Role of Munc18-1 in the biological functions and pathogenesis of neurological disorders (Review)

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Abstract. The release of neurotransmitters following the fusion of synaptic vesicles and the presynaptic membrane is an important process in the transmission of neuronal information. Syntaxin-binding protein 1 (Munc18-1) is a synaptic fusion protein binding protein, which mainly regulates synaptic vesicle fusion and neurotransmitter release by interacting with soluble N-ethylmaleimide sensitive factor attachment protein receptor. In addition to affecting neurotransmitter transmission, Munc18-1 is also involved in regulating neurosynaptic plasticity, neurodevelopment and neuroendocrine cell release functions (including thyroxine and insulin release). A number of previous studies have demonstrated that Munc18-1 has diverse and vital biological functions, and that its abnormal expression serves an important role in the pathogenesis of a variety of neurological diseases, including epileptic encephalopathy, schizophrenia, autism, Parkinson's disease, Alzheimer's disease, multiple sclerosis, Duchenne's muscular dystrophy and neuronal ceroid lipofuscinosis. The present review summarizes the function of Munc18-1 and its possible relationship to the pathogenesis of various neurological diseases.

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1. Introduction

The release of neurotransmitter caused by the fusion of synaptic vesicles and the presynaptic membrane is an important process in the transmission of neuronal information (1). This process is regulated by a series of key proteins, including soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE), protein transport protein Sec1 (Sec1)/ Mammalian uncoordinated (Munc) 18, N-ethylmaleimide sensitive factor (NSF), soluble NSF adaptor protein (SNAP), synaptotagmin-1 and Munc13, among others (2). SNARE protein is the core complex that establishes membrane fusion, which is mainly composed of SNAP25, vesicular-associated membrane protein and syntaxin-1. SNAP25 and syntaxin-1 form the target membrane of the vesicle protein (T-SNARE), which binds to the synaptic vesicle protein (3). SNARE complex assembly is an important step in the fusion process of synaptic vesicles and the presynaptic membrane (4). SNARE protein-mediated synaptic vesicle fusion and secretion are regulated by a variety of proteins, of which Sec1/ Munc18 (SM) serves an important role. Previous studies have revealed that the absence of the SM protein can inhibit membrane fusion in different systems, such as the endocrine and the vascular system (5-8).

The SM protein family is an indispensable regulatory protein for membrane fusion. It is a highly conserved polypeptide chain with a molecular weight of 60-70 kDa and a length of ~600 amino acids (9). The human genome contains 7 homologous SM proteins, of which syntaxin-binding protein (Munc18)-1, Munc18-2 and Munc18-3 are involved in secretion, with Munc18-1 being highly expressed in neurons and neuroendocrine cells (10). Munc18-1 is a synaptic fusion protein binding protein and is encoded by the syntaxin-binding protein 1 gene (STXBP1), which is located on chromosome 9q34.11 and contains 20 exons (11). The protein encoded
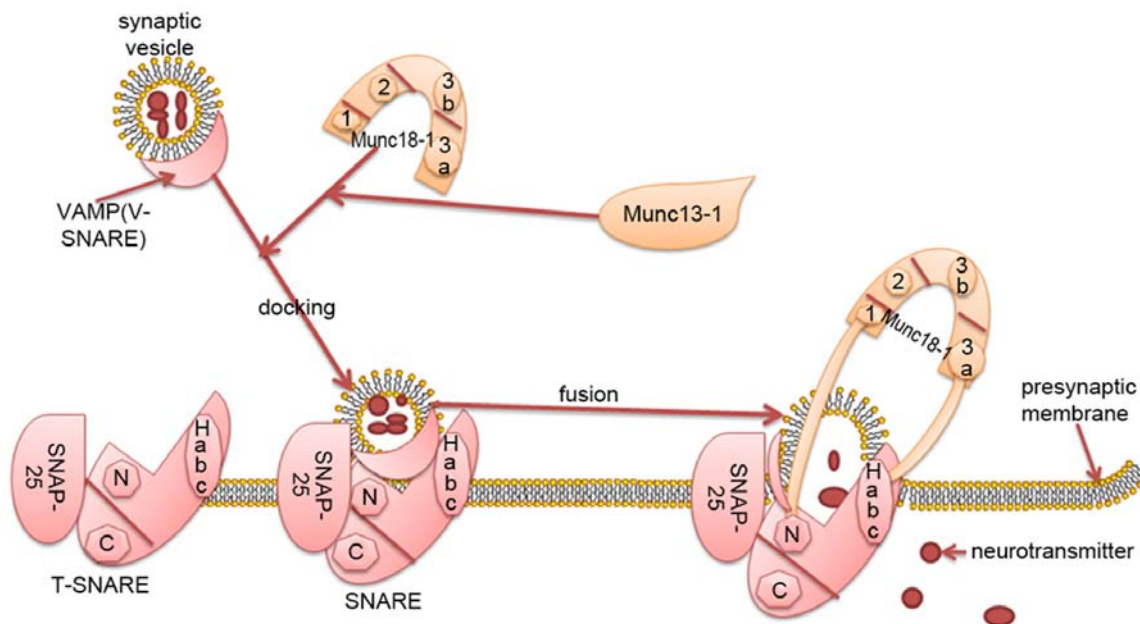
Figure 1. Munc18-1regulates neurotransmitter transmission by interacting with the SNARE complex. The SNARE protein is mainly composed of SNAP-25, vesicular-associated membrane protein and syntaxin1. SNAP-25 and syntaxin1 form the target membrane of vesicle protein (T-SNARE), which binds to synaptic vesicle protein (VAMP). Munc18-1 has a complex, arched, tertiary structure. The arch consists of four closely connected domains named 1, 2, 3a and 3b. Domain 1 and 3a form an arched gap. Syntaxin-1 is the core protein of SNARE. Munc18-1 primarily regulates vesicle fusion by interacting with syntaxin-1 (Habc domain; N-terminal short peptide). Domain 3a is in close contact with the Habc domain of syntaxin-1, domain 1 is located on the other side of the arched gap and binds to the N-terminal short peptide of syntaxin-1. Munc13-1 bridges synaptic vesicles and presynaptic membrane fusion to coordinate the assembly of SNARE with Munc18-1. Munc18-1 regulates vesicle docking and fusion by interacting with the SNARE complex, affects the released vesicles and participates in the transmission of neurotransmitters. Munc, mammalian uncoordinated; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor; SNAP25, synaptosomal-associated protein of 25 kDa; T-SNARE, target membrane of soluble N-ethylmaleimide-sensitive factor attachment protein receptor; V-SNARE, synaptic vesicle protein of soluble N-ethylmaleimide-sensitive factor attachment protein receptor; VAMP, vesicular-associated membrane protein; N, N-terminus; C, C-terminus; 1, domain 2; 2, domain 2; 3a; domain 3a; 3b, domain 3b.

by the STXBP1 gene is highly conserved in evolution. The occurrence of missense, nonsense, frameshift and splicing mutations can lead to insufficient protein haploids, impaired protein stability and cortical excitation imbalances that affect learning and memory (12-14).

Syntaxin-1 is the core protein of SNARE and Munc18-1 primarily regulates vesicle fusion by interacting with syntaxin-1 (Habc domain; N-terminal short peptide) (15). The tertiary structure of Munc18-1 is complex, exhibiting an arched structure, consisting of four closely connected domains named 1, 2, 3a and 3b (domains 1, 2, 3a and 3b form the arch), domains 1 and 3a form an arched gap, domains 3a contacts with the Habc of syntaxin-1. Domain 1 is located on the other side of the arched gap and binds to the N-terminal peptide of syntaxin-1 (Fig. 1) (16).

2. Function of Munc18-1

Munc18-1 regulates neurotransmitter transmission by interacting with the SNARE complex. Munc18-1 promotes or inhibits the assembly of the SNARE complex primarily by combining with syntaxin-1 (Habc domain; N-terminal short peptide) in the 'open' and 'closed' conformations (17). Munc18-1 regulates vesicle fusion by interacting with the SNARE complex, affects the number of vesicles released and the supply of vesicles, and participates in the transmission of various neurotransmitters, including dopamine, glutamate and γ-aminobutyric acid (GABA) (Fig. 1) (18,19).

Previous studies have demonstrated that the N-terminal peptide and Habc domain of syntaxin-1 serve an important role in synaptic vesicle fusion. The N-terminal peptide is essential for vesicle fusion itself, whilst the Habc domain regulates this fusion by forming a closed syntaxin-1 conformation (20,21). The current hypothesis is that Munc18-1 and syntaxin-1 have several binding modes: i) Munc18-1 binds to syntaxin-1 (via the Habc domain) in a 'closed' conformation, stabilizing the conformation of syntaxin-1 and inhibiting the assembly of the SNARE complex (16); ii) Munc18-1 combined with 'closed' conformation syntaxin-1 serves as a template for SNARE assembly, with Munc13-1 helping to open the syntaxin-1 'closed' conformation. Munc13-1 Bridges synaptic vesicles and presynaptic membrane fusion, and Munc18-1 and Munc13-1 coordinate the assembly of the T-SNARE complex together in an NSF-SNAP resistant manner (22); and iii) Munc18-1 combined with the 'open' conformation ofsyntaxin-1 (N-terminal short peptide) initiates and stimulates SNARE-mediated membrane fusion (16). The regulatory mechanism of Munc18-1 is complex, involving promotion and inhibition of SNARE assembly; however, the promotion process is the more dominant of the two.

Munc18-1 participates in the regulation of synaptic plasticity, neurodevelopment and neuroendocrine cell function. Munc18-1 can function to regulate brain-derived neurotrophic factors, participate in the development of synapses and affect cognitive functions (23,24). Synaptic plasticity is the basic

Table I. Abnormal expression and pathogenic mechanism of Munc18-1 in different neurological disorders.

First author, year	Neurological disorders	Munc18-1 expression	Pathogenic mechanism	(Refs.)
Romaniello et al, 2015; Ortega-Moreno et al, 2016; Miyamoto et al, 2017	Epileptic encephalopathy	Decreased	GABA-ergic disorder Impaired synaptic plasticity	(17,43,73)
Toonen et al, 2006	Autism	Decreased	Glutamatergic and GABA-ergic disorder Neurodevelopmental disorders	(71)
Ramos-Miguel et al, 2015 Behan et al, 2009 Urigüen et al, 2013	Schizophrenia	Increased	Dopaminergic disorder	(19,46,75)
Lanoue et al, 2019	Parkinson's disease	Decreased	Regulates α-synaptic protein replication and aggregation Impaired synaptic plasticity	(78)
Ramos-Miguel et al, 2015	Alzheimer's disease	Decreased	Highly phosphorylated Tau protein Impaired synaptic plasticity GABA-ergic disorder	(82)
Linker et al, 2009	Multiple sclerosis	Increased	Glutamate excitotoxicity	(48)
Murphy et al, 2015	Duchenne's muscular dystrophy	Decreased	Unclear	(49)
Sleat et al, 2017	Neuronal ceroid lipofuscinosis	Decreased	Unclear	(50)

GABA, γ-aminobutyric acid.

process responsible for learning and memory (25). Previous studies have determined that the expression of Munc18-1 was decreased in the hippocampus of STXBP1 heterozygous knockout mice. This impaired synaptic plasticity, synaptic vesicle release rate and vesicle pool production, leading to impaired learning and memory (26-28). Activation of the diacylglycerol/protein kinase C (PKC) pathway can enhance neuronal synaptic transmission (29,30). In addition to the phosphorylation of Munc18-1, which transfers synaptic vesicles to active regions, the PKC pathway further accelerates the assembly of the SNARE complex (31,32). Synaptotagmin-1 is a synaptic-associated protein, the phosphorylation of which is an important step in the PKC pathway of synaptic transmission (33). Together with Munc18-1 phosphorylation, they represent an important aspect of synaptic plasticity, enhancing synaptic transmission and promoting neurotransmitter release (34,35).

The functional destruction of Munc18-1 affects the development of the cerebral cortex, which may cause neurodevelopmental disorders by affecting brain-derived neurotrophic factor and the downstream tropomyosin receptor kinase B pathway (36). While the functions of the exocytotic proteins SNARE and Munc18-1 have garnered increasing attention, studies have demonstrated that Munc18-1 promotes insulin secretion by interacting with SNARE, and the decline of its expression leads to insulin secretion disorders (37,38). Thyroid hormone is very important for normal brain development. Previous studies have determined that in mice with mild spatial cognitive impairment, the spatial learning and memory

of old and middle-aged mice decreases. Additionally, the expression of serum thyroxine is decreased in these animals, which increases Munc18-1 levels, demonstrating a negative correlation and indicating that Munc18-1 negatively regulates the role of thyroxine in brain development (39-41).

3. Association between Munc18-1 and neurological disorders

Munc18-1 is a key regulatory protein of transmission and its physiological function has been increasingly studied (6). The abnormal expression of Munc18-1 is involved in the pathogenesis of various neurological diseases and is closely associated with epileptic encephalopathy, autism, schizophrenia (SCZ), Parkinson's disease, Alzheimer's disease (AD), multiple sclerosis (MS), Duchenne's muscular dystrophy and neuronal ceroid lipofuscinosis (Table I) (42-50).

Munc18-1 and epileptic encephalopathy. Epileptic encephalopathy is a severe brain dysfunction with poor prognosis that is commonly observed in infants and children, and is often characterized by frequent seizures, intellectual disability and dyskinesia (51). The etiology of epileptic encephalopathy is not completely understood, and it has been reported that ~70% of epileptic encephalopathy cases are associated with genetic factors (52,53). An increasing number of studies have reported that STXBP1 exhibits multiple mutation types in severe epileptic encephalopathy, including missense and splicing mutations, which lead to single protein insufficient ploidy, impaired

protein stability, unbalanced cortical excitement, impaired synaptic plasticity and GABA-ergic transmission barriers that adversely affect learning and memory (28,43). The treatment of epileptic encephalopathy is challenging. However, studies have reported that drugs such as Keppra and adreno-cortico-tropic-hormone can prevent epilepsy to a certain extent, exerting no significant effect on other clinical symptoms, such as psychomotor disability and cognitive dysfunction (54-57).

Ohtahara syndrome, also known as epileptic encephalopathy in early infants, is a severe seizure related to stunting and mental disability. Its other neurological characteristics include autism spectrum disorder and dyskinesia (14). STXBP1 is the pathogenic gene of Ohtahara syndrome. Saitsu *et al* (58) identified that STXBP1 was mutated in children with this syndrome in 2008. With research on genetic diagnosis, an increasing number of studies have identified gene mutations in Ohtahara syndrome (59,60). A previous study demonstrated that the clinical manifestations in children <3 months were accompanied with tonic spasm, and the electroencephalograms of most children exhibited burst suppression in Ohtahara syndrome (61). The main clinical manifestations of West syndrome include nodding spasm, high irregularities in electroencephalograms and severe intellectual disability. Since Otsuka *et al* (62) first reported the STXBP1 mutation in West syndrome in 2010, following studies then identified STXBP1 nonsense and splice site mutations through clinical studies (63). In addition, STXBP1 mutations were identified in Dravet syndrome and Lennox-Gastaut syndrome, although evidence of these mutations in Ohtahara syndrome is not sufficient (64,65). Rett syndrome (RTT) is a neurodevelopmental disorder typically caused by mutations of methyl-CpG binding protein 2 (MECP2). However, Yuge *et al* (66) reported in 2018 that a STXBP1 mutation was detected in a case diagnosed with RTT. Moreover, a 9-year-old female with RTT and severe cognitive impairment exhibited no MECP2 mutation, but a STXBP1mutation was identified. MECP2 gene mutations result in the decrease of glutamate and gamma-aminobutyric acid receptor densities (17). It is hypothesized that deficits in synaptic plasticity may, to some extent, indicate an association between MECP2 and the pathogenicity of STXBP1 mutations (17).

Munc18-1 and autism. Autism is a common neurodevelopmental disorder with a high disability rate and strong genetic basis (67). At present, while almost all patients with STXBP1 mutations exhibit certain clinical phenotypes, including seizures and mental disabilities, ~20% have autism and can demonstrate increased aggressive behavior (68,69). A study by Dachtler *et al* (45) used a synaptic adhesion protein α-neurexin II-deficient mouse model of autism, and through protein quantitative analysis, detemined that Munc18-1 encoded by STXBP1 was significantly reduced in the hippocampus of mice. Synaptic adhesion proteins serve a key role in the formation and maintenance of synapses, and are involved in mediating synaptic plasticity (70). Decreased expression of Munc18-1 regulates the expression of α-neurexin II, impairs synaptic plasticity and participates in the occurrence of autism (71). In a mouse model of heterozygous STXBP1 knockout, it was revealed that the number of vesicles in the synaptic active region was reduced, and

glutamatergic and GABA-ergic transmission were impaired. These mice demonstrated increased anxiety and impaired emotional learning (72). In addition, Miyamoto *et al* (73) reported that the normalization of excitatory synaptic transmission in STXBP1 knockout mice, which exhibit various degrees of cognitive impairment and aggressiveness, improved Munc18-1 haploid deficiency-related aggression. It is speculated that Munc18-1 may be involved in the clinical phenotype of autism, potentially providing novel treatment methods.

Munc18-1 and SCZ. SCZ is a severe mental disorder with unclear causes. The main hypotheses suggest that it is mediated by overactive dopamine activity, 5-hydroxytryptamine and norepinephrine neural pathway dysfunction, excitatory amino acid dysfunction and the neuropeptide hypothesis. Neuropeptide protein is involved in the process of neurodevelopment, and low expression is involved in SCZ through the release of glutamate and γ-aminobutyric acid) (74). A proteomic analysis of the brain tissue of patients with SCZ by Behan *et al* (46) determined that Munc18-1 was highly expressed. Compared with patients who did not use anti-SCZ drugs, Munc18-1expression in patients who used these drugs decreased. Additionally, in a mouse model of SCZ utilized by Urigüen *et al* (75), a high expression of Munc18-1 was identified in the gray matter of the brain. Similarly, a high expression of Munc18-1 was detected in STXBP1 transgenic mice, which exhibited symptoms of SCZ, decreased expressions of dopamine receptors, decreased expressions of membrane transporters and decreased brain gray matter volume (75). Munc18-1 participates in the pathogenesis of SCZ by regulating excitatory neurotransmitters; however, the cause and mechanism of associated brain volume reduction is unclear.

Munc18-1 and neurodegenerative diseases. Parkinson's disease is a degenerative disease of the nervous system. Previous studies have demonstrated that the abnormal expression of α-synuclein and self-replication aggregation serve a key role in the degenerative changes of Parkinson's disease neurons (76,77). Munc18-1 serves other biological functions in addition to regulating neurotransmitter transmission. For example, Lanoue *et al* (78) revealed that Munc18-1 participates in various neurodegenerative diseases, including Parkinson's disease, by controlling the self-replication and aggregation of α-synuclein. Neurodevelopment in epileptic encephalopathy may also be affected by synuclein (78). The physiological functions of α-synuclein are complex; α-synuclein self-replication and aggregation inhibit exocytosis, affect vesicle secretion and regulate synaptic plasticity (76,79). A previous study revealed that Munc18-1 acts as a chaperone to regulate the replication and aggregation of α-synuclein, the decreased expression of Munc18-1 increases the aggregation tendency of α-synuclein, while the increased expression of Munc18-1 reverses this affect (80). At present, the specific pathways underlying the Munc18-1-associated regulation of α-synuclein are unclear, and further research is required to further understand and effectively treat Parkinson's disease.

AD is a progressive neurodegenerative disorder with an insidious onset. Its etiology includes the deposition of abnormal amyloid β extracellular plaques and intracellular

neurofibrillary tangles containing hyperphosphorylated Tau protein, which lead to impaired synaptic plasticity (81). Previous studies have demonstrated that at the GABA-ergic presynaptic inhibition terminal, the expression of the Munc18-1 long splice variant is closely associated with cognitive function. Furthermore, the deletion of the Munc18-1 long splice variant increases the risk of AD (47,82). In a study assessing the effect of pomegranate on an AD mouse model, it was determined that pomegranate can enhance synaptic plasticity by increasing the expression of Munc18-1, SNAP25 and synaptophysin, improving spatial learning impairment in mice (83). Additionally, a study has determined that when comparing the brain tissue of healthy individuals and deceased patients with AD of the same age, brain tissue following AD-associated death is rich in highly phosphorylated Tau protein and Munc18-1 is abnormally expressed (84). The current treatment methods used for patients with AD remain poor, and further understanding of Munc18-1 may provide a novel target.

Munc18-1 and other diseases, including MS, Duchenne muscular dystrophy and neuronal ceroid lipofuscinosis. The most common type of white matter injury is demyelination, which includes myelin loss and axonal abnormalities (85). MS is an autoimmune disease characterized by the inflammatory demyelination of white matter in the central nervous system (86). A previous study reported that in a murine model of multiple sclerosis, the expression of Munc18-1 and axon structural protein α-internexin increases, and the expression of glutamate decarboxylase decreases, mediating immunity to myelin damage (48). An increased expression of α-internexin, axon disintegration, the Munc18-1 regulation of glutamatergic transmission and glutamate excitotoxicity further increase axon damage (87). However, there are few studies that assess Munc18-1 in MS and the specific underlying pathogenic mechanism remains unknown.

Duchenne muscular dystrophy is a disease that affects the neuromuscular system. Progressive skeletal muscle atrophy is the main complication, and developmental cognitive deficits and behavioral abnormalities are its main clinical features (88). Murphy *et al* (49) performed proteomics on the brain tissue of mice with Duchenne muscular dystrophy, the results of which revealed that the expression of Munc18-1 was significantly decreased. A further study revealed that the expression of Munc18-1 was significantly decreased in the brains of children with neuronal ceroid lipofuscinosis (50). These studies indicate that Munc18-1 may be involved in the pathogenesis of Duchenne muscular dystrophy and neurodegenerative lysosomal diseases. Combined with the characteristics of these diseases, impaired synaptic plasticity and neurodevelopmental pathways serve an important role in these diseases. Munc18-1 may participate in the pathogenesis of disease by regulating synaptic plasticity and neurodevelopment; however there are few related studies to confirm this, and the specific underlying mechanisms require further exploration.

4. Conclusion and future perspectives

Munc18-1 has powerful biological functions and can regulate the balance of synaptic excitatory neurotransmitter and inhibitory neurotransmitter transmission, and mediate disease processes at increased or decreased expressions. Decreased Munc18-1 expression also damages synaptic plasticity and affects learning and memory. Neuronal transmission requires the participation of multiple neurotransmitters, and Munc18-1 regulates neurotransmitter transmission by regulating the SNARE complex. This process is complicated and requires the participation of multiple regulatory proteins. The relationship between the expression of Munc18-1 and specific neurotransmitter regulation mechanisms remains unclear and requires further study. An enhanced understanding of the function of Munc18-1 may elucidate novel therapeutic strategies for the treatment of neurological diseases.

Availability of data and materials

Not applicable.

Authors' contributions

FT and DX conceived the present study. FT, DX, LC and XL provided the software. FT, DX, HG and XL provided the study resources. HG and XL generated the figure. FT and DX drafted the manuscript. FT, DX, HG, HG and XL reviewed and edited the manuscript. XL supervised the study. DL and XL confirm the authenticity of all the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

All authors declare that they have no competing interests.

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