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REVIEW ARTICLES

SLC6A1 and Neuropsychiatric Diseases: The Role of Mutations and Prospects for Treatment with Genome Editing Systems

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Abstract—*SLC6A1* (Solute Carrier Family 6 Member 1) is a gene encoding GAT1, the gamma-aminobutyric acid (GABA) transporter protein. GAT1 is responsible for GABA reuptake from the synaptic cleft and intercellular space. Mutations in the *SLC6A1* gene can lead to impaired GABA regulation and are associated with epilepsy and a number of mental disorders. In this review, we discuss the role of GAT1 protein in GABAergic regulation and the association of mutations in *SLC6A1* gene with epilepsy, autism spectrum disorders, intellectual disability, and schizophrenia, as well as prospects for their treatment with genome editing systems.

Keywords: *SLC6A1*, GAT1, GABAergic system, de novo mutations, autism spectrum disorders, intellectual disability, epilepsy, schizophrenia

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INTRODUCTION

SLC6A1 (Solute Carrier Family 6 Member 1) is a gene encoding GAT1 transporter protein (GABA transporter 1). The function of GAT1 protein is to remove excess gamma-aminobutyric acid (GABA) from the synaptic cleft and terminate GABAergic neurotransmission [1]. GABA is the key inhibitory neurotransmitter in the central nervous system (CNS). GAT1 transporter plays an important role in the control of extrasynaptic GABA concentration, modulating both phasic and tonic inhibition [2–5]. Furthermore, GABA is a neurotrophic factor, essential for the early stages of brain development and proliferation of neural stem cells [6, 7].

The clinical manifestations of mutations in *SLC6A1* include a wide range of diseases [8, 9]. Epilepsy is the most common disease associated with *SLC6A1* mutation. These mutations are frequently seen in severe cases of epilepsy [10, 11]. Epilepsy is often accompanied by autism spectrum disorders (ASD), intellectual disability (ID), and various neurological symptoms, such as ataxia or unsteady gait, tremor, and impaired fine motor skills [10]. The search for rare and de novo mutations in patients with ASD detected the connection between *SLC6A1* mutations and autism [12, 13]. The direct relationship between these mutations and ASD remains hard to assess since autism is comorbid with epilepsy [14] and is also associated with GABAergic system dysfunction [15]. The discovery of mutations in *SLC6A1* appeared to be the key result of the studies on search for de novo mutations in patients with schizophrenia; moreover, all mutation carriers did not manifest any epilepsy- or ASD-related symptoms suggesting an independent role of *SLC6A1* in the pathogenesis of schizophrenia [16]. It was shown that GAT1 protein dysfunction was typical of patients with schizophrenia, affecting a number of brain regions, including the prefrontal cortex [17], limbic system [18], and cerebellum [19]. According to the GWAS catalog (www.ebi.ac.uk/gwas/genes/SLC6A1) at the time of writing, the polymorphisms in *SLC6A1* were found to be associated with a number of diseases, e.g. myopia [20], uterine cancer [21], and alcohol abuse [22]. The investigations of genetic susceptibility to alcoholism discovered that *SLC6A1* polymorphisms were the second major signal among the obtained results, which is not a surprise considering that ethanol influences the body via GABA receptors. In addition, associations of the *SLC6A1* gene with attention deficit hyperactivity disorder (ADHD) were found in a large candidate trial [23] and early GWAS [24], however, no associations were replicated in later GWAS [25].

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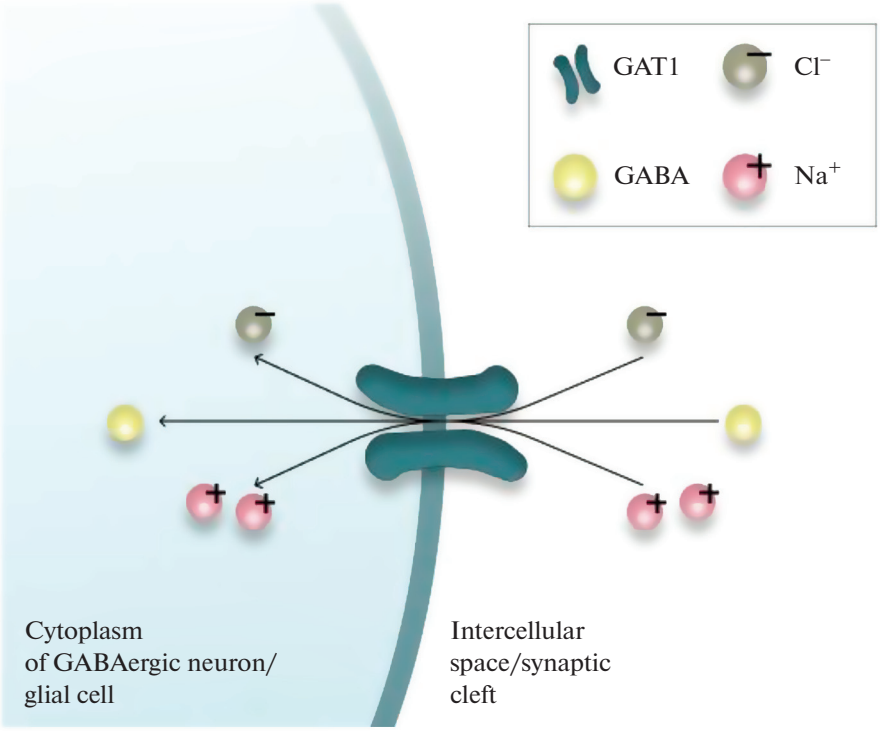

Fig. 1. Function of GAT1 GABA transporter. GABA is co-transported with sodium and chloride ions.

Therefore, the investigations of the role of genetic traits related to *SLC6A1* mutations in the pathogenesis of different diseases will help to thoroughly understand the mechanisms of their development and help to elaborate novel approaches for diagnosis and potential gene therapy.

SLC6A1 GENE

The *SLC6A1* gene belongs to a family including 19 paralogous genes encoding the carriers of simple chemical compounds. In humans, it is localized on chromosome 3 (3p25.3) and consists of 47 061 base pairs, according to the Ensembl Genome Browser (http://www.ensembl.org, v103). *SLC6A1* contains 18 exons and has several annotated alternative promoters expressing 41 alternative transcripts. The expression of different variants of *SLC6A1* is poorly studied. Some researches link various alternative transcripts with epilepsy [26] and the development of ADHD [27]. At the time of writing, 277 orthologs are known for the *SLC6A1* gene. A significant number of genetic studies on GAT1 were performed on orthologous genes in laboratory animals.

SLC6A1 is mainly expressed in the CNS. In the brain, GAT1 is mostly located on the presynaptic axonal membrane of GABAergic neurons [28]. Experiments performed with animal models have also demonstrated the presence of the transporter on the membranes of astrocytes [29], oligodendrocytes [30], and microglial cells [31]. Marked expression of *SLC6A1* has been observed outside the brain in the liver tissues, although GAT1 protein itself is sparse in the liver, according to the Human Protein Atlas (www.proteinatlas.org) [23]. Furthermore, GAT1 can be found in the male reproductive system, where the protein is apparently required for normal spermatogenesis [33].

THE ROLE OF GAT1 PROTEIN IN GABAERGIC REGULATION

GAT1 is responsible for GABA cotransport with sodium and chloride ions from the synaptic cleft to the terminals of presynaptic neurons and nearby glial cells, thus promoting the termination of GABA signaling (Fig. 1) [34].

GABA transport is an active process that requires the electrochemical gradient for sodium ions provided by Na^+/K^+ ATPase. GABA transmitters cotransport GABA, sodium, and chloride ions in a ratio of $1GABA : 2Na^+ : 1Cl^-$ down the concentration gradient of sodium and chloride ions [35, 36]. Neurotransmitter reuptake happens within milliseconds of its release, therefore preventing GABA from activation surrounding synapses [37]. However, GAT1 as an ion channel participates in the generation of, at least, two currents that are stoichiometrically unrelated to the transmission of GABA, sodium, or chloride ions through the membrane: the GABAergic current of

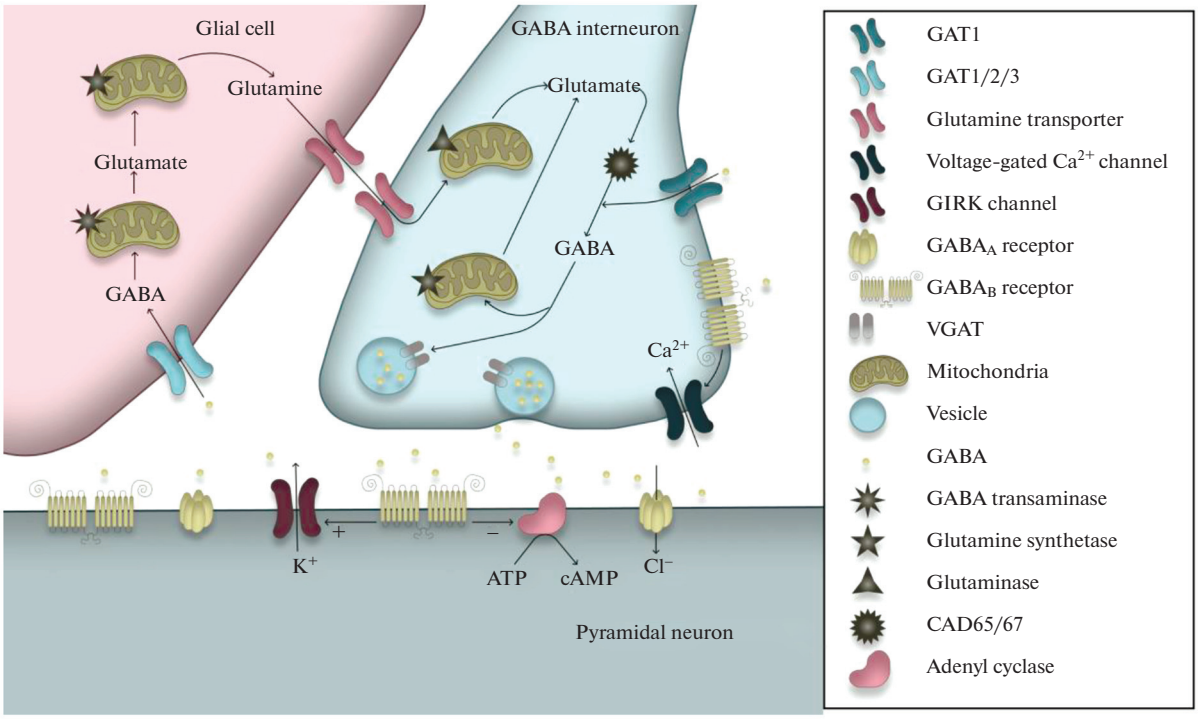

Fig. 2. GABA metabolism. GABAergic neurons release GABA, which in turn influences postsynaptic $GABA_A$ and $GABA_B$ receptors. The neurotransmitter excess is transferred to glial cells predominantly by GAT2-mediated reuptake and to the presynaptic terminal mainly by GAT1 transporter. GABA metabolism occurs intracellularly and involves mitochondria. Inside glial cells GABA transforms into glutamate, then glutamine, which is further transferred by glutamate transporters into GABAergic synapses. There glutamine undergoes a reverse transformation into GABA via glutamate and is packed in vesicles. Excitation of the GABAergic neuron results in another GABA release. This way the cycle is closed. Extrasynaptic $GABA_A$ and $GABA_B$ receptors are activated by GABA which is always present in the intercellular space at low concentrations.

sodium ions in the intracellular space [38] and GABA-independent cation leak currents [39]. Because of these currents, the activation of GAT1 is able to create local changes in the membrane potential [37].

The key function of the GABAergic system is to modulate the ongoing activity of neural networks. By affecting ionotropic and metabotropic receptors, GABA controls the generation of action potentials and the temporal structure of activity patterns formed by whole populations of neurons [40–43]. In order to do this, the time of GABA release from the presynaptic terminal and GABA clearance from the extracellular matrix should be tightly regulated. Adequate functioning of GABA receptors providing a high signal-to-noise ratio requires a steady low concentration of the neurotransmitter in the extracellular medium. This can be achieved only by reuptake, because GABA metabolism is intracellular, occurring inside neurons and glial cells (Fig. 2) [44–46]. In addition, the operation of GABA transporters restricts GABA outflux from active synapses, i.e. spillover, and, therefore, controls the spatial specificity of GABAergic transmission [47–49].

A number of experiments on mice with knockout of *SLC6A1* gene revealed such manifestations as decreased aggressiveness [50], anxiety, depression [51], hypoalgesia [52], and reduced behavioral reactions to ethanol [53]. These results can be associated with elevated extracellular GABA levels due to GAT1 dysfunction and a resulting enhancement of inhibition because GAT1 is the main GABA transporter in CNS. However, tremor, ataxia, irritability, and increased GABA-induced excitation in the cerebellum were observed in studies by Chiu et al. on *SLC6A1* knockout mice [54, 55]. This phenotype resembles the side effects from treatment with tiagabine, a highly selective GAT1 inhibitor and an antiepileptic drug [56].

To understand such phenotypic manifestations, we will now address the potential consequences of GAT1 dysfunction from the perspective of the structure of the GABAergic system.

The family of GABA transporters. GABA transporters belong to the SLC6 (Solute Carrier 6) transporter family. The SLC6 family consists of 19 proteins and is divided into four groups depending on the type of transmitted substance: GABA, amino acid, monoamine, and essential amino acid transporters (Fig. 3) [57]. These transporters maintain low extracellular levels of GABA and excitatory amino acids, thus regulating their functional activity and intracellular metab-

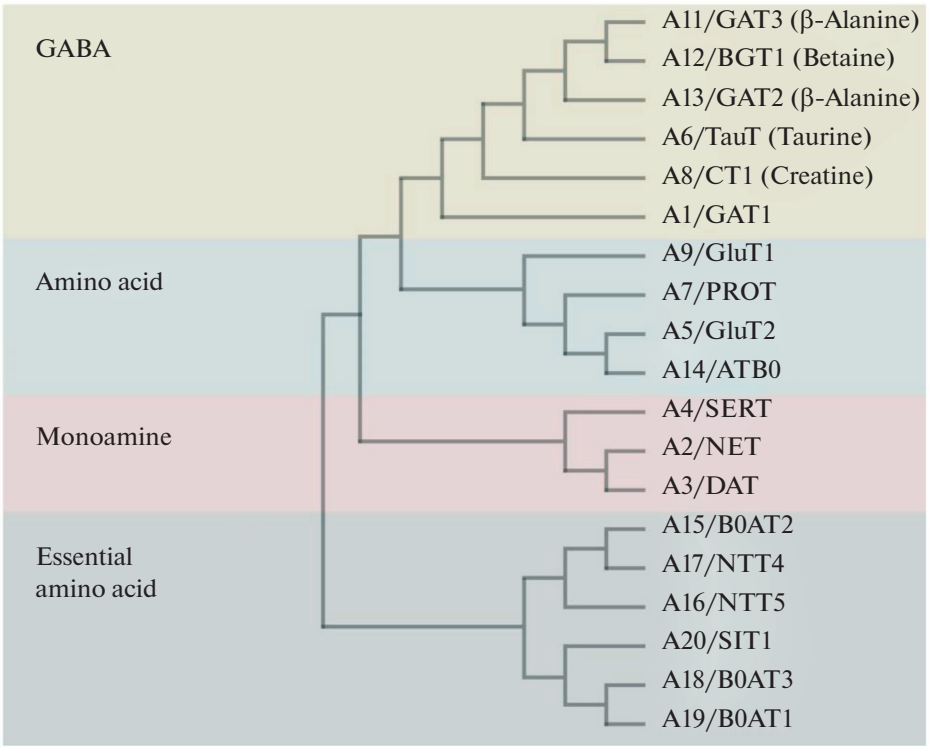

Fig. 3. Molecular phylogenetic analysis of the family of SLC6 cotransporters. The SLC6 family is divided into four groups: GABA (green), amino acid (blue), monoamine (pink), and essential amino acid (gray) transporters. Other substances that can be transmitted by GABA transporters are given in parentheses.

olism. The transporters are characterized by various features regarding their transport functions, i.e. neurotransmitter reuptake, and their ability to act like ion channels [58].

The group of GABA transporters includes six proteins: A1/GAT1, A13/GAT2, A11/GAT3, A12/BGT1, A8/CT1, and A6/TauT, each of them capable of transporting GABA and other molecules, such as β-alanine (GAT2 and GAT3), taurine (TauT), creatine (CT1), and betaine (BGT1) [59]. The following proteins play the biggest role in the processes of GABA reuptake among the aforementioned transporters: GAT1, GAT2, GAT3 (especially GAT1) [8, 45, 59, 60]. GAT1 is a highly selective sodium- and chloride-dependent GABA transporter, predominantly localized on the presynaptic membrane of GABAergic neurons and, to a lesser extent, on the astrocyte membranes surrounding synapses [36, 45, 58].

The results of immunohistochemical studies in rats showed weak expression of GAT2 in the CNS compared to the peripheral organs and high abundance of GAT1 and GAT3 all over the brain, [61]. The largest levels of GAT1 expression were seen in the hippocampus, olfactory bulbs, the molecular layer of cerebral cortex (L1), the piriform cortex, superior colliculus, interpeduncular nucleus, and spinal trigeminal nucleus. The highest levels of GAT3 expression were observed in the olfactory bulbs, thalamus, hypothalamus, pons, medulla oblongata, globus pallidus, basal ganglia, substantia nigra, cerebellar nuclei, and spinal trigeminal nucleus [61]. The level of GAT1–3 expression in the cerebral cortex depends on the layer. GAT1 is mainly expressed in L2–L4 (external granular, external pyramidal, and internal granular layers); GAT2 can be found predominantly in the molecular layer, and GAT3 is accumulated in L3 (external pyramidal layer), and the upper part of L5 (internal pyramidal layer) [61, 62].

A comparison of GABA transporter expression in the CNS on the cellular and subcellular levels demonstrates that GAT1 expression is more prominent on presynaptic membranes of GABAergic neurons and less pronounced in glial cells [62]. The level of GAT3 expression in the CNS is generally lower than the level of GAT1, mostly occurring in glial cells [28]. The expression of GAT2 in the CNS is also lower compared to GAT1 and, similarly to BGT1, it is predominantly expressed outside the CNS, i.e., in the liver and kidneys [45, 63]. Therefore, GAT1 is the key GABA transporter in the CNS.

GAT1 protein structure. GABA transporters of the SLC6 family have a common structure consisting of 12 transmembrane domains (Fig. 4). Each transporter differs in its affinity to GABA, localization in tissues,

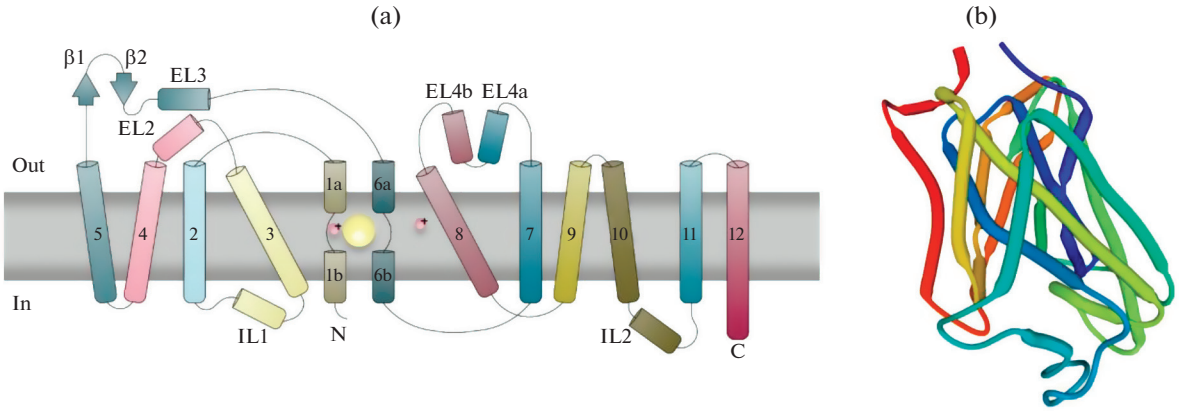

Fig. 4. The structure of GAT1 protein. (a) Schematic structure of GAT1 of Aquifex aeolicus LeuTAa. The transporter consists of 12 transmembrane domains. The N- and C-terminals reside in the cytoplasm. There are two extracellular β-chains in GAT1 (sage green arrows), as well as four extracellular (EL2, EL3, EL4a, and EL4b) and two intracellular helices (IL1 and IL2). Sodium ions are represented as two pink spheres. The substrate molecule (Leu) is illustrated as a large yellow sphere. Adapted from [59]. (b) A model of the 3D structure of GAT1 protein based on the X-ray structure of the dopamine transporter of Drosophila 4xp9.1.A [66].

and substrate specificity [45]. GAT1 protein with a molecular mass of 67074 Da consists of 599 amino acids [64]. GAT1 has the strongest CNS expression of all GABA transporters [45]. These features made GAT1 a target for the development of selective antiepileptic drugs [65].

To understand the processes occurring in defective GABA reuptake, let us introduce the types of GABAergic neurons, the structure of GABA receptors, and types of inhibition in the CNS.

GABAergic interneurons (types, localization, and participation in maintenance of balance between excitation and inhibition). GABA is the major inhibitory neurotransmitter in CNS. In the prefrontal cortex, GABAergic interneurons inhibit pyramidal neurons, thus controlling the excitatory inputs from the afferent structures and generation of action potentials [41, 67]. GABAergic interneurons can be classified according to their morphology, electrophysiological properties, and histological markers [68, 69]. Referring to the most common classification, the following types of interneurons can be distinguished by the expression of typical markers: parvalbumin-positive interneurons (calcium-binding protein parvalbumin (PV), and interneurons expressing ionotropic 5HT3a serotonin receptor (5HT3aR) [70]. PV interneurons, the most common type of GABAergic interneurons, can be further divided into two subtypes: basket cells that innervate soma and proximal dendrites, and chandelier cells that form synaptic contacts with the initial segments of axons [71, 72].

A number of studies discovered that basket interneurons are involved in the generation of γ-oscillations of 30–80 Hz associated with cognitive functions and information processing in different species [73–75]. The presence of significant changes in γ-oscillations can be seen in diseases related to cognitive impairments, such as schizophrenia and ASD [76, 77]. To make these processes efficient, the inhibition and excitation levels should be maintained at a certain ratio that is around 20/80% (excitation/inhibition) for the prefrontal cortex [78, 79]. The balance between the processes of excitation and inhibition originates from the early stages of development of the nervous system and continues to be supported by homeostatic mechanisms throughout the lifetime [80, 81]. Accumulated data indicate that the imbalance between excitation and inhibition contributes to the etiology and clinical manifestations of a number of diseases associated with neural development, including schizophrenia, ASD, and epilepsy [82].

The defects of working memory in patients suffering from schizophrenia are related to the dysfunction of the dorsolateral prefrontal cortex [83]. Deficit of the GABA synthesizing enzyme glutamate decarboxylase (GAD67) is typical of schizophrenia [84–86]. Although the majority of GABA interneurons in the prefrontal cortex of these patients express the normal level of GAD67 mRNA, approximately 25–35% of GABA interneurons, mostly PV neurons [87], do not contain the detectable level of this transcript. However, the disturbances in PV neurons alone cannot fully explain the deficiency of GAD67 mRNA expression, since these changes were also registered in cortical layers I, II, and V, where the amount of PV neurons is scarce [88, 89]. Therefore, this dysfunction is, at least partially, related to defective processes of phasic, i.e. synaptic, and tonic, or extrasynaptic, inhibition of signal inputs of pyramidal cells by SST-containing and CCK-containing GABA interneurons [83].

GABA receptors. Until now three types of GABA receptors have been described, two of them, $GABA_A$ and $GABA_c$, being ligand-gated ion channels, and $GABA_B$, a G protein-coupled metabotropic receptor [90].

Ionotropic receptors consist of five subunits. The structure of $GABA_C$ receptors contains ρ1–3 subunits [91]. This type of receptor was initially related to the $GABA_A$ subtype. Their functions are related to the processing of visual information, regulation of sleep-wake cycles, pain perception, memory, learning, hormonal regulation, and gastrointestinal secretion. These receptors are located in retina, thalamus, hippocampus, pituitary gland, and gastrointestinal tract [92]. $GABA_A$ receptors are especially interesting in the context of mental and neurological disease development. They produce a number of isoforms with various combinations of 16 types of subunits (α1–6, β1–3, γ1–3, δ, ε, π, and θ) [93, 94]. Subunit composition influences various traits of $GABA_A$ receptors, such as activation kinetics (affects their desensitization [95]), and localization (GABA receptors carrying α1 and γ2 subunits are concentrated on postsynaptic segments, mediating phasic inhibition there [96, 97], while GABA receptors with α4 and δ subunits in the forebrain are selectively located on extrasynaptic segments [96–98]). Extrasynaptic receptors are highly sensitive to GABA and participate in tonic inhibition [83]. The activation of presynaptic $GABA_A$ receptors suppresses the release of neurotransmitter by inhibiting voltage-gated calcium channels. In turn, postsynaptic $GABA_A$ receptors are responsible for the generation of inhibitory postsynaptic potentials (IPSPs) and the development of rapid hyperpolarization of the postsynaptic membrane [99]. Nevertheless, it was also shown that prolonged activation of postsynaptic $GABA_A$ receptors may lead to the development of long-lasting depolarizing postsynaptic potentials [100]. The subunit composition of $GABA_A$ receptors can be crucial for disease pathogenesis; for instance, the composition of $GABA_A$ receptor subunits in neurons can vary during epileptogenesis; with these changes affecting the pharmacodynamics of drugs [101, 102].

Metabotropic $GABA_B$ receptors consist of two subunits (GBR1 and GBR2) [103] and can be located on both pre- and postsynaptic membranes [104, 105]. The interaction between $GABA_B$ receptor and ligand (GABA) induces a cascade of reactions leading to the opening of G protein-gated potassium channels and a slow IPSP lasting hundreds of milliseconds [106–108]. As a result, long-term hyperpolarization develops after rapid hyperpolarization provoked by the activation of ionotropic receptors [99]. Extrasynaptic $GABA_B$ receptors can be activated by the low GABA concentrations constantly maintained in the intercellular matrix and GABA outside the synaptic cleft due to GABA spillover [106]. The activation of presynaptic $GABA_B$ receptors suppresses GABA neurotransmission of inhibitory interneurons [105].

The diversity of GABAergic inhibition types. There are different types of GABAergic inhibition. Depending on the type of inhibited neuron, there is inhibition of GABAergic interneurons (which leads to enhanced excitation) and inhibition of excitatory neurons (which leads to the suppression of excitation) [109]. According to the localization of inhibitory synaptic terminals, inhibition occurs either in axodendritic synapses (control over input currents travelling from the dendrites towards soma), axosomatic synapses, or at the initial segment of axon (control over output currents or the generation of action potentials) [110]. As for the pathologies related to GABA reuptake disturbance, we would like to take a closer look at the classification of inhibition by the type of synaptic transmission. Phasic inhibition is mediated by the action of high GABA concentration on postsynaptic GABA receptors ensuing from discrete neurotransmitter release from the presynaptic terminal. However, a small amount of GABA is constantly present in the intercellular space due to spillover of neurotransmitter outside the synaptic cleft and reversed functioning of GABA transporters [111–113]. Low GABA concentrations affect extrasynaptic GABA receptors and their activation induces tonic inhibition [114, 115]. This type of inhibition supports a certain membrane potential and modulates cellular excitability by reducing the threshold for the generation of action potentials [114]. A number of studies demonstrated that long-term stimulation of extrasynaptic GABA receptors can modify their functioning and promote the development of depolarization resulting from their activation, rather than hyperpolarization [116–118], which can possibly occur after disruption of GABA reuptake.

Enhanced tonic inhibition and prolonged decay time of phasic inhibition mediated by postsynaptic GABA receptors are seen in mice with GAT1 deficiency, while the frequency, amplitude, and kinetics of GABA-induced postsynaptic currents remain unchanged [55, 119]. However, other studies involving GAT1 deficient mice discovered a decrease in IPSP frequency possibly related to the increased expression of enzymes (GAD65/67) participating in GABA synthesis on presynaptic terminals of inhibitory neurons [120, 121].

Together these results suggest numerous molecular and cellular functions of GAT1. Disturbed functioning of the GABA transporter explains the sophisticated pathogenesis of diseases in patients carrying *SLC6A1* gene mutations.

GABA and neurogenesis. Differentiation, migration, and integration of neurons are managed by several molecular processes.

GABA is a neurotrophic factor [122]. During the embryonic stage GABA acts like an excitatory neurotransmitter [123, 124]. Tonic excitation mediated by GABA receptor activation stimulates and directs the migration of projection neurons [122]. The stimulation of $GABA_B$ and $GABA_C$ receptors promotes the migration of neurons through the cortical plate [125],

while the activation of $GABA_A$ receptors terminates this process [126].

Defective GABA signalization at the early developmental stages modifies cellular migration and the cortical architecture [127–132].

What is more, GABA is involved in neurogenesis in the adulthood, too. Though other neurotransmitters can also regulate various processes of neuron maturation from neural stem cells, GABA is the most valuable. Thus, the development and functioning of granular cells in adult brain are controlled by the GABAergic system [133].

Brain-derived neurotrophic factor BDNF and other neurotrophins promote the migration of cells, synaptogenesis, dendritic growth, and maintenance of synaptic function throughout life [125, 134–136]. Changes in BDNF level can impair the migration of GABAergic interneurons in the developing brain [137–139]. In addition, BDNF functions are still critical even at the later stages of brain development during adolescence, when synaptic contacts reinforce or get pruned [140, 141]. The predisposition to mental illnesses is strongest during this period [142].

BDNF is a growth factor synthesized and secreted by pyramidal neurons and its receptors are present on different types of GABA interneurons [143]. GABA regulates BDNF activity by switching from activation to inhibition of the BDNF gene during switching of GABA signalization from excitatory to inhibitory [144]. During development, GABA-induced membrane depolarization causes the opening of L-type calcium channels and BDNF release, thus assisting neural differentiation [145]. Increased expression of chloride potassium cotransporter 2 (KCC2) at birth changes the gradient of chloride ions which leads to the switch from depolarizing to hyperpolarizing GABA [146, 147]. With the development of hyperpolarization [148], activation of L–type calcium channels stops which arrests synthesis and release of BDNF [144].

Therefore, BDNF and GABA closely cooperate and impaired GABA reuptake can result in the reduction of neurotrophic processes both at the early developmental stages and in the mature brain, therefore, favoring the development of certain neurological disorders.

SLC6A1 GENETICS AND NEUROPSYCHIATRIC DISEASES

Mutations in protein-coding genes are significant genetic risk factors for the development of mental and neuropsychiatric diseases. Now we will move to some phenotypic manifestations of *SLC6A1* gene mutations.

Epilepsy. Epilepsy is a chronic neurological disease characterized by recurrent seizures [149]. Mutations in the *SLC6A1* gene were primarily detected in patients with myoclonic-atonic seizures [10]. Nevertheless, the range of phenotypic manifestations of mutations in this gene turned out to be wider. Johannesen et al. analyzed 34 cases (24 probands, 6 family members, and clinical data from 4 previously described subjects) with mutations in the *SLC6A1* gene [8]. Epilepsy was diagnosed in 31 out of 34 subjects and manifested in the following forms: absence, atypical absence, atonic seizures, and generalized tonic-clonic seizures less frequently (5/31). It should be mentioned that pronounced intellectual disability of different extents from mild to moderate was common for all patients, and was accompanied by speech impairment. Moreover, 3 out of 34 subjects had a mild intellectual disability in the absence of epilepsy. Additionally, half of patients showed behavioral disturbances, such as hyperactivity, aggressive behavior, ADHD, and autism in different combinations. Similar symptoms were earlier observed in animal models, particularly in mice with *slc6a1* mutations [150].

As previously discussed, GAT1 dysfunction can contribute to the dynamics of excitatory and inhibitory processes via numerous mechanisms. Disturbances of reuptake processes lead to an increase in GABA concentrations both in the synaptic cleft and outside the synapse [119]. This can cause the hyperstimulation of extrasynaptic $GABA_A$ and $GABA_B$ receptors responsible for the development of tonic inhibition [151]. Sore et al. demonstrated enhancement of $GABA_A$-mediated tonic inhibition in thalamocortical neurons of GAT1 knockout mice, causing hyperpolarization of thalamocortical neurons and defective generation of action potentials [151]. In addition, long-term activation of $GABA_B$ receptors, which induces the opening of T–type voltage-gated calcium channels, may provoke circulating excitation in the thalamocortical system by generating continuous action potentials [152, 153]. Previous studies discovered that the activation of $GABA_B$ receptors initiated seizures in mice and rats and preliminary administration of a $GABA_B$ receptor agonist could diminish their duration or interrupt them [154, 155]. Reduced GAT1 function can also cause the reduction of GABA concentration in synaptic terminals and, hence, the disturbance of GABA release and phasic inhibition. In addition, phasic inhibition performed by $GABA_A$ receptors can be interrupted by modifications in their subunit composition [156]. *In vivo* studies involving GAT1 knockout mice revealed significant enhancement of tonic inhibition in the hippocampus with unchanged or reduced IPSP amplitudes on postsynaptic membranes [119, 157]. To sum up, a decrease in GAT1 function can lead to the development of epilepsy both by excessive activation of extrasynaptic $GABA_A$ and $GABA_B$ receptors and reduction of synaptic $GABA_A$-mediated signalization [158].

GAT1 is one of the key targets for epilepsy treatment [26]. For example, tiagabine, a selective GAT1 inhibitor, is broadly used for managing focal epilepsy

[26]. It is fair to question if mutations in the *SLC6A1* gene and dysfunction of the GAT1 protein is one of the causative factors for epilepsy, why should GAT1 inhibitors be used for epilepsy treatment? This can be explained by the fact that GAT1 mutations associated with epilepsy do not necessarily manifest in a decrease of its concentration, or that GAT1 knockout models partially lack the symptoms of epilepsy such as seizures.

Therefore, it is important to define the influence of *SLC6A1* gene mutations on GABAergic signaling and brain development, as well as the consequences of treatment with GAT1 inhibitors.

Autism spectrum disorders. Autism, or broadly speaking, ASDs are genetic disorders characterized by disturbances of neuropsychiatric development ordinarily manifesting in childhood. The genetic architecture of ASDs includes rare de novo or inherited variations in hundreds of genes and polymorphisms in thousands of loci. Genetic studies on rare and common variabilities related to ASD demonstrate that the mutations involved in autism pathogenesis generally impact the initial stages of brain development, including the processes of cell differentiation in the cerebral cortex [159–161]. The relationship between defects in functioning of the GABAergic system and ASD is well shown in physiological studies and animal models of autism [162]. Frequently genes, mutations of which are associated with ASD and epilepsy, are involved in the functioning of GABAergic system [163, 164]. It should be emphasized that ASD and epilepsy are often concomitant demonstrating the similarity of their pathogenesis [165]. A number of studies investigated relationships between mutations in *SLC6A1* gene and autism development [8, 9, 166–168]. The imbalance between the processes of excitation and inhibition, which may also ensue from the dysfunction of GAT1 transporter, is one of the hypothetical mechanisms of ASD development [15, 82, 169]. Furthermore, GABA is a neurotrophic factor and plays an important role in proliferation of neural stem cells at the early stages of brain development [6, 7]. The damage to this process can be one of the possible causes of ASD.

Intellectual disability (ID). ID is the disorder characterized by mental retardation and associated deficiency of cognitive abilities, speech, and motor skills, leading to the loss of social capacity [170]. The ID is seen in the majority of patients with *SLC6A1* gene mutations, which may develop spontaneously or be associated with another mental or physical disorder [8]. Previous studies reported that one of the most typical manifestations of *SLC6A1* mutations is ID preceding epilepsy in mild or moderate form, usually with speech impairment [10]. Behavioral tests were conducted in the study by Gong et al. on *SLC6A1* knockout mice [5]. It was found that the changes in GABAergic regulation in hippocampus are associated with disturbances in learning, memorizing, and synaptic plasticity. Furthermore, GABA plays a pivotal role in the processes of embryonic development of neural tissue [7], disruption of this process due to GAT1 malfunctioning may also result in ID.

Schizophrenia. Schizophrenia is a polygenic polymorphic mental illness characterized by the loss of mental integrity [171]. The diagnosis of schizophrenia comprises a number of individual syndromes differing in the genetic background and expressiveness of positive, negative, and cognitive symptoms [169, 171]. Currently, pharmacological treatment with antipsychotic drugs is the most successful approach for schizophrenia management and its variable efficiency ensues from the clinical heterogeneity of the disease. The cognitive symptoms, e.g. deterioration of concentration, short-term memory, associative connections, and the absence of integral and sequential thinking are poorly controlled by antipsychotic drugs [172–174]. They occur in all forms of schizophrenia and develop before the positive (hallucinations, delusions, speech and behavior disorganization) and negative (blunted affect, social withdrawal, suppressed desires, anhedonia, and alogia) symptoms [175].

Probably, the clinical heterogeneity of this disease has a genetic origin. Genetic architecture of schizophrenia is very sophisticated [176, 177]. Genetic predisposition majorly contributes to the development of this disorder. It can be related to a certain set of single-nucleotide polymorphisms (SNPs) which are detected with the method of Genome-Wide Association Studies (GWAS) and reflect the polygenic risk score (PRS) of the disease development [98, 178–185]. Genetic predisposition can be also associated with rare mutations inherited from parents and identified by different sequencing methods [16, 186–188]. Interestingly, de novo mutations located in genes associated with schizophrenia were also found in patients with low genetic predisposition estimated by polygenic risk score [16, 189]. In the recent study, Rees et al. have investigated the data of exome sequencing by searching for de novo mutations in 613 trios (patients with schizophrenia and their parents) and combined them with the literature data (3444 trios in total). The additional evidences found in this study demonstrate that certain classes of de novo mutations, including *SLC6A1*, can increase the risk of schizophrenia. The combination of exome sequencing and GWAS results showed that the carriers of de novo mutations related to schizophrenia are at lower polygenic risk than non-carrying patients. These statistically independent results prove probability of disease development in the absence of genetic predisposition [16]. In addition, the correlation between de novo mutations in *SLC6A1* gene and schizophrenia was independently discovered by Schizophrenia exome meta-analysis consortium (SCHEMA) [190].

As already mentioned, gene mutations can result in dysfunction of GAT1 protein and subsequent dysregu-

lation of GABA level in the synaptic cleft [8]. The abundance of neurotransmitter interacting with $GABA_A$ receptors can provoke membrane hyperpolarization of postsynaptic neurons and refractoriness [157]. In turn, the activation of $GABA_B$ receptors with excessive GABA is mediated by the opening of T-type slow voltage-gated calcium channels and, therefore, periodic excitations in the thalamocortical system [191]. The thalamocortical system is involved in the regulation of cognitive processes and short-term memory [192]. Moreover, since direct and indirect excitatory projections on midbrain dopaminergic neurons originate from the prefrontal cortex, a defect of GABAergic regulation in the prefrontal cortex can lead to hyperdopaminergy and thus provoke psychosis [174].

In study [175], a systematic analysis of transcriptomes associated with GABA in the prefrontal cortex of patients suffering from schizophrenia and a control group was performed. In subjects with schizophrenia, deficient expression of GAT1 was detected in PV neurons innervating the initial axonal segments of pyramidal neurons. In addition, $GABA_A$ receptors on postsynaptic membranes of initial axonal segments of pyramidal cells had an increased amount of α2 subunits which was not associated with an increase in the number of receptors. These results reflect the modification of subunit composition of $GABA_A$ receptors with the elevation of neurotransmitter concentration in the synaptic cleft. Normally, the α2 subunit can be seen only in $GABA_A$ receptors located in somatodendritical synaptic contacts [193]. GABA-mediated regulation of the dendritic area of pyramidal neurons is important for filtering excitatory input currents from various cortical and subcortical areas, whereas GABA-regulation of the perisomatic area, i.e. the initial segment of axons and soma, is crucial for the control of action potential generation in time and for synchronization of pyramidal neurons [68, 194]. Disturbed filtering of output signals can ensue from the alteration of subunit composition [156]. Furthermore, this study also described changes in GABA neurotransmission associated with the dysfunction of SST-containing and CCK-containing basket GABA interneurons which form synaptic contacts, predominantly, with distal dendrites and somas of pyramidal neurons, respectively. Changes in the subunit composition of $GABA_A$ receptors were also identified in these areas (a decrease in the amount of α1 and γ2 subunits constituted in postsynaptic receptors responsible for phasic inhibition and α4 and δ subunits of extrasynaptic receptors involved in tonic inhibition) resulting in an alteration of phasic and tonic inhibition on the dendrites of pyramidal neurons.

Thus, in the abovementioned study, modified GABA regulation was found in patients with schizophrenia which affected both input and output segments of pyramidal neurons of the prefrontal cortex, which confirms the results of in vivo studies that showed that the development of cognitive symptoms of schizophrenia involves disturbances in GABAergic signalization in the prefrontal cortex [195] and an imbalance of excitation and inhibition [196]. These changes affect information processing in the prefrontal cortex, thus contributing to the cognitive impairment in patients with schizophrenia [169].

Numerous behavioral abnormalities were seen in GAT1 knockout mice, associated with positive, negative, and cognitive symptoms [157]. GAT1 deficiency did not influence the dopamine level in the striatum, though it substantially enhanced tonic inhibition in the prefrontal cortex.

Significant changes in γ-oscillations, which are involved in cognitive functions and information processing, can be detected in patients with schizophrenia, which correlates with the level of functional disturbances of working memory [197].

GAT1 dysfunction and dysregulation of extrasynaptic GABA concentration can cause the cognitive symptoms of schizophrenia, because of the excitatory role of GABA and its participation in neurogenesis along with BDNF at the early stages of brain development [122].

Therefore, the defects in GABA reuptake are able to provoke different impairments, e.g. changes in subunit composition of $GABA_A$ receptors, or disturbance of phasic and tonic inhibition and neurogenesis, which altogether can induce such diseases as epilepsy, ASD, ID, and schizophrenia.

Neuropsychiatric diseases caused by SLC6A1 mutations: prospects for treatment with genome editing systems. Referring to our assumption that *de novo* mutations in the *SLC6A1* gene are directly related to the whole spectrum of neuropsychiatric disorders, one might wonder whether humanity can introduce methods for treating these pathologies at the current state of its development, preferably eliminating the source of problem rather than only alleviating the symptoms.

Gene therapy is one of the approaches based on the delivery of a full correct *SLC6A1* gene copy to the neurons of the brain, thus potentially recovering the lost functions of GAT1 transporter protein. At present, a number of technologies have come into common use, delivering genes into target cells also with the help of AAV vectors based on adeno-associated viruses [198]. This method allowed elaboration of several drugs for managing spinal muscular atrophy [199], mucopolysaccharidosis [200], Parkinson disease [201], and so on. AAV vectors proved themselves as an effective delivery system that is safe in terms of immunogenicity and oncogenicity [202]. The DNA of the vector does not integrate into the cell genome and persists in the form of episomes, which is a great advantage of AAV vectors, helping to avoid the risk of insertion mutagenesis, unlike lenti- and retroviral vectors. However, there is a chance that this approach might be inefficient in treating *SLC6A1* mutation-related diseases.

First of all, the majority of described *SLC6A1* mutations are heterozygous [9], meaning that the genotype of carriers initially has a non-mutant gene allele, which may be expressed. However, despite this fact, typical symptoms are still observed in these patients. Secondly, the majority of described high penetrant mutations in the *SLC6A1* gene are not knockout, therefore it is unclear how mutant GAT1 protein disturbs the functioning of the nervous system, hence no understanding of how the delivery of a healthy gene copy can help in these cases.

The second approach is based on the modern methods of genome editing, including the use of CRISPR/Cas9. Over the past 8 years, since the first publications on its application in mammalian cells, this system has been actively improving and spreading widely [203], and received worldwide recognition with the Nobel Prize in Chemistry in 2020. Currently, several dozens of gene-therapy drugs based on the CRISPR/Cas9 system are undergoing clinical trials [204]. Theoretically, using this method the sequence of defective *SLC6A1* gene can be returned to the natural wild type, which is more likely to normalize the functioning of nervous system. The evidence of the operating principle of CRISPR/Cas9 system in the neurons in the brain has been shown in several studies [205, 206]. Nevertheless, there are several problems discrediting the application of CRISPR/Cas9 as well as previous systems, like ZFN or TALEN. The first problem is the low efficiency of point target-specific editing of DNA sequences in cells. The main effect of Cas9 protein is a DNA double-strand break in the desired gene locus. This system can be effectively used for gene knockout in cells, because after such a break the NHEJ (non-homology-end-repair) DNA repair systems often make mistakes and include short deletions or insertions, leading to frameshift of the protein coding sequence, if the guide RNA of CRISPR/Cas9 system is made for the protein coding part of the gene [207]. However, targeted changes in *SLC6A1* mutant gene sequences require the HDR (homology-direct-repair) repair system and simultaneous delivery of a DNA donor inside cells, a single-strand or double-strand DNA sequence, which serves as a matrix for synthesis of the correct gene sequence. However, these repair systems compete in mammalian cells and the efficacy of HDR is significantly lower than that of NHEJ and makes up only several percent of all activity [208]. Nowadays, it is topical to elaborate new approaches to fixing point mutations without HDR recombination such as Prime Editing [209]. This system is based on a chimeric RNA guide (pegRNA) which carries the sequence for the correct edited segment on the 3' end and the chimeric mutant protein (PE) nCas9 (a nickase with the single catalytic center of DNA cleavage removed) with a viral reverse transcriptase synthesizing the new correct DNA strand on the spot using the available RNA template. The efficacy of this complex can reach 30–70% depending on the edited genomic region. However, the application of this system is complicated by its delivery since a minimum of two components, pegRNA and PE, must be simultaneously inserted inside cells. The delivery of a large amount of copies, plasmid DNA, or viral vectors into the brain is still a sophisticated problem requiring precise stereotaxic injections for homogenous distribution of particles in a relatively large volume. After the physical delivery of vectors coding the system elements inside brain, the effective penetration of vectors through the cellular membrane should occur, which is another substantial barrier. For example, the efficiency of transfection under the ideal conditions of Petri dish by plasmid vectors with the CRISPR/Cas9 system elements is around 10–15% (unpublished data of the review authors), i.e. the vast majority of cells do not accept plasmids. The size of genetic cassettes is also a challenge, because a single Cas9 gene requires about 4200 base pairs, and the size of Prime Editor cassette reaches up to 7200 base pairs in combination with additional elements, i.e. reverse transcriptase, the promoter, and the WPRE signal. Unfortunately, this fact is another barrier for Prime Editor delivery inside cells using AAV vectors, since the maximal cassette size for them is about 4200–4500 base pairs [210]. The last but not least classical problem of all genome editing systems is off-target effects. Different researchers differently estimate the risk of using CRISPR/Cas9 systems in terms of undesirable mutations [211], though cases of detection of such off-target mutations are scientifically proven. This issue will have to be faced in the process of security proof for treatment methods involving genome editing.

Therefore, it can be stated that multiple obstacles currently prevent the development of treatment methods for *SLC6A1* mutations with genome editing systems, which complicates the easy resolution of this problem in the near future. Nevertheless, it should be emphasized that the abovementioned challenges are mostly technological, which is why the discovery of novel effective methods of delivery and novel effective small-sized genome editors may break the impasse.

CONCLUSIONS

Mental and neurological polygenic diseases have a complex genetic architecture. Currently, no polygenic mental disorder has a comprehensive genetic profile. However, a growing number of studies is dedicated to the analysis of GWAS data [98, 212], sequencing results [213, 214], and approaches to their processing with bioinformatic methods [89, 215]. The samples are expanding; new loci and candidate genes are being discovered. All of the above approximates the creation of genetic portfolios for polygenic diseases.

De novo mutations in the *SLC6A1* gene cause the dysfunction of the GAT1 GABA transporter, leading to impaired regulation of GABA levels and signifi-

cantly enhancing the risk of epilepsy and several mental illnesses, including schizophrenia. The investigation of *SLC6A1* mutations is also important for the comparison of genetic maps of these diseases. Early detection of these mutations with the help of next generation sequencing and development of approaches for their correction may become the first step towards personalized treatment of polygenic diseases.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interests.

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