The role of DTNBP1/dysbindin-1 in schizophrenia

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SVEUČILIŠTE U RIJECI
ODJEL ZA BIOTEHNOLOGIJU

Preddiplomski sveučilišni studij
Biotehnologija i istraživanje lijekova

Ema Belužić

Uloga *DTNBP1*/dysbindin-1 u shizofreniji

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Abstract

Schizophrenia is complex neurological disease that is present in 1% of the general population. It is known as a multifactorial disease because of the simultaneous influence of genetic and environmental factors. Some of the symptoms that are recognized are hallucinations, delusions, loss of energy and motivation and cognitive dysfunctions like working memory dysfunction and dysfunctions in learning. A major problem in treating schizophrenia is its heterogeneity and patients do not respond the same to the same treatment. There is also little to no effect in treating cognitive dysfunction with available medications. We still have limited knowledge of the core of pathology of disease and more research are needed to find the best treatment. In the past years lots of research was performed on genetic risk factors for schizophrenia, and one of the most researched genetic factors is the *DTNBP1* gene. The *DTNBP1* gene encodes for dysbindin-1 protein. In the brain, dysbidnin-1 is mostly expressed in the hippocampus and prefrontal cortex. Studies on post-mortem schizophrenia cases found that there is a decrease in dysbindin-1 expression in brain. These suggest the involvement of dysbindin-1 in the pathology of disease. Dysbindin-1 is known to be a component of the BLOC-1 complex in brain and therefore regulates several functions in the brain including vesicular trafficking following by regulation of neurotransmitter release, neural growth, and transcriptional regulation. Studies on "sandy" mice, with a mutation in the *Dtnbp1* gene also show promising results in its involvement in developing schizophrenia-like symptoms. Researching dysbindin-1 and other genetic risk factors functions in the brain and their binding partners is important in finding new potential targets for future treatment of schizophrenia.

Keywords: DTNBP1, schizophrenia, BLOC-1, neurotransmission, "sandy" mice

Sažetak

Shizofrenija je složena neurološka bolest koja je prisutna u 1% opće populacije. Poznata je kao multifaktorijalna bolest zbog istodobnog utjecaja genetskih čimbenika i čimbenika okoliša. Neki od simptoma koji definiraju ovu bolest su halucinacije, zablude, gubitak energije i motivacije te kognitivne disfunkcije poput disfunkcije pamćenja i disfunkcije u učenju. Glavni problem u liječenju shizofrenije jest njena heterogenost i pacijenti ne reagiraju jednako na isti tretman. Liječenje dostupnim lijekovima je također slabo učinkovito na poboljšanje kognitivnih funkcija. Još uvijek postoji ograničeno znanje o srži patologije bolesti i potrebno je više istraživanja kako bi se pronašlo najbolje liječenje. U posljednjih nekoliko godina provedeno je mnogo istraživanja za otkrivanje genetskih čimbenika, a jedan od najistraženijih genetskih čimbenika je gen *DTNBP1*. Disbindin-1 se najviše eksprimira u hipokampusu i prefrontalnom korteksu. Studije na postmortalnim slučajevima shizofrenije otkrile su da dolazi do smanjene ekspresije disbindin-1 proteina u mozgu. Zbog tih rezultata sugerira se utjecaj disbindina-1 u patologiji bolesti. Poznato je da je disbindin-1 komponenta BLOC-1 kompleksa u mozgu i stoga regulira nekoliko funkcija u mozgu, uključujući transport vezikula, što direktno utječe na regulaciju oslobađanja neurotransmitera. Također regulira razvoj neurona i transkripciju. Studije na "sandy" miševima s mutacijom na genu *Dtnbp1* također pokazuju obećavajuće rezultate u njegovoj ulozi u razvoju simptoma sličnih shizofreniji. Istraživanje disbindina-1 i drugih genetskih čimbenika te njihovih funkcija u mozgu i proteina za koje se vežu, važno je u pronalaženju novih potencijalnih meta za buduće liječenje shizofrenije.

Ključne riječi: DTNBP1, shizofrenija, BLOC-1, neurotransmisija, “sandy” miševi

Table of contents

1. Introduction

Schizophrenia is a complex and serious psychiatric disorder that affects patients' behaviour and cognitive functions. It is present in 0.5-1% of the general population. It is distinguished by symptoms, which can be divided into positive symptoms, such as delusions, disorganized speech, and hallucinations, negative symptoms such as decreased expression, decreased productions of speech, loss of energy and interests, and impairments in cognition, which include working memory, attention, motor speed, learning and executive functions (1). Many of these symptoms are connected to dysfunctions of the dorsolateral prefrontal cortex and abnormalities in hippocampus, followed by dysfunctions in neurodevelopment (2). Dopaminergic neurotransmission dysregulation and glutamatergic neurotransmission dysregulation are also important factors in the mechanisms involved in cognitive dysfunctions. There is still very little known about aetiology of schizophrenia that could help in treatment. Currently, the treatments that is the most commonly used are antipsychotics, which are only effective in dealing with positive symptoms of disease (2). The major problem in treating schizophrenia is the variety of causes that lead to development of disease. There is a variety in symptoms between patients due to these different causes, which leads to considerable differences in their response to treatment, and therefore no treatment is highly effective for every patient.

It has been demonstrated that both environmental and genetic factors have a role in the development of this complex disorder. Some of these environmental risk factors are immigration, urban residence, cannabis use, gender and perinatal occurrences (such as maternal infections), stress, and famine. However, genetics is the single most important known risk factor for schizophrenia (3). As predicted many years ago, based on genetic epidemiological findings, schizophrenia is highly polygenic, with hundreds of characteristic genetic loci involved. Most of the potential candidate genes

were discovered through linkage analysis. Linkage studies were done in the affected families using a polymorphic marker for genome screening to determine chromosome and potential location of risk gene (4). Some of these potential candidate genes are *AKT1, COMT, DISC1, PRODH, DRD3, HTR2A, DTNBP1, G30/G72, HTR2A, NRG1, RGS4, SLC6A4* and *ZDHHC8*. The collected data showed most promise for *DISC1, DTNBP1, NRG1* and *RGS4* because there is linkage evidence for each of these genes and they are supported by multiple studies (5). More recently, new methods for discovering risk genes, such as copy number variation (CNV) studies and genome-wide association studies (GWAS) have provided significant evidence indicating that both common and rare variants have a major part in the development of schizophrenia. GWAS have established more than 100 loci of which are around 70% are genes that encode proteins (6). CNV studies have established 11 rare copy number variants that have been related to a higher risk of schizophrenia. Despite this, the majority of the previously mentioned risk genes have yet to be verified by these association studies.

One of the most researched and promising schizophrenia candidate gene is the dystrobrevin-binding protein 1 gene (*DTNBP1*). The *DTNBP1* gene is located on chromosome 6 on shorter (p) arm at 22.3 position. It encodes the dysbindin-1 protein. Dysbindin-1 is normally expressed in the hippocampus and dorsolateral prefrontal cortex, which both play important roles in cognition. Normally, dysbindin-1 is a part of several protein complexes in brain, and it regulates brain development, as well as, neuronal growth, neurotransmitter release and gene transcription. As a potential risk factor for schizophrenia, it was firstly identified in a report from the Irish Study of High-Density Schizophrenia Families. They did analysis using single nucleotide polymorphism (SNP) markers and found that, on chromosome 6p22.3, there were several SNPs that seemed to be associated with schizophrenia. They cloned a cDNA from this locus and identified the sequence as the *DTNBP1* gene (7). Other studies also show that there is an

association between SNPs of the *DTNBP1* gene and phenotypes in schizophrenia patients. SNP rs1997679 and SNP rs9370822 were researched and associated with visual hallucinations in schizophrenia patients, SNP rs909706 was associated with loss of attention and SNP rs9370822 is associated with glutamatergic or dopaminergic neurotransmission dysfunction (8). A connection to schizophrenia is also indicated by dysbindin-1 expression levels in the brain. Expression levels of both dysbindin-1 and *DTNBP1* mRNA is decreased in the dorsolateral prefrontal cortex, which was shown by post-mortem studies looking into levels of dysbindin-1 in brain of patients with schizophrenia (8). In addition to these connections, a study with mice was performed, that had a deletion mutation in the *Dtnbp1* gene ("sandy" mice), which showed some promising results. In this study, behavioural analyses were preformed, and the results showed abnormalities in dopaminergic and glutamatergic transmission followed by lower levels of dopamine (9). These finds show that there is significant connection between mutations of *DTNBP1* gene and schizophrenia disease that needs to be researched further.

2. Purpose of review

The goal of this review is to look at all the normal functions and cellular roles of dysbindin-1 in the brain that could be linked to schizophrenia symptoms or disease pathology. Dybindin-1 has numerous activities in the brain, as well as important interactions with complexes and cellular proteins involved in schizophrenia development pathways. Given the lack of promising treatments, investigating genetic risk factors is important to help in understanding illness aetiology. Identifying the normal activities of risk factors, conducting more research on animals with specific gene mutations, and lastly linking the results to the symptoms of schizophrenia could each lead to finding better treatments. The purpose of these review is to state every possible association of schizophrenia disease and mutations in the *DTNBP-1* gene that may be useful in finding the new targets for medication in the future treatments of disease.

3. Dysbindin-1 protein

Dystrobrevin-binding protein is a protein that is encoded by the *DTNBP1* gene which is around 140 kb long on 6p22.3 position and has 10 exons. The protein is composed of around 350 amino acids and has two coiled-coil domains. In the brain, dysbindin-1 is mostly expressed by neurons in the hippocampus and the dorsolateral prefrontal cortex (8). As a result of alternative splicing of *DTNBP1* mRNA, dysbindin-1 has 3 isoforms, 1A, 1B and 1C (Figure 1). Dysbindin-1A and 1C share a domain rich with proline (P), glutamic acid (E), serine (S) and threonine (T) (the PEST domain), while dysbindin-1B lacks this domain. In addition, dysbindin-1C lacks 81 amino acids at its N-terminus (Figure 1). All three of isoforms are expressed in neuronal cells: dysbindin-1A and 1B are mostly expressed in the nucleus, while dysbindin-1C is expressed in the cytosol. Dysbindin-1A is found at postsynaptic densities, isoform 1B in synaptic vesicles and 1C is expressed in both. In schizophrenia patients, it was discovered that the levels of dysbindin-1B and dysbindin-1C are reduced, while levels of dysbindin-1A were the same as in healthy patients. Out of all the isoforms, dysbindin-1C has been linked most often to neurogenesis and neurodevelopment (10)(11).

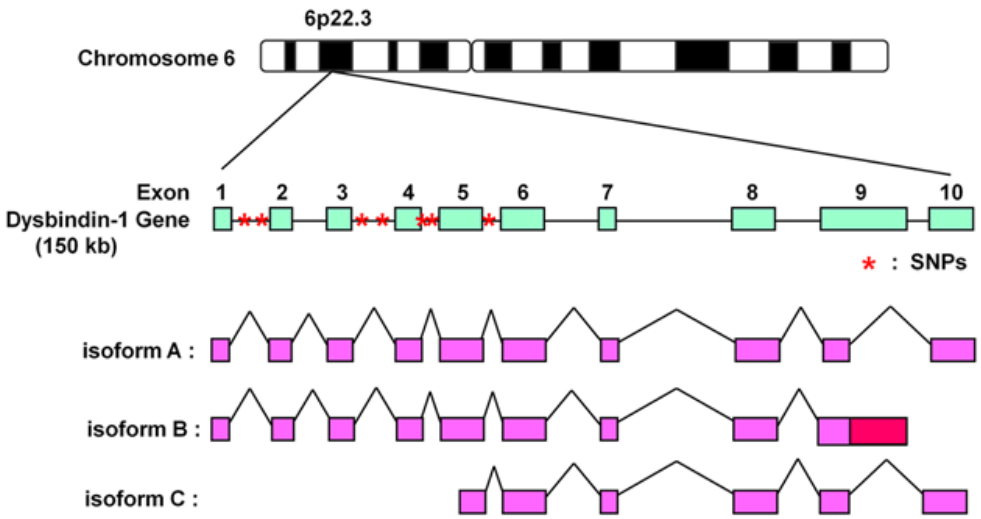

Figure 1. Dysbindin-1A, 1B and 1C isoforms as a result of mRNA alternative splicing. The red parts represent some of the major SNPs that are

associated with schizophrenia-like symptoms. Taken from Oyama et al 2009 (12)

3.1. Deletion of dysbindin-1 gene

In order to investigate which brain functions are disrupted by mutation of the dysbindin-1 gene, many studies did research on mice with deletion of the *Dtnbp-1* gene or with mutations on same gene. Sandy (sdy) mice do not express dysbindin-1, because of a 38,129 nucleotide deletion in the *Dtnbp1* gene. It was initially discovered when the mutation occurred spontaneously in the DBA/2J mouse strain, which has defects in lysosomes and melanosomes. Since then, this strain has been used for research due to its dysbindin-1 mutation. Several tests were performed to analyse cognitive and behavioural abilities of these mice. Differences in overall health and appearance, beside the colour that was different due to melanosome mutation, were not seen between the wild-type mice and sdy mice, but the tests showed some abnormal behaviours. Firstly, in the open field test it was discovered that the locomotor activity and exploratory behaviour of sdy mice were reduced, compared to wild-type mice. This decreased activity of sdy mice can be connected to negative symptoms of schizophrenia. Next, they found that sdy mice had impaired motor learning, which is connected to the cognitive deficits and learning deficits in schizophrenia patients. Other cognitive deficits that sdy mice showed were impaired memory preservation and impaired working memory, which is directly connected to schizophrenia since almost all of patients show disturbed memory function (13). The main cause of cognitive deficits in these dysbindin-1 knockdown mice is thought to be abnormal glutamate transmission regulation in the mossy fibre and dentate gyrus, which are thought to play an important role in working memory.

Some studies show that the DBA/2J genetic background and its associated mutations could have an impact in behaviour, lead to deficits in locomotor activity and other deficits, making it harder to understand the specific

effects of loss of dysbindin-1. Therefore, they transferred the *Dtnbp1* mutation onto mice with C57BL/6J background (dys-/-). In these mice, the results were similar, showing clear schizophrenia-like symptoms. Firstly, they did the open filed test, and the dysbindin-1 deficient mice travelled a greater distance then the wild- type mice. This showed hyperactivity, which is connected to disruptions in dopaminergic transmission. This was also the only significant difference in a behaviour between DBA/2J and C57BL/6J background mice. The cause of hyperactivity is thought to be a dopamine dysfunction. Some other finds in this study are that Morris water maze test showed that learning and spatial memory are impaired in dys-/- mice which is also connected to disruption in hippocampal function and also suggests a role of dysbindin-1 in cognitive functions (14).

These finds show that there is a direct connection between lower levels of dysbindin-1 protein in the brain and symptoms common in patients with schizophrenia. Research on dysbindin-1 knockdown mice support the idea that disruption of neurotransmission, which can be seen in the patients with schizophrenia, is directly related to expression levels of dysbindin-1, as well as the importance of the cellular interactions that dysbindin-1 protein is a part of.

3.2. Dysbindin-1 interactions with proteins

It is known that dysbindin-1 interacts with many proteins, which indicates its variety of biological functions in the brain and other tissues. It interacts with key complexes to affect neuronal and muscle morphologies, neurotransmitter release and signal transmission, as well as neuronal growth and gene transcription. It was initially found to be a component of the dystrophin-associated protein complex (DPC) by its interaction with α-dystrobrevin and β-dystrobrevin (15). It is also a component of the biogenesis of lysosome-related organelles complex 1 (BLOC-1) by interacting with several other components of this complex (16). Besides

these complexes, dysbindin-1 interacts with DNA-dependent protein kinase (DNA-PK), nuclear factor-kappa B (NF-κB), histone deacetylase 3 (HDAC3) and disrupted in schizophrenia 1 (DISC1) (8). All these interactions are essential for normal neurodevelopment and neuronal activity.

3.2.1. Dystrophin-associated protein complex

Initially, dysbindin-1 was found to be a component of the dystrophin-associated protein complex (DPC). DPC is a complex located mostly in skeletal and cardiac muscle, as well as the brain. It is required for the maintenance of muscular integrity and normal muscular function (17). It is formed of the sarcoglycan, dystroglycan, and cytoplasmic complexes, which include α- and β-dystrobrevin and the α-, β1-, and β-syntrophins (Figure 2). Based on immunocytochemical localization of some components of DPC, it was found that DPC-like complexes are localized in the brain as well. In the brain they are involved with cognitive impairment, that is seen in patients with Duchenne muscular dystrophy. Dysbindin-1 binds to both α- and β-dystrobrevin in muscle. In brain, β-dystrobrevin is localized in postsynaptic densities and in axon and neuronal nucleus. Preforming co-immunoprecipitation of dysbindin-1 and β-dystrobrevin in brain suggested that they form a complex in axons, which shows dysbindin-1 involvement in DPC-like complexes function in the brain (17).

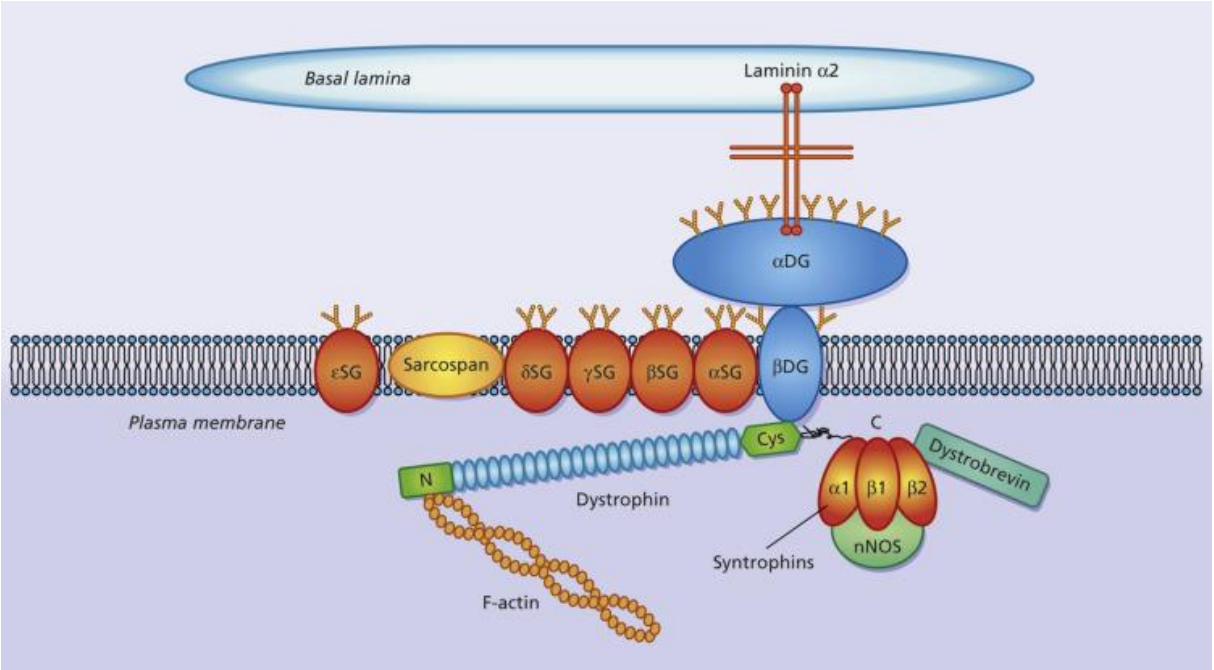

Figure 2. Structure of the Dystrophin-associated protein complex, composed of sarcoglycan and dystroglycan proteins as membrane complexes and dystrobrevin and syntrophins as cytoplasmic complexes. Taken form Brown et al 2017 (18).

3.2.2. Biogenesis of Lysosome-related Organelles Complex-1

Most of the dysbindin-1 molecules in the brain are part of the Biogenesis of Lysosome-related Organelles Complex-1 (BLOC-1), which is a protein complex that is required for the normal function of endosomal trafficking. As a part of this complex, dysbindin-1 is found in axonal or synaptic terminals. BLOC-1 is located on transferrin-receptor positive endosomes, where it is important for the transport of membrane proteins to lysosomes, synaptic vesicles, and lysosome-related organelles (19)(Figure 3). It was also discovered to regulate cell surface expression of the D2 dopamine receptor, as well as neurite development and, because of these functions, it has been associated with risk of neurodevelopment disorders. The BLOC-1 complex has eight subunits: BLOS-1, BLOS-2, BLOS-3, dysbindin-1, snapin, cappuccino, muted and pallidin. Some of these subunits are connected into two larger units; the first unit consists of dysbindin-1, snapin and BLOS-2, and the second unit is formed by pallidin, cappuccino and BLOS-1. These are necessary for its function, and lack of any subunit results in the absence of all BLOC-1 protein complex subunits in the brain (20). The connection between each subunit was confirmed in a study where they performed experiment on sandy mice, as well as, on the mice with a null mutation in the gene encoding pallidins and the results showed that the levels of other BLOC-1 proteins were lower. As expected, “sandy” mice showed behavioural abnormalities, but it was also reported that mice with pallidin gene mutations show impairment of social recognition memory, which show us that impairment of BLOC-1 subunits can lead to changes in cognitive behaviour and greater susceptibility to neurodevelopmental disorders and present risk of development of schizophrenia (21). Loss off dysbindin-1, which directly leads to loss of the BLOC-1 complex, can directly affect several cell functions including membrane protein sorting and membrane fusion.

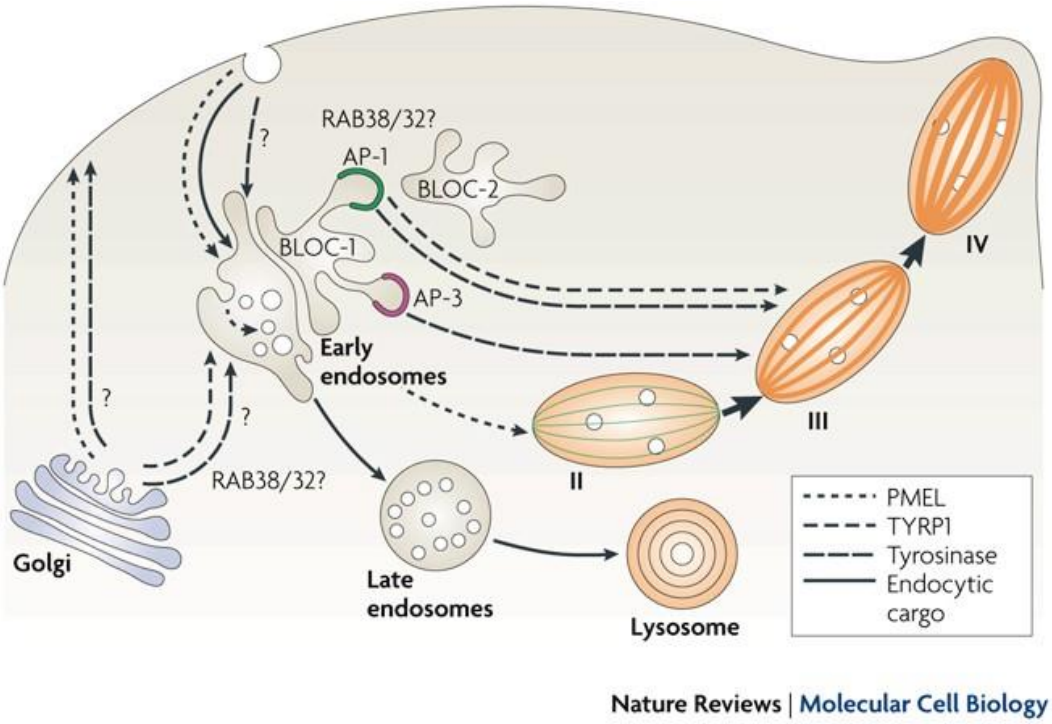

Figure 3. Process of trafficking proteins to lysosome-related organelles showing all the proteins and the complexes that are involved in this process. BLOC-1 is important in the early stage of trafficking proteins from the early endosome toward the late endosome. Taken from Raposo et al 2007 (22)

3.2.3. BLOC-1 binding partners

BLOC-1 interacts with subunits of the adaptor protein 3 complex (AP-3). AP-3 is important for sorting signals in membrane proteins, which are transferred to lysosomes and lysosome-related organelles. Their connection was seen in BLOC-1 deficient mice, which showed decreased AP-3 expression, directly indicating influence of dysbindin-1 on protein sorting mechanisms (23). This decreased AP-3 expression was associated with incorrect sorting of vesicles, leading to disruptions in neurotransmission, which are in turn connected to disturbed levels of neurotransmitters in patients with schizophrenia (24). BLOC-1 also interacts with SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins, which have a role in neurite outgrowth. SNARE proteins are important in regulating normal fusion and docking of vesicles to plasma membrane in

the process of exocytosis. It was determined that BLOC-1 can bind to SNAP-25 and syntaxin 13, which are members of SNARE proteins and are involved in the fusion that happens during neurite outgrowth. In BLOC-1 deficient neurons of mice, disruption in the ability of neurons to extend neurites during development was seen. This findings confirm that deficiency in dysbindin-1, and therefore deficiency in BLOC-1, can lead to disruption in neurite outgrowth (25).

4. Dybindin-1 binding partners

It is known that dysbindin-1 interacts with several other proteins besides dystrobrevin and BLOC-1 components. Its interactions in the brain are thought to be a main reason for its association with pathology of schizophrenia disease. By interacting with other proteins, dysbindin-1 can directly regulate cell functions such as neurotransmitter release, neuronal plasticity and inflammatory response. While most of dysbindin-1 in the cell is localized in the cytosol as a part of the major BLOC-1 complex required for normal endosomal trafficking, dysbindin-1 has also been found that interacts with proteins in the nucleus. Research showed that dysbindin-1 is a nucleocytoplasmic shuttling protein, which means that its nuclear import and export are regulated. It is found in nucleus as a result of leptomycin B, which functions as a nuclear export inhibitor (26). The study showed that dysbindin-1 is degraded faster in nucleus than in cytosol, by the ubiquitin-proteasome pathway (27). Its stability can be enhanced by interacting with Disrupted-in-schizophrenia 1 (*DISC1*). *DISC1* is one of the major susceptibility risks factors for schizophrenia, along with dysbindin-1. It plays a role in neuronal proliferation, synapse formation and neurodevelopment. Researching potential connection between dysbindin-1 and *DISC1*, showed that they form a functional complex. It was previously determined that *DISC1* can regulate ubiquitylation of some of its binding substrates, including dysbindin-1. The level of dysbindin-1 was measured after knockdown of *DISC1,* and results showed that concentrations of dysbindin-1 were lower without *DISC1* interaction (28).

4.1. Synapsin 1

Dysbindin-1 in the nucleus regulates several proteins, one of which is the synaptic vesicle phosphoprotein synapsin 1. Synapsin 1 regulates the kinetics of neurotransmitter release by controlling synaptic vesicle fusion (26). It has been found that in the hippocampus of schizophrenia patients there is lower expression of synapsin 1 then in the healthy population. One study showed that dysbindin-1 in the nucleus increases expression of synapsin-1 by interacting with the promoter of synapsin 1. In "sandy" mice, it was also seen that the levels of synapsin 1 mRNA were lower. This proves that a decrease in dysbindin directly causes decreases in synapsin 1, and so regulates neurotransmission (26).

4.2. NF-κB

Regulating the transcription of many proteins involved in normal brain development, dysbindin-1A interacts with nuclear factor-κB (NF-κB). NF-κB is a major transcription factor that regulates inflammatory responses. In neurons, it is important for cell survival, neuronal growth, and synaptic plasticity. Dysbindin-1 interacts with p65, which is a subunit of NF-κB and through that promotes the transcription activity of NF-κB. It was found that, with deletion of dysbindin-1, the concentrations of TNF-α, which is the main target of NF-κB, are significantly lower. In addition, expression of Protein Kinase cAMP-Activated Catalytic Subunit Alpha and Matrix Metallopeptidase 9, which are regulated by NF-κB and have a role in neuronal plasticity, was decreased in the absence of dysbindin-1 (27). This data shows us that dysbindin-1 in the nucleus can regulate NF-κB activity and have an important role in neuronal growth and plasticity. Interacting with NF-κB, dysbindin-1 affects the transcription of Myristoylated Alanine-Rich Protein Kinase C Substrate (MARCKS) (26). MARCKS has many functions in development, brain plasticity and immune response. It shows an impact on

neurotransmission and vesicular transport as well. It was discovered that dysbindin-1 with NF-κB can regulate MARCKS transcription by binding to the promoter region. In dysbindin-1 knockdown cells levels of MARCKS expression were increased, which means that neurotransmission is increased and can relate to increased dopaminergic transmission in brains of schizophrenia patients (29).

4.3. DNA-dependent protein kinase

In the nucleus, dysbindin-1 also interacts with DNA-dependent protein kinase (DNA-PK). DNA-PK is a large enzyme that repairs double strand DNA breaks. It has catalytic subunit and Ku heterodimer that consists of p68 (Ku80) subunit and p70 (Ku70) subunit. It was found, by using immunoprecipitation, immunocytochemical staining, and subcellular fractionation methods, that dysbindin-1 in brain binds to the Ku80 and Ku70 subunits. While interacting with subunits of kinase, dysbindin-1 is a phosphorylation target. Results showed that DNA-PK phosphorylates the dysbindin-1A and 1B isoforms, while isoform 1C was not found in the nucleus (12). Another target of this kinase in the nucleus is Histone Deacetylase 3 (HDAC3). HDAC3 is a transcription regulator that deacetylates histone tails. In patients with schizophrenia, it was found that expression levels of this enzyme were higher in prefrontal cortex than in healthy patients. In addition, it was also discovered that mice without HDAC3 in the hippocampus had enhanced long-term memory, which suggests that higher levels of HDAC3 in the hippocampus have a negative effect on long-term memory. In the nucleus, dysbindin-1 interacts with HDAC3 and the two form a complex (30). This interaction may be important for the activity of HDAC3, as research showed that in presence of dysbindin-1, phosphorylation of HDAC3 is increased. In this way, dysbindin-1 can directly affect activity of HDAC3 and its functioning in brain.

Putting all of these interactions together, dysbindin is involved in major processes in brain and as a part of each pathway it can influence many neuron and synaptic functions. Dysbindin-1 can directly regulate neurotransmission by binding to synapsin-1 which is important in synaptic vesicle fusion. It also regulates transcription of many proteins that have a role in plasticity and neuronal growth, by binding to NF-κB. Lastly, it was found that dysbindin-1 can regulate phosphorylation of the HDAC3 enzyme, which is important for long-term memory in schizophrenia patients. With decreased levels of dysbindin-1 in the hippocampus and prefrontal cortex, dysfunction of these protein pathways can lead to severe cognitive deficits and synaptic disturbances.

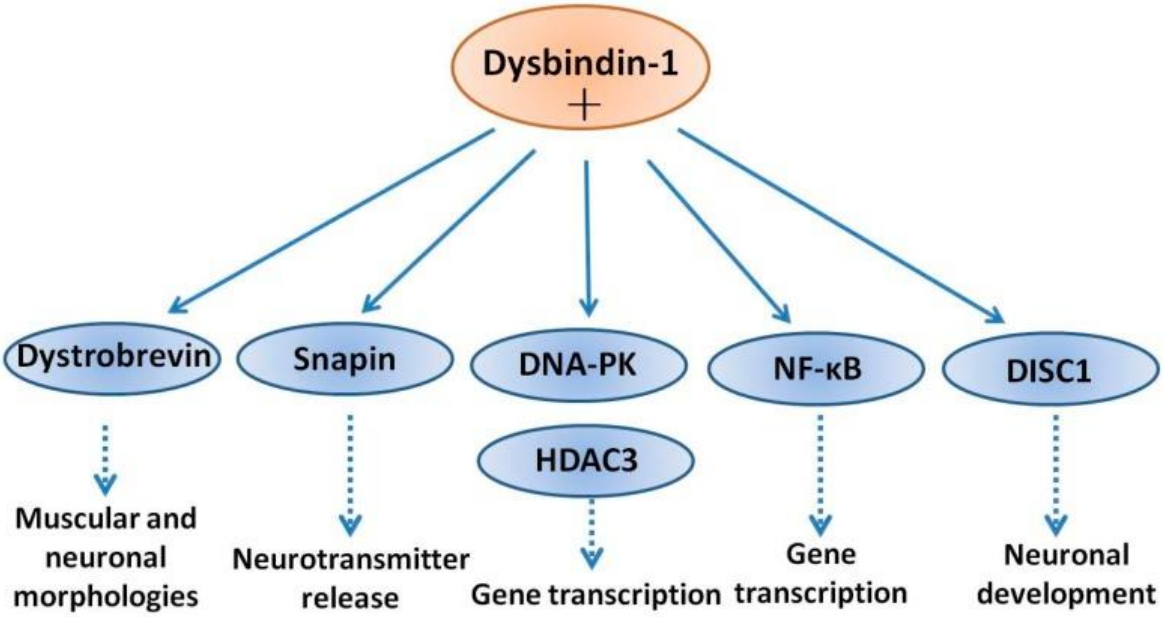

Figure 4. Proteins that dysbindin-1 interacts with, and the functions associated with these pathways. Taken from Wang et al 2007 (8).

5. Neurotransmission regulation

Normal neurotransmission between neurons is needed for normal function of the central nervous system, including motor function and cognitive processes such as learning, memory, and attention. Dysfunction of neurotransmission or receptor function can cause disruption in cognition and schizophrenia-like symptoms. Schizophrenia was before mostly considered a disease of dopamine dysfunction, leading to the dopamine hypothesis of schizophrenia. Typical antipsychotics for treating schizophrenia are also directed toward dopamine pathway, with antagonists of D2 receptors being widely prescribed for psychotic symptoms. Since treatments are not effective enough and dopamine antagonists are not effective in treating cognitive deficits, new targets for treatment are researched and studies revealed the dysregulation in glutamate and gamma-aminobutyric acid transmission in patients with schizophrenia as well (2). The major role that dysbindin-1 has in pathology of schizophrenia is its localization on the presynaptic and postsynaptic sites and therefore it can regulate dopamine neurotransmission following by the regulation of other receptors that are found to be impaired in patients with schizophrenia. The role of dysbindin-1 in these pathways is not completely understood, but some studies suggest involvement in lysosome related pathways by being a component of BLOC-1 is a connection between levels of dysbindin-1 in brain and expression of receptors involved in neurotransmission.

5.1. Dopamine receptors

Dopamine signalling was shown to be important for regulating motor and cognitive functions, as well as human emotion, behaviour, and motivation. Increased dopamine release can lead to psychosis and, because of this, dopamine signalling dysfunction is connected to the aetiology of schizophrenia. Studies with knockdown of dysbindin-1 show that it leads to increased cell expression of dopamine D2 receptors resulting in increased release of dopamine in brain. Using methods for quantification of D2 receptors on membranes, a study showed that, following dysbindin-1 expression, there was decrease in D2 membrane receptors. The reason for this increased expression of D2 receptors in patients with schizophrenia is thought to be impaired internalization of protein. After internalization, the D2 receptor is trafficked for degradation through the lysosome pathway (31). The explanation for the impairment in this process might be that dysbindin-1 is connected to the BLOC-1 complex, which is important for protein trafficking to lysosomes, and in this way it can regulate internalization and degradation of D2 receptors (32). By regulating D2 receptor internalization, dysbindin-1 also regulates dopamine signalling and several other signalling pathways. Through regulation of D2 receptors, dysbindin-1 reduces the phosphorylation of Akt and ERK1/2 and has an control over these signalling cascades (32)(Figure 4).

In a study with the dys -/- mice, the open filed test showed hyperactivity of dys-/- mice. The cause of hyperactivity is thought to be a dopamine dysfunction. As stated previously, lack of dysbindin-1 leads to an increase in D2 receptor cell surface expression and therefore an increase in dopamine release in brain in hippocampus (14).

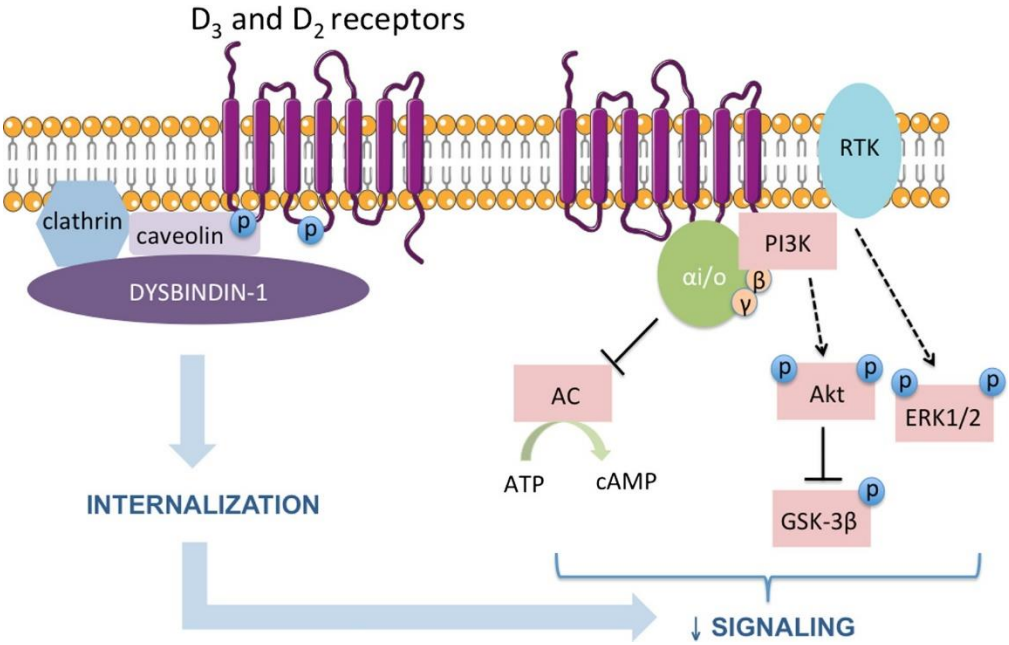

Figure 4. Dysbindin-1 regulates cellular expression of D2 receptors by regulating internalization and degradation through lysosome pathway. In addition, dysbindin-1 also regulates the dopamine induced signalling cascades. Taken from Schmieg et al 2016 (32).

5.2. Glutamate release

Glutamate is one of most prevalent neurotransmitters in the human brain and is one of the primary excitatory neurotransmitters. The N-methyl-D-aspartate (NMDA) receptor is the glutamate receptor that is important in excitatory neurotransmission and synaptic plasticity (33). It consists of two NR1 subunits and two NR2 subunits. In post-mortem studies in patients with schizophrenia, it was found that there is disturbance in glutamate transmission and reduced NMDA receptor expression. This state, with impaired glutamate transmission, can lead to psychosis and schizophrenia-like symptoms. Dysbindin-1 is found in the axonal terminal of glutamatergic neurons and is involved there in normal glutamate trafficking and transmission (Figure 5). In research using sandy mice it was found that mRNA expression of the NR1 subunit is decreased, leading to decreased expression in NMDA receptors. This decrease in NMDA receptors can lead to decreased activity of the prefrontal cortical network, which can affect working memory. Such deficits in the working memory are reported in schizophrenia patients (34).

On the other hand, dysbindin-1 has a presynaptic impact as well. It was found that it regulates vesicular glutamate transporter-1 (VGluT-1) expression and that, with decreased levels of dysbindin-1, there is increase in VGlut-1. It was found that VGlut-1 is increased by 75% schizophrenia patients. It is suggested that this happens because of disruption in the VGlutT-1 expression and degradation processes in which dysbindin-1 is involved through its interaction with BLOC-1 components and its connection to AP-3 complex in lysosome pathway (35). Patients using NMDA receptor antagonists showed symptoms similar to symptoms of schizophrenia. This result implies that dysfunction of glutamatergic transmission could present a functional target in future treatment of schizophrenia, because of a possibility of treating cognitive deficits unlike the D2 receptors antagonists which are not effective on cognitive functions (36).

In sandy mice, a study showed the cognitive deficits associated with lack of dysbindin-1 protein in brain, the main cause of cognitive deficits is abnormal glutamate transmission regulation in the mossy fibre and dentate gyrus which are thought to play an important role in working memory. Dysbindin-1 regulates the expression of pre-synaptic proteins synapsin 1 and SNAP25, which are involved in vesicle trafficking and neurotransmitter release. Following this, dysbindin-1 regulates exocytosis of glutamate by regulating these proteins. The impairment leads to dysfunction of glutamatergic transmission in the dentate gyrus and has an effect on cognitive functions (13).

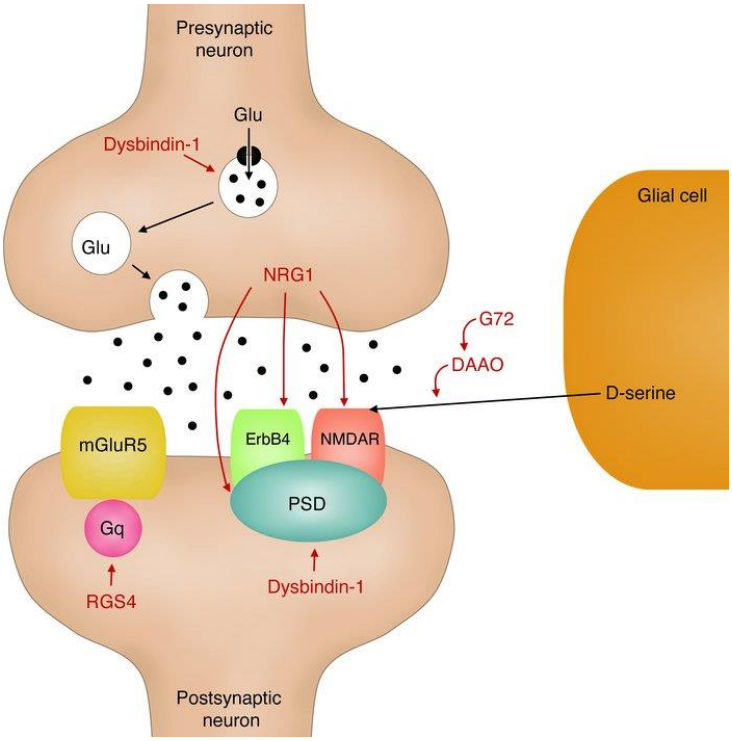

Figure 5. The presynaptic and postsynaptic impact of dysbindin-1 in glutamatergic neurotransmission. Taken from Owen et al 2014 (37).

5.3. GABA receptors

In schizophrenia patients, levels of Gamma-aminobutyric acid (GABA) synthesizing enzyme GAD-67 and of the GABA transporter have been shown to also be decreased in prefrontal cortex in schizophrenia patients where the dysbindin-1 is normally expressed (38). GABA is the primary inhibitory neurotransmitter in the central nervous system (CNS). GABAergic interneurons are important for stabilizing neuron activity and decreasing excitability. This balance between inhibition and excitation is crucial for normal cognitive functions of the brain (39). Disruptions in GABAergic transmission may be due to decreased frequency of miniature inhibitory postsynaptic currents (mIPSCs) and spontaneous inhibitory postsynaptic currents (sIPSCs). The disruptions in these gamma-band oscillations had been connected to cognitive deficiencies and hallucinations symptoms in schizophrenia (40). A study with the dysbindin-1 mutant mice showed that the amplitude and frequency of sIPSCs was reduced in prefrontal cortex leading to impaired gamma-band oscillations. The mutation on dysbindin-1 gene also showed decreased GABAergic interneuron excitability (41). In several studies it was also shown that GABA agonists are effective in the treatment of schizophrenia symptoms (42).

6. Conclusion

Schizophrenia is a complex disease because of its many risk factors, both genetic and environmental. Research into genetic risk factors is the most promising way to get some new insights in the pathology of disease which will help in the developing of the new treatment. To this day there is still not enough knowledge of which genes are involved in development of schizophrenia, and what are their main neurological functions that can be compromised. It is not enough to only concentrate on one risk factor or symptom because of the complexity of disease and simultaneous involvement of different factors.

DTNBP1, along with a few of other gene risk factors is for now one of the most researched factors for its involvement in the disease. The dysbindin-1 protein itself is a part of some major processes that have a role in normal regulation of neuron development and neurotransmission. It became a promising risk factor because of its expression levels in brains in patients with schizophrenia, which are decreased. In addition, several SNPs on the *DTNBP1* gene were found to have a connection to schizophrenia symptoms, including hallucinations and lack of attention. Its involvement in the important complex BLOC-1, which has a role in regulating vesicular trafficking, is thought to have an impact on the dysfunctions in neurotransmission that happens in the brains of patients with schizophrenia.

There are several treatments that are used today, including older typical antipsychotics that target D2 receptors and more recent atypical antipsychotics that target serotonin and dopamine neurotransmission. The effectiveness of these treatments on cognition deficits in people with schizophrenia remains an issue, and some patients do not respond to the treatment. In this review, I have described functions of dysbindin-1 and shown dysfunctions with the mutation on the dysbindin-1 gene show some new potential targets for treatment of schizophrenia. With more research

into some of its binding partners, and into the mechanisms behind these interactions there is a way to find new molecules that could potentially be antagonist or agonist for the same binding spots and have a positive effect on schizophrenia symptoms that occur due to lack of the dysbindin-1 protein. In order to research further into the functions of dysbindin-1 that are dysfunctional in schizophrenia, there needs to be more of the studies with animal models that have mutation of the *Dtnbp-1* gene, and only of that gene, so that specific effects can be seen. Following this, studies with several mutations on risk genes for the development of schizophrenia could show some new insights on the interactions between different proteins, which could be important in increasing the risk of disease. In addition, it is important to include some of the environmental risk factors into this research because of the multifactorial nature of disease and concentrating on only geniting factor may not give the expected results.

This insight into functions of one of the risk genes has a purpose in showing that there is still plenty of research to be done for solving the problem of treating schizophrenia. With each new piece of research done on other proteins involved in pathology of schizophrenia there is a chance in discovering new potential targets for treatment in the future.

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German

Listening	Reading	Spoken production	Spoken interaction	Writing
B1	B1	B1	B1	B1

French

Listening	Reading	Spoken production	Spoken interaction	Writing
A1	A1	A1	A1	A1

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A project that encourages the education of younger generations in the basics of natural sciences by demonstrating simple and interesting experiments.

01/10/2019 – CURRENT

Project "Student mentor"

University of Rijeka, Department of Biotechnology
Assistance to new students in the Department of Biotechnology in finding their way and adjusting to their studies.

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