19), migration (2.10) and cannabis use (OR: 5.17), and these stress factors lead to the sensitization of the subcortical dopamine system[11].

Many genes relevant to schizophrenia, especially immune genes, can be altered by air pollution. Children with greater exposure to traffic-related air pollution had increased levels of proinflammatory cytokines. It is not yet clear whether air pollution itself causes brain changes or inflammatory changes caused by air pollution contribute to the pathology of schizophrenia[15].

A study of the roles of both genetic and environmental influences on the development of schizophrenia is necessary to explain the fact that in approximately 40%-55% of cases, monozygotic twins do not share a diagnosis of schizophrenia[34]. How genetic and environmental factors interact and the related neurobiological mechanisms that induce schizophrenia are not yet known.

Stress and schizophrenia

The vulnerability-stress model of schizophrenia proposes that when stress exceeds the vulnerability threshold, an individual is likely to develop a psychotic episode[35]. Stressful life events or psychological stress, especially in key periods of neurodevelopment, increase the risk of schizophrenia. These events include physical or mental abuse, lower socioeconomic status, urban environment, and neglect. The molecular mechanisms connecting these stressful situations with schizophrenia remain unclear. It was proven that patients with schizophrenia have altered cortisol function, and its release is linked to the inflammatory response rather than the anti-inflammatory response. Observation of HPA axis activation and cortisol release as a result of stress events in individuals with schizophrenia has produced inconsistent results; however, HPA axis dysfunction has been observed[15].

Neurons are extremely sensitive to redox imbalance during neurodevelopment and differentiation, mostly because of their high lipid content and metabolic rate. Increased reactive oxygen species (ROS) production and/or lowered antioxidant system capacity are considered risk factors for schizophrenia development. Increased protein and lipid oxidation and lowered levels of vitamin C and E, catalase, glutathione peroxidase and superoxide dismutase have been detected in schizophrenia patients. A study revealed that participants with low vitamin D3 Levels in the first year of life were at two times higher risk of schizophrenia. Glutamate-cysteine ligase is the rate-limiting biosynthetic enzyme of glutathione. One allelic variant of the GCLC gene is linked to the decreased

activity of glutamate-cysteine ligase and schizophrenia. NMDA receptors are regulated by the redox state, and glutathione deficiency induces NMDA receptor hypofunction, which leads to cortical oxidative stress and glutathione decrease[36,37].

Neurodevelopmental model

The neurodevelopmental model postulates that an increased risk of schizophrenia development is the result of abnormal brain neurodevelopment caused by genetic and environmental factors years before the onset of the disease[38]. The hypothesis is based on clinical, epidemiological, brain imaging, and genetic studies[39,40]. Schizophrenia is supposed to be a developmental disorder of the brain, and changes in brain neuroplasticity are involved. The disconnection hypothesis[41] presumes the involvement of abnormal synaptic connections in the pathophysiology of schizophrenia. Impaired synaptic plasticity and synaptic efficacy, mainly in areas of the brain responsible for learning, memory, and emotion, participate in schizophrenia pathophysiology. Modulation of ascending neurotransmitter systems and consolidation of synaptic connections during learning are implicated in schizophrenia neuropsychology, especially in impaired adaptive behavior and disintegrative aspects [42].

The unitary hypothesis of schizophrenia includes different types of pathophysiological models[43]; according to the hypothesis, early brain insults can lead to dysplasia of selective neural circuits, which is responsible for premorbid cognitive and psychosocial dysfunction in patients with schizophrenia. The onset of psychosis in adolescence may be associated with the excessive elimination of synapses with subsequent dopaminergic over activity. Decreased glutamatergic neurotransmission can predispose the brain to these processes. After the onset of the disease, these neurochemical changes can lead to further neurodegenerative processes. Brain plasticity includes both synaptic and nonsynaptic plasticity. The dysplastic model of schizophrenia suggests that impaired neuroplasticity during brain development may underlie cognitive and deficit symptoms and may lead to reorganization in other neuronal circuits, which may lead to affective and psychotic symptoms[44].

The multiple hit theory of schizophrenia[45] presumes that schizophrenia can be conceptualized as a process involving multiple vulnerability factors across numerous neurodevelopmental windows in which some hits are applied prenatally, in childhood, in adolescence, and in adulthood. Thus, the development of schizophrenia is driven by the interactions between genetic vulnerability and environmental influences (including prenatal vitamin D, nutrition, childhood trauma, viral infections, IQ, smoking, cannabis use, and social defeat), which are cumulative and interact with each other. The neurodevelopmental phase involves changes in synaptogenesis, synaptic enhancement, and myelination, leading to excessive elimination of synapses and loss of neuroplasticity.

An extension of the neurodevelopmental model[46] proposes that the abnormal formation and maturation of connectomes (an extensive network of interconnected neurons) is central to the etiology of the disease. That is, abnormal anatomical architecture and functional organization of the connectome may be a final common pathway leading to the manifestation of schizophrenia symptoms. To further refine the developmental hypothesis of schizophrenia, progress in our understanding of brain connectivity during development and dysconnectivity resulting from genetic and environmental factors is necessary.

Oxidative stress and apoptosis

Disconnection of the prefrontal cortex in schizophrenic patients is associated with abnormalities in white matter, oligodendrocytes, and myelin. Myelin is produced by mature oligodendrocytes, and oligodendrocyte precursor cells are extremely sensitive to oxidative stress. A redox-induced prefrontal oligodendrocyte precursor cell-dysfunctioning hypothesis of cognitive symptomatology in schizophrenia has been proposed[47]. According to this hypothesis, the combination of environmental factors and genetic predisposition causes oxidative stress due to the excessive generation of ROS and reactive nitrogen species in oligodendrocyte precursor cells. Oxidative stress can lead to the downregulation of myelin-related genes in oligodendrocytes, decreased expression of myelin basic protein, and a reduced number of oligodendrocytes in the rat brain. During adolescence, a high concentration of ROS impairs the proliferation and differentiation of oligodendrocytes and their precursors. This leads to their dysfunction and hypomyelination and consequently to the disruption of connectivity in the prefrontal cortex. The resulting cognitive symptoms coincide with the onset of schizophrenia.

Additionally, oxidative stress induces dysregulation of the immune system and favors a proinflammatory response. Inflammation and disruption of immunity are other factors contributing to the pathogenesis of schizophrenia, as described in the following sections.

Mitochondria play a major role in cellular bioenergetics, oxidative stress, and apoptosis. According to the mitochondrial hypothesis of schizophrenia, mitochondrial dysfunction leads to distorted neuronal activity and plasticity, causing imbalanced brain circuitry and finally abnormal behavior[48]. Massive loss of white matter oligodendrocytes is a hallmark of schizophrenia. Therefore, it has been hypothesized that mitophagy is increased in oligodendrocytes in schizophrenia, which contributes to disease-related white matter neuropathology.

The intrinsic pathway of apoptosis is activated by intracellular signals generated during cellular stress and is triggered by the release of proapoptotic factors from mitochondria. Thus, consistent with the mitochondrial hypothesis, the apoptotic hypothesis postulates that apoptosis contributes to the pathophysiology of schizo-phrenia. The data indicate a dysregulation of apoptosis in several cortical areas in schizophrenia. The potential involvement of nonlethal localized apoptosis in the early stages of the disease is presumed[49].

NEUROCHEMICAL HYPOTHESES

Dopamine hypotheses

According to the classic (receptor) dopamine hypothesis of schizophrenia, psychotic symptoms are related to dopaminergic hyperactivity in the brain. Hyperactivity of dopaminergic systems during schizophrenia is the result of increased sensitivity and density of dopamine 2 (D_2) receptors. This increased activity can be localized in specific brain regions[50,51]. The dopamine hypothesis does not assume that dopamine hyperactivity fully explains schizophrenia. Over activation of D_2 receptors appears to be only one effect of the overall dysregulation of chemical synapses in this disease.

The modified dopamine hypothesis assumes that schizophrenia is characterized by abnormally low prefrontal dopamine activity (causing negative symptoms) that leads to excessive dopamine activity in mesolimbic dopamine neurons (causing positive symptoms). Thus, this hypothesis presumes the co-occurrence of high and low dopamine activity in different neuronal circuits, which could explain the concurrent presence of positive and negative symptoms[52].

The unifying dopamine hypothesis of schizophrenia, called "the final common pathway", proposes that multiple environmental, genetic, and other risk factors (such as stress, drugs, or frontotemporal dysfunction) interact and result in striatal dopamine dysregulation, which alters signal transmission and leads to psychosis[53]. This hypothesis combines dopamine dysfunction with other risk factors, including pregnancy and obstetric complications, stress and trauma, drug abuse, genetic predis-position and environment–gene interactions, with both increased presynaptic striatal dopaminergic function and other brain functions that underlie negative and cognitive symptoms.

A model has been presented of how genes and environmental factors may sensitize the dopamine system so that it is vulnerable to acute stress, leading to progressive dysregulation and the onset of psychosis[13]. The main steps of this model are as follows: genetic risk factors lead to impaired glutamatergic regulation, followed by increased striatal dopamine release, aberrant salience, and psychotic symptoms. Acute psychosocial stress can activate increased striatal dopamine release both directly and indirectly *via* blunted cortical dopamine release and impaired glutamatergic regulation. The dopaminergic system interacts also with muscarinic cholinergic system and closely related muscarinic hypothesis of schizophrenia.

Glutamate hypotheses

The glutamate hypothesis assumes that schizophrenia is caused by developmental abnormalities in glutamate synapse formation at specific sites, particularly at GABA interneurons in the cerebral cortex. These abnormalities may lead to subsequent excessive glutamate signaling to the ventral tegmental area (VTA), and excessive activation of this pathway may result in an excess of dopamine in the ventral striatum *via* the mesolimbic pathway[54]. The role of dysregulation of glutamatergic neurotrans -mission in the pathophysiology of schizophrenia is supported by evidence from genetics, pharmacological, postmortem, and brain imaging studies[55]. The conver-

gence of GABA impairment and glutamate neurotransmission in the dorsolateral prefrontal cortex could explain the impairment of certain cognitive functions in schizophrenia[56].

The NMDA receptor hypofunction hypothesis[57] assumes that genetic and other risk factors induce epigenetic alterations leading to NMDA receptor hypofunction in schizophrenia. NMDA receptor hypofunction induces a cascade of downstream disturbances in neuronal activity, calcium entry, and epigenetic machinery, leading to abnormal synaptic development and dopaminergic and GABAergic dysfunction. These changes in neurotransmission result in the cognitive and social deficits found in schizophrenia. According to this hypothesis, changes in the dopamine system are secondary to NMDA receptor hypofunction.

Antagonists of NMDA receptors (*e.g.*, phencyclidine) have been shown to cause symptoms similar to the positive and negative symptoms and cognitive defects in schizophrenia[58]. According to increasing evidence, deficits in NMDA transmission are linked to cognitive defects and negative symptomatology[59].

Serotonin hypothesis

There are 3 interconnected pathways hypothetically associated with hallucinations and delusions: (1) Dopamine hyperactivity at D_2 dopamine receptors in the mesolimbic pathway, which extends from the VTA to the ventral striatum; (2) NMDA receptor hypoactivity on GABAergic interneurons in the prefrontal cortex; and (3) Serotonin (5-HT) hyperactivity of 5-HT_{2A} receptors on glutamate neurons in the cerebral cortex. All 3 pathways can lead to hyperactivity of the mesolimbic dopamine pathway[54].

According to the serotonin hypothesis[60], the basic cause of schizophrenia is stress-induced serotonergic hyperfunction in the cerebral cortex, especially in the anterior cingulate cortex and the dorsolateral frontal lobe. The serotonin hypothesis assumes hyperfunction of 5-HT_{2A} receptors on glutamate neurons in the cerebral cortex. This overactivation of 5-HT_{2A} receptors may be due to an excess of serotonin, upregulation of 5-HT_{2A} receptors, or the effects of 5-HT_{2A} receptor agonists. Subsequent release of glutamate in the VTA may activate the mesolimbic pathway, resulting in excess dopamine in the ventral striatum[54].

Cannabinoid hypothesis

According to the cannabinoid hypothesis[61-63], changes in the endocannabinoid system may contribute to the pathogenesis of schizophrenia. This hypothesis proposes that increased activation of the endocannabinoid system through CB_1 receptors on GABAergic interneurons in the ventral tegmental area, basolateral amygdala, and medial prefrontal cortex may lead to a hyperdopaminergic and hypoglutamatergic status, which may cause schizophrenia. The hypothesis was supported by evidence that cannabis use in adolescence is an independent risk factor for schizophrenia development (OR: 3.90)[31] and by the confirmation of interactions between the cannabinoid and dopamine systems that may be related to the processes associated with drug addiction or schizophrenia[64].

BLOOD BRAIN BARRIER

The pathophysiology of many central nervous system (CNS) disorders, including schizophrenia, includes altered function of the blood brain barrier (BBB), as shown by evidence from neuroimaging studies, research of both cerebrospinal fluid (CSF) and blood-based biomarkers, and postmortem studies[65]. It remains to be elucidated whether BBB dysfunction is the cause or consequence of schizophrenia pathology[65]. P-glycoprotein is highly expressed in capillary endothelial cells. P-glycoprotein limits the accumulation of psychotropic drugs in the brain and is responsible for the efflux of drugs from the CNS by using the energy from ATP hydrolysis to return the compound to the bloodstream.

According to increasing evidence, malfunction of the BBB and microvascular abnormalities contribute to the pathophysiology of schizophrenia[66]. In a postmortem study, the cellular expression of ABCB1 [the gene encoding P-glycoprotein 1 (P-gp); multidrug resistance protein 1] was examined in patients with schizophrenia. A reduced density of P-gp-expressing neurons was found in the medial habenula of patients with schizophrenia compared to that of controls[66]. Furthermore, polymorphisms of *ABCB1* have been associated with changes in drug disposition and pharmacotherapy response[67].

P-gp is not the only efflux protein; multiple drug resistance (MRP) and breast cancer resistance protein (BCRP) also facilitate the efflux of ATP-dependent substrates. In addition to P-gp, BCRP (ABCG2) and the multidrug resistance proteins MRP1 (ABCC1) and MRP2 (ABCC2) are ATP-dependent efflux transporters present in the BBB[68].

Claudin-5 is a component of tight junctions and is specifically expressed in endothelial cells in the CNS. Polymorphism of claudin-5 has been associated with schizophrenia risk[65], and serum claudin-5 Levels were decreased in patients with schizophrenia[69]. The expression of claudin-5 in the hippocampus was reduced in patients with schizophrenia; the levels of claudin-5 correlated with the duration and age of onset of the disease[70]. The BBB impedes the transfer of many drugs, including antipsychotics, as well as some inflammatory molecules, such as cytokines, which play an important role in the pathophysiology of schizophrenia (see below).

Other explanations of pharmacoresistance in schizophrenia involve abnormal structure of the BBB, downregulation of genes encoding ion transport proteins, impaired immune system, dysfunctional glutamatergic transmission, *etc*.

NEUROINFLAMMATION

Based on the observation that schizophrenia is often associated with chronic neuroinflammation in the CNS[71], the vulnerability-stress model has been expanded into the vulnerability-stress-inflammation model[72], which suggests that the symptoms of schizophrenia are associated with specific changes in dopaminergic, serotonergic, noradrenergic, and glutamatergic neurotransmission following neuroinflammation and microglial activation. The hypothesis is based on the following findings: (1) Stress can increase proinflammatory cytokines and may even contribute to a chronic proinflammatory condition; (2) The typical changes in neurotransmission observed in schizophrenia have also been found in low-level neuroinflammation; (3) Risk factors for schizophrenia include genes whose expression promotes inflammation, environmental stressors, alterations of the immune system, severe infections, and autoimmune disorders; and (4) Antipsychotics also provide anti-inflammatory and immunomodulatory effects.

The vulnerability-stress-inflammation model of schizophrenia suggests that genetic vulnerability and infection during pregnancy may induce a proinflammatory response in the mother, causing deleterious effects on the neurodevelopment of the fetus and increasing the risk of developing schizophrenia. The development of the glutamate system may be disrupted. Re-exposure to stress at a later age may be followed by increased cytokine release, astrocyte activation or loss, dopaminergic hyperactivity, and NMDA antagonism, leading to the positive, negative, and cognitive symptoms of schizophrenia. Immune conditioning and immune sensitization can elicit a repeated response to stress leading to the symptoms of the disease.

Immunologic processes in schizophrenia

Currently, the immune system, immunological processes and inflammation are believed to have a significant role in the neurobiology of schizophrenia[73]. Evidence of immune etiology in schizophrenia comes from GWAS, where a significant association between schizophrenia and the expression of MHC, located on chromosome 6, was observed[16,74].

The relationship between neurotransmitters and mediators of the inflammatory response can be reciprocal; an immunoregulatory function of dopamine has been described. Increased expression of dopamine D_3 receptors and increased synthesis of interferon gamma (IFNγ) in lymphocytes were observed in nonmedicated patients suffering from schizophrenia[75]. An important finding from PET studies of inflammation with elevation of proinflammatory cytokines produced by microglia was an elevated microglial activity in subjects with subclinical symptoms and patients with schizophrenia[64].

Numerous studies have found immune dysregulation in patients with schizophrenia compared to healthy controls, and several meta-analyses have concluded that patients with schizophrenia exhibit signs of low-grade peripheral inflammation characterized by upregulated proinflammatory cytokines and acute phase proteins[76-78]. A recent meta-analysis of postmortem brain studies evaluating histological alterations of cellular composition and those assessing molecular parameters strengthened the immunologic hypothesis of schizophrenia[79]. The authors found significant increases in the density of microglia (especially in the temporal cortex) and

the overall expression of pro-inflammatory genes but no difference in the expression of anti-inflammatory genes in patients with schizophrenia compared to those in controls. However, it is important to note that these immunological alterations have been found only in a subgroup of patients with schizophrenia: approximately 40% of studied patients have exhibited some level of inflammation[80,81]. As schizophrenia is seen as a syndrome consisting of several disease phenotypes with different underlying pathologies, it is crucial to define robust immune biomarkers that would help in the identification of patient groups that might benefit from anti-inflammatory therapy[76, 82]. Cytokines represent a broad category of signaling molecules produced by a wide range of cells, including immune cells such as B and T lymphocytes, macrophages, and mastocytes, as well as endothelial cells and fibroblasts. A meta-analysis of 18 studies found alterations in both proinflammatory and anti-inflammatory cytokines, and these disturbances were stage dependent[83]. In patients with first-episode psychosis, elevated levels of proinflammatory cytokines were found, whereas the level of interleukin (IL)-4 was significantly reduced. In acutely ill patients, increased pro-inflammatory cytokines were observed, and lower levels of IL-4 and IL-10 Levels were found than in controls. In chronically ill patients, augmented levels of IL-1β, sIL-2R, IL-6, and tumor necrosis factor alpha (TNF-α) and reduced IFNγ levels were observed compared to those in controls. Details are summarized in Table 1.

Moreover, a study evaluating the gene expression of cytokines in peripheral blood mononuclear cells reported increased mRNA levels of IL-6, IL-8 and TNF-α and decreased anti-inflammatory IL-2 mRNA[81]. Alterations in these cytokines were also found in CFS[84], and a meta-analysis of 16 studies found significantly higher CSF levels of IL-1β, IL-6 and IL-8 in patients with schizophrenia compared to healthy controls. Interleukins (*e.g.*, IL-1β and IL-6) play important roles in neurotransmitter systems in schizophrenia. A relationship exists between increased concentrations of IL-6 in childhood and a higher risk of subclinical psychotic symptoms in young adulthood. Increased concentrations of IL-6 and other proinflammatory cytokines, such as TNF-α, IL-1β, and IFNγ, are normalized in episodes of remission after antipsychotic treatment. Some studies have suggested an association among increased serum concentrations of cytokines, including IL-6, severity of the disease, and duration and antipsychotic therapy[74].

Alterations in the immune system influence the neurotransmission of dopamine, 5-HT, norepinephrine, and glutamate. The immune system can activate indoleamine 2,3-dioxygenase, an enzyme involved in tryptophan/kynurenine metabolism[73]. Kynurenic acid acts as a naturally occurring NMDA antagonist in the human brain. Increased levels of kynurenic acid were found in the CSF of patients with schizophrenia[85,86]; however, no changes in kynurenic acid levels were observed in the peripheral blood of patients with schizophrenia[73]. Proinflammatory cytokines increase the concentration of kynurenic acid. Approximately 10% of nonmedicated patients in acute episodes of schizophrenia produce NMDA receptor antibodies, and this finding supports the hypothesis of NMDA receptor antagonism in schizophrenia[72,73].

C-reactive protein (CRP) appears to be the most promising theranostic marker for inflammation, and patients with increased CRP might benefit from anti-inflammatory therapy[76,87]. CRP is synthesized in the liver in response to IL-1β, IL-6 and TNF-α and is released from macrophages and adipocytes[76]. CRP has been reported to correlate both with positive and negative symptoms of schizophrenia[88] and with cognitive dysfunction[89]. Recently, "ultraresistance" to treatment in schizophrenia (defined as current clozapine treatment and a mean positive and negative syndrome scale (PANSS) score ≥ 70) was found to be associated with abnormal CRP levels (> 3 g/L), providing further justification for treating ultra-resistant patients with anti-inflammatory agents[90]. Additionally, other acute phase proteins, including haptoglobin, alpha-1 antitrypsin, and alpha-2-macroglobulin, were also found to be elevated in a subgroup of individuals with schizophrenia and other psychoses[91,92].

Additional biomolecules and metabolites essential for inflammation and endothelial cell function (*e.g.*, creatine kinase m/B, angiotensin-converting enzyme, matrix metalloproteinase, thyroid-stimulating hormone, thyroxine-binding globulin, intercellular adhesion molecule 1, cortisol, α-2-macroglobulin, and thrombopoietin) have been found in lower concentrations in drug-naïve patients than in controls[93]. The authors commented that most of these proteins are involved. Additionally, upregulation of leucocyte adhesion molecules was described in psychotic disorders, increased levels of soluble L-selectin were detected in the serum of drug-naïve patients with schizophrenia, and the serum levels of L-selectin and P-selectin in patients with schizophrenia did not differ from those in healthy controls[94]. In another study, P-selectin plasma levels were found to be increased in patients with acute psychosis[95].

Table 1 Possible diagnostic/theranostic immunologic biomarkers in schizophrenia

Parameter	Serum/plasma/peripheral blood	CSF
Pro-inflammatory cytokines	↑ IL-6, IFN-γ, IL-1RA, IL-1β, IL-6, IL-8, IL-12, sIL-2R, TGF-β, and TNF-α	↑ IL-1β, IL-6 and IL-8
Anti-inflammatory cytokines	↓ IL-10 and IL-4	
Acute phase proteins	↑ CRP, haptoglobin, α-1 antitrypsin, and α-2 macroglobulin	
Antibodies	↑ Anti-cardiolipin IgG and anti-NMDA receptor titers	
Immune cells	↑ CD4+, CD3+ and CD56+	
Other biomolecules/metabolites	↓ Creatine kinase m/B, MMP3, ACE, cortisol, TBG, α-2 macroblobulin, thrombopoietin, TSH, and ICAM-1, P-selectin	

ACE: Angiotensin-converting enzyme; CRP: C-reactive protein; CSF: Cerebro-spinal fluid; ICAM: Intercellular adhesion molecule; IFNγ: Interferon gamma; IL: Interleukin; MMP3: Matrix metalloproteinase 3; NMDA: N-methyl-D-aspartate; TBG: Thyroxine-binding globulin; TGF-β: Transforming growth factor-beta; TNF-α: Tumor necrosis factor-alpha; TSH: Thyroid-stimulating hormone.

Certain studies have also found changes in the number of immune cells in patients with schizophrenia compared to healthy controls. A meta-analysis of 16 studies evaluating blood lymphocyte counts found a significant increase in the percentages of CD4+ (T-helper lymphocytes) and CD56+ (natural killer cells) lymphocytes in acutely relapsed inpatients and a significant increase in the absolute numbers of total lymphocytes and CD3+ (T-lymphocytes) and CD4+ cells[96]. However, a significant decrease in the percentage of CD3+ cells was found in drug-naïve patients in the first episode of schizophrenia. Additionally, some autoimmune responses were also found in schizophrenic patients, but their clinical relevance remains elusive. Regarding the pathogenesis of schizophrenia, different autoantibodies, as well as antibodies against diet antigens, *e.g.*, gliadin and casein, were investigated in different parts of the brain, serum, and CSF. A systematic quantitative review of 81 studies found significantly increased anti-cardiolipin IgG and anti-NMDA receptor autoantibody titers in patients in the first episode of schizophrenia[97]. The authors also reported increased titers of anti-cardiolipin IgG and IgM and nerve growth factor in patients with schizophrenia compared with controls.

As mentioned, there is a strong link between oxidative stress and the immune system; therefore, by counteracting oxidative stress, antioxidants reduce inflammation and the overactive immune response. Glutathione is an antioxidant that is essential in the myelinization and maturation of white matter and can be supplemented as the amino acid precursor N-acetyl cysteine. N-acetyl cysteine possesses antioxidant properties and mild anti-inflammatory effects and regulates synaptic NMDA receptors. NAC supplementation for 6 mo ameliorated positive symptoms and improved neurocognition (processing speed) in patients with schizophrenia with high peripheral oxidative stress[98]. Omega-3-type polyunsaturated fatty acids have also exerted antioxidative capacity and anti-inflammatory effects[99].

Non-steroidal anti-inflammatory drugs in pharmacotherapy

Anti-inflammatory agents have shown some benefits as adds-ons to antipsychotic treatment in schizophrenia, as reported by two recent meta-analyses[100,101]. A meta-analysis of 62 double-blind randomized clinical trials studying aspirin, celecoxib, omega-3 fatty acids, estrogens, pregnenolone, minocycline, N-acetyl cysteine, and erythropoietin in 2914 patients found an overall significant effect of a decrease in the PANSS score[100]. Additionally, cognitive improvement was significantly associated with minocycline and pregnenolone therapy. Another meta-analysis of 70 randomized clinical trials including 4104 subjects investigated either primarily non-steroidal anti-inflammatory drugs (NSAIDs), minocycline and monoclonal antibodies, or drugs with potential anti-inflammatory properties (N-acetyl cysteine, melatonin, neurosteroids, estrogens, fatty acids, statins, and glitazones) as adjunctive therapies to antipsychotics. The analysis also found a decrease in the PANSS score[101]. Small but significant effect sizes were observed on both negative and positive symptoms, general psycho-pathology and working memory. Interestingly, primarily anti-inflammatory drugs were not found to be superior to potential anti-inflammatory drugs. However, the authors highlighted that the reported effects might be overestimated due to the many small study samples included in the analysis.

In conclusion, changes in the frequencies of immune cells, the levels and expression of cytokines, and the levels of acute phase proteins in the blood and CSF were observed in patients with schizophrenia compared to healthy controls, and CRP seems to be a promising theranostic biomarker for schizophrenia. Moreover, larger studies with longer treatment durations and the inclusion of only schizophrenic patients with proven inflammation (CRP levels > 3 g/L) are warranted to elucidate the efficacy of anti-inflammatory treatment in schizophrenia.

The challenges of drug development include the development of novel molecules affecting the immune system and of immunotherapy using autoantibodies as well as the stratification of patients with schizophrenia according to their immune phenotypes to enable the selection of effective pharmacotherapeutic agents[74].

MICROBIOME-BRAIN AXIS MODEL

The microbiome-gut-brain axis model postulates that bidirectional communication between the central and enteric nervous systems ensures the connection of the brain with peripheral intestinal functions[102,103]. It is presumed that the gut microbiome can program brain function during early development; in other words, active signals from the microbiome play a critical role in brain development. It is thought that the microbiome may affect brain development through epigenetic mechanisms[104]. Signal pathways from the gut microbiome to the brain include: (1) The direct activation of the vagus nerve; (2) The production or induction of various metabolites that may cross the BBB to regulate neurological functions; and (3) An immune system whose cytokines affect neurophysiology[105].

Probiotics modulate the immune responses of the host and could be beneficial for schizophrenia patients[106]. The immunomodulatory effects of probiotic supplementation were examined after 14 wk in chronic patients with schizophrenia, and *Lactobacillus* and *Bifidobacterium* were administered as adjuvant treatments. Increased levels of brain-derived neurotrophic factor, chemokine ligand 5 (RANTES), monocyte chemotactic protein and macrophage inflammatory protein-1 beta were found[106]. Gut-brain communication suggests the direct secretion of some neuroactive substances, and some intestinal bacteria can produce mediators, *e.g.*, GABA, acetylcholine.

INNOVATIVE DRUG APPROACHES AND TARGETS

All antipsychotics for schizophrenia treatment are based on dopamine agonism/antagonism, and no nondopaminergic antipsychotics have yet been developed or approved (Figure 1). Lumateperone is characterized as a partial D_2 receptor agonist/antagonist and a $5-HT_{2A}$ antagonist, and it is a serotonin reuptake inhibitor that indirectly modulates glutamatergic transmission (effect on NMDA receptor subtype 2B, NR2B)[107,108]. It has no metabolic or extrapyramidal adverse effects and positively affects cognition[109]. Another study found that lumateperone was well tolerated with minimal extrapyramidal and cardiometabolic adverse effects and maintained or reduced the symptoms of schizophrenia after a switch from a previous antipsychotic[110].

A promising therapeutic drug strategy for schizophrenia is the use of antipsychotics without D_2 receptor binding[111]. Agonists of trace amine-associated receptor 1 (TAAR1) seem to selectively affect dopamine and may represent a new class of psychotropic drugs[112,113]. A novel compound, SEP-363856, was described as a TAAR1 and $5-TH_{1A}$ agonist and was tested in a pilot trial with 120 patients with an acute exacerbation of schizophrenia. The reduction in the PANSS score of the treatment group was significant compared with that of the placebo group; longer and larger trials are necessary to prove the efficacy and safety of this TAAR1 agonist[113]. Other TAAR1 agonists, *e.g.*, RO5263397, have been developed and are being tested for use in schizophrenia. RO5263397 was found to be safe, but a great variety in metabolism and plasma levels was found that depended on ethnicity and genotype[112,114].

Novel drugs indirectly targeting glutamate neurotransmission are among the strategies being pursued for antipsychotic development. Inhibitors of glycine transporters (*e.g.*, sarcosine and bitopertin), coadministration of NMDA agonists, allosteric modulators of AMPA receptors (ampakines), and allosteric modulators of $mGluR_5$ are being tested; novel drugs affecting the glutamate ionotropic and meta-
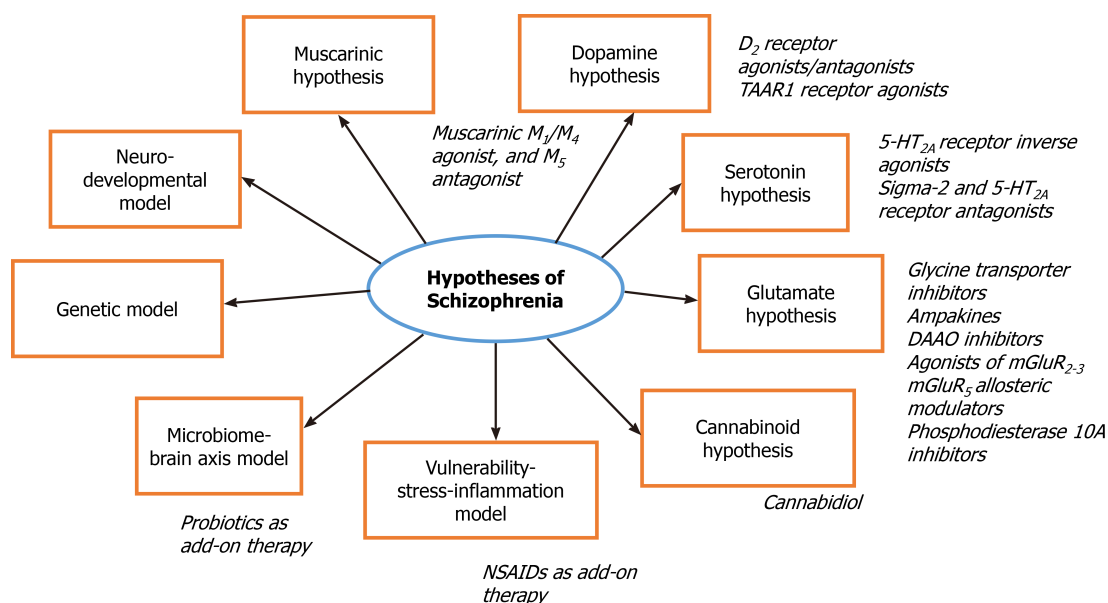
Figure 1 Illustration of current hypotheses and novel approaches in treatment of schizophrenia. DAAO: D-Amino acid oxidase; TAAR1: Trace amine-associated receptor 1; NSAIDs: Non-steroidal anti-inflammatory drugs.

botropic receptors mGluR$_{2-3}$ have been developed[115].

Agonists of mGluR$_{2-3}$ have anxiolytic properties and reverse the effects of stress; agonists of the mGluR$_3$ receptor antagonize the effect of phencyclidine and amphetamine. Novel drugs directly affecting receptors were found to be ineffective and/or to have adverse effects. Pomaglumetad methionil is an mGluR$_{2-3}$ agonist[58]. It was tested as an adjunctive therapy for patients with negative symptoms of schizophrenia, and the study did not find a difference between the pomaglumetad group and the placebo group[116]. Post hoc analyses suggested the efficacy of pomaglumetad in patients suffering from schizophrenia for less than or equal to 3 years or in patients previously treated with antipsychotics predominantly acting as D$_2$ antagonists [117,118]. Thus, the potential of novel mGluR$_{2-3}$ agonists is suggested for treating psychosis and aggression/agitation associated with neurodegenerative diseases (Parkinson's disease, Alzheimer's disease, dementia with Lewy bodies, *etc.*)[117].

D-Amino acid oxidase (DAAO) inhibitors were found to modulate NMDA transmission. Sodium benzoate, a DAAO inhibitor, has been tested as an add-on therapy and improved symptoms in clozapine-resistant patients[119]. In another study, sodium benzoate was administered to patients with schizophrenia as an add-on therapy compared to placebo. The adjunctive therapy was well tolerated, and chronic schizophrenia patients showed improved function, especially neurocognition[120].

Cannabidiol seems to be a promising candidate for the treatment of schizophrenia. Increased function of the endocannabinoid system was observed in schizophrenic patients; cannabidiol was found to decrease mesolimbic dopaminergic activity. There is evidence that chronic and acute administration of cannabidiol led to improvement of schizophrenia symptomatology[121].

Based on the serotonin hypothesis, pimavanserin was developed and characterized as an inverse agonist at 5-HT$_{2A}$ receptors, and it has binding affinity for sigma-1 receptors. This antipsychotic has been FDA-approved for the treatment of psychosis associated with Parkinson's disease. Further randomized controlled trials are needed to consider pimavanserin as a drug for schizophrenia treatment[122].

Antagonism at sigma-2 receptors was described for roluperidone, which was developed as a novel antipsychotic affecting 5-HT$_{2A}$ receptors and sigma-2 receptors [58]. Roluperidone has been found to be effective in the treatment of negative symptoms[123].

Xanomeline is a muscarinic M$_1$/M$_4$ agonist and M$_5$ antagonist that was originally developed for Alzheimer´s disease. Currently, it is being tested in combination with trospium, which reduces peripheral adverse effects (nausea and vomiting), as a new antipsychotic with a novel mechanism of action.

Phosphodiesterase inhibitors have been investigated as drugs to enhance cognition in schizophrenia[124]. Phosphodiesterase 10A inhibitors likely modulate D$_1$ (directly) and D$_2$ (indirectly) striatal pathways and regulate glutamate receptors[112]. TAK-063, a

phosphodiesterase 10A inhibitor, was tested, but the clinical trial did not meet the primary endpoint, and extrapyramidal syndromes occurred more often in the TAK-063 group than in the placebo group[125].

CONCLUSION

Genetic predisposition and neurodevelopmental and environmental risk factors for schizophrenia were summarized. Nevertheless, the understanding of schizophrenia pathophysiology is limited, and current pharmacotherapy is complicated by adverse effects, pharmacoresistance, and low compliance of patients. Novel targets and approaches of antipsychotic treatment are being developed with the aim of covering the wide range of schizophrenia symptoms, especially negative symptomatology, cognitive impairment, and residual and treatment-resistant symptoms. Novel drug targets based on current schizophrenia hypotheses include molecules indirectly targeting glutamate neurotransmission, DAAO inhibitors, 5-HT$_{2A}$ receptor inverse agonists, sigma-2 receptor antagonists, phosphodiesterase inhibitors, *etc.* In pharma-coresistant patients, possible comorbidities may be related to inflammation or a disrupted microbiome-gut-brain axis, and augmentation of antipsychotic treatment *via* NSAID or probiotic administration can be considered. Further research on schizo-phrenia pathophysiology, genetic predisposition (based on GWAS), regulatory mechanisms in impaired mediator transmission and other factors is needed to improve the clinical outcomes of pharmacotherapy.

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