REVIEW

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Neuromuscular junction disorders: Experimental models and pathophysiological mechanisms

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Located between skeletal muscle fibers and motoneurons, the neuromuscular junction is a chemical synapse essential for the transmission of information from nervous system to skeletal muscle. There are many diseases related to neuromuscular junction dysfunction, including myasthenia gravis, Lambert-Eaton myasthenic syndrome, congenital myasthenic syndromes, amyotrophic lateral sclerosis, and spinal muscular atrophy. The pathophysiological mechanisms of these diseases have been investigated using many animal models. Among them, mouse models are the most commonly used and have provided the majority of current data. Moreover, advances in human induced pluripotent stem cell technology has resulted in new opportunities to study neuromuscular junction disorders from both patients and healthy individuals. Currently, patient-specific induced pluripotent stem cells derived from motor neurons have begun to be studied. These studies will help us achieve a more comprehensive understanding of diseases related to neuromuscular junction disorders. We will describe the research models of neuromuscular junction disorders and provide an overview of recent key findings.

Key words: neuromuscular junction, animal model, induced pluripotent stem cell, myasthenia gravis, Lambert-Eaton myasthenic syndrome, congenital myasthenic syndromes, amyotrophic lateral sclerosis, spinal muscular atrophy

INTRODUCTION

The neuromuscular junction (NMJ), a chemical synapse between skeletal muscle fibers and motor neurons, was first described by Wilhelm Friedrich Kuhne who discovered it through examining many species using light microscopy (Sanes and Lichtman, 1999). The NMJ is a specialized region where muscle and nerve communicate (Wu et al., 2010). It consists of 3 major elements that include: the presynaptic region, containing the nerve terminal; a synaptic space surrounded by a basement membrane; and the postsynaptic muscle membrane (Verschuuren et al., 2016; Engel, 2018) (Fig. 1A). At the nerve terminal, neurotransmitters are released. In adults, the NMJ is where a motor neuron innervates a piece of contractile myofiber by a single axon (Larsson and Ansved, 2016). When the action potentials arrive, the end of the motor neuron releases acetylcholine (ACh), which induces voltage-gated calcium channels (VGCCs) to open. ACh activates the ACh receptor (AChR) of the muscle fiber, depolarizes the muscle cell, and triggers the release of calcium from the sarcoplastic reticulum, resulting in a local depolarization or endplate potential. When the endplate potential reaches a threshold, sodium channels open, then the action potential propagates along the muscle fibers to induce muscle contraction (Li et al., 2018).

The NMJ is vital to our physical activity and daily life. Its indispensable role in the contraction of muscle depends on the functioning and accurate location of many proteins (Fig. 2). Muscle-specific kinase (MuSK), agrin (AGRN), and low-density lipoprotein receptor-related protein 4 (LRP4) are especially important. Spe-

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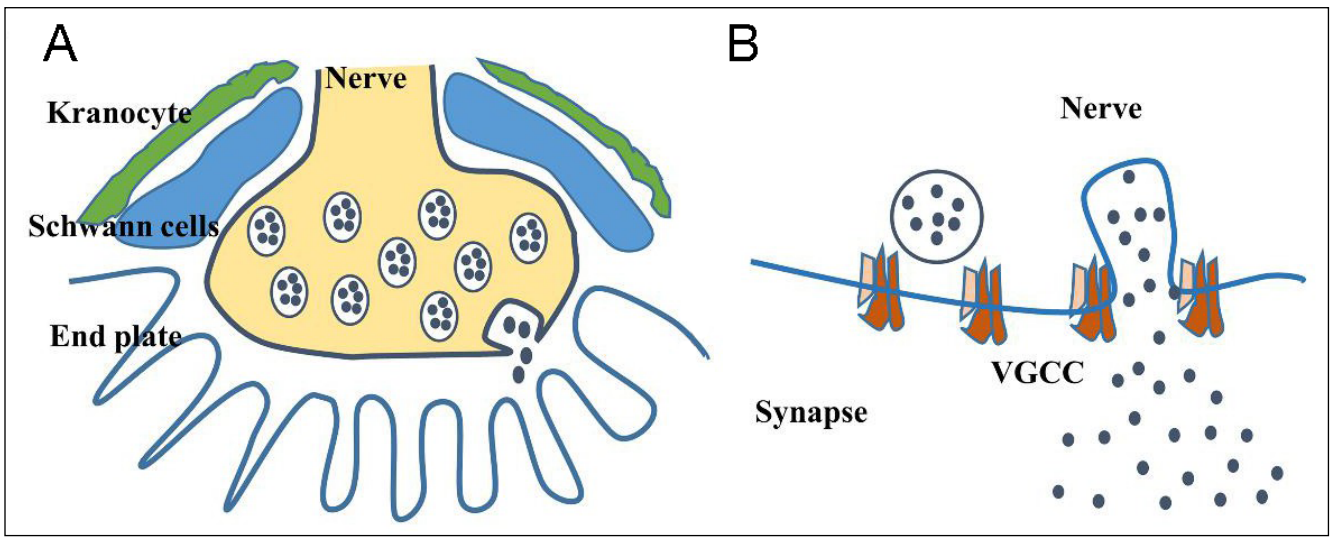

Fig. 1. Schematic diagram of neuromuscular synaptic release of neurotransmitter (Verschuuren et al., 2016). (A) the cellular composition of the NMJ and (B) the activation of presynaptic voltage-gated calcium channels and acetylcholine release.

cifically, MuSK is a critical transmembrane protein for maintaining the functional integrity of the NMJ. AGRN is a protein released from nerves that binds to LRP4 and MuSK. LRP4 is the actual receptor of AGRN, in a complex with MuSK (Meriggioli and Sanders, 2009; Koneczny et al., 2013). Other proteins that are vital to NMJ function include rapsyn, a downstream protein of tyrosine kinase-7 (DOK7); collagen Q (ColQ); and the proteins of the extracellular matrix (ECM) (Webster, 2018). The mutation of antibodies to these proteins can result in weakness and fatigability typical of congenital myasthenic syndromes (CMS) (Kummer et al., 2018). Moreover, when the sodium channels demonstrate an impaired function or the endplate potential exhibits a low amplitude, the risk of neuromuscular transmission failure increases, likely resulting in a myasthenic syndrome (Meager et al., 1997; Buckley et al., 2001; Abicht et al., 2012). Additionally, presynaptic or postsynaptic defects are often a cause of diseases related to NMJ (Finlayson et al., 2013; Ohkawara et al., 2014; Zhang et al., 2014), such as

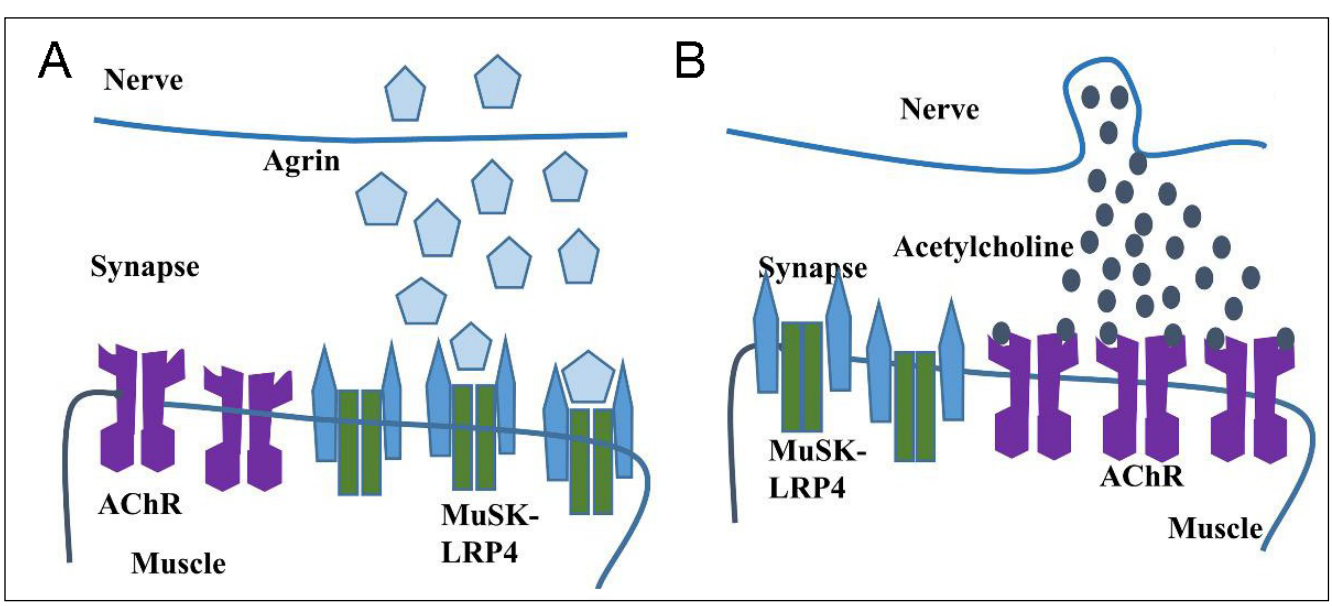

Fig. 2. Critical proteins for NMJ function (Verschuuren et al., 2016). (A) that agrin binding to the LRP4-MuSK complex activates multiple signaling pathways and transforms the aggregation of AChR and neuromuscular synaptic and (B) that ACh binding to AChR induces the central ion channel to open briefly, leading to membrane depolarization and a muscle action potential that causes the muscle fiber to contract.

myasthenia gravis (MG) and Lambert-Eaton myasthenic syndrome (LEMS). NMJ degeneration is also considered a key and early pathological feature of motor neuron loss in amyotrophic lateral sclerosis (ALS) patients (Raff et al., 2002, Wishart et al., 2006).

Here we will focus on the mouse models and relevant research studies that will enrich our understanding of the mechanisms involved in NMJ disorders. These models include animal models – primarily transgenic mice, expression of mutant genes in different cellular models, and induced pluripotent stem (iPS) cell-derived models. We focus on models and research mostly related to LEMS, MG, ALS, CMS, and spinal muscular atrophy (SMA).

Lambert-Eaton myasthenic syndrome

LEMS is an autoimmune disease that results from the autoantibody-mediated removal of a P/Q-type Ca^{2+} channel subset that participates in neurotransmitter release. In LEMS, normal neurotransmission at the NMJ is disrupted, causing limb muscle weakness and decreased reflexes (Tarr et al., 2015). We will describe experimental models and research progress for LEMS.

Antibodies against VGCCs were first identified by Fukunaga in 1983 which facilitated research on LEMS. The following research shows that the most common antibodies found are against P/Q-type VGCCs, which cause most of the clinical symptoms of LEMS. Mouse passive transfer models were used to reveal a paucity of presynaptic membrane active zones. In LEMS, the active zone particles induce the release of quantal transmitters under the signal of nerve impulses, which further supports the idea that these particles are Ca^{2+} channels that mediate membrane lesions in LEMS by IgG (Fukunaga et al., 1983). Another mouse passive transfer model experiment demonstrated that IgG antibodies from LEMS patients may bind to the determinants of nerve terminals, and these substances participate quantal and nonquantal ACh release (Lang et al., 1984). Meanwhile, LEMS autoantibodies certainly influence the voltage-gated P/Q-type Ca^{2+} channels in mammalian NMJ and regulate the release of ACh in motor nerves. Passive LEMS transfer to mice as a result of repetitive plasma administration from LEMS patients can lead to a drop in the perineurial P/Q-type current amplitude and unmask a dihydropyridine (DHP)-sensitive L-type Ca^{2+} current at the nerve terminal. Therefore, ACh release from the L-type Ca^{2+} channel only contributed to passive LEMS transfer after the quantum duration release, and ACh release has been identified as adaptive in LEMS (Flink and Atchison, 2002).

It can be concluded that autoantibodies of LEMS can influence the voltage-gated P/Q-type Ca^{2+} channels in mammalian NMJs, then modulate the release of ACh in motor nerves, ultimately causing membrane lesions in LEMS.

Myasthenia gravis

MG is an acquired autoimmune disorder in which a reduced number of autoantibodies or autoantibodies with impaired function act against the postsynaptic AchRs, resulting in muscle fatigue and weakness (Wu et al., 2013). The experimental autoimmune myasthenia gravis (EAMG) models are produced by administration of autoantigen, and models have been generated for LRP4, MuSK, and AChR. A most common animal EAMG model is produced by immunizing the mice or rats with AChR in complete Freund's adjuvant (CFA). Furthermore, mice or rats are immunized by MuSK in CFA to produce models of MuSK-MG; these model animals demonstrated a more debilitating and severe course of disease characterized by significantly greater weight loss compared to the EAMG model induced by AChR immunization (Kusner et al., 2015). Studies based on immunization with different AChR-subunit antigens have introduced different EAMG models (Table 1) (Wu et al., 2020).

Active immunization and passive transfer models have provided an understanding of the pathogenicity

Table 1. AChR-related EAMG models.

Animal species	Animal strains	Method of immunization	AChR- antibodies	NMJ
Mouse	C57Bl/6J	Native whole *Torpedo* AChR in CFA	Present	Present
	C57Bl/6J	Native whole *Torpedo* AChR in LPS	Present	Present
	HLA-DQ8 transgenic B10 B6	*E. coli* plasmid with recombinant human AChR α-subunit in CFA	Present	Present
Rat	HLA-DQ8 transgenic B10	*E. coli* plasmid with recombinant human AChR γ-subunit in CFA	Present	Present
	Lewis rat	*Torpedo* AChR in CFA	Present	Present

of MuSK autoantibodies. MuSK IgG4 autoantibodies are more prone to bind to the first IgG-like domain of MuSK *in vitro*, and such binding interferes with the association between MuSK, LRP4, and ColQ. Such binding was also correlated with the severity of the disease. Even so, the exact pathophysiological mechanisms of MuSK-MG remained largely known.

Fortunately, research in recent years has yielded promising results. Kawakami et al. (2011) found that MuSK IgG inhibits ColQ binding to MuSK. No weakness or weight loss was described in the mouse passive transfer model group injected with purified total IgG from a MuSK-MG patient, but anchoring of ColQ was compromised, and a less prominent effect on MuSK and AChR expressions was observed at the endplates (Kawakami et al., 2011). Additionally, Klooster and colleagues showed that purified IgG4 from MuSK-MG patients induced severe paralysis in mice by binding to mouse NMJs. The IgG4 (MuSK) antibodies are functionally monovalent and do not activate complement (Klooster et al., 2012).

Antibodies against AGRN and LRP4 (the receptor of AGRN) are crucial for the formation and maintenance of NMJs in MG patients. Clinical studies have investigated whether these two antibodies can be new biomarkers for MG (Tezuka et al., 2014; Barik et al., 2014; Yan et al., 2018). To determine the prevalence and clinical manifestations of double-seronegative myasthenia gravis (DNMG) patients who are positive for both LRP4 and AGRN antibodies, Rivner et al. (2020) studied 181 DNMG patients from 16 US sites. Among the patients, 27 (14.9%) were positive for either LRP4 or agrin antibodies, and 23 (12.7%) were positive for both. The patients with positive results for LRP4 or agrin antibodies demonstrated a higher prevalence of generalized symptoms compared with their antibody-negative counterparts, and 24 of the 27 patients (89%) developed generalized MG (Rivner et al., 2020). Thus, we can conclude that AGRN and LRP4 antibodies have the potential to serve as new biomarkers for MG.

Although there are many EAMG models produced by the administration of MuSK and AChR, the exact pathophysiological mechanism of MG remains unclear, likely due to the intricate antibody system underlying MG. Among them, AGRN and LRP4 antibodies may provide new biomarkers for MG.

Amyotrophic lateral sclerosis

Mouse models in ALS

ALS is a fatal neurodegenerative disease characterized by the premature death of brain and spinal cord motor neurons and an extremely heterogeneous clinical and genetic phenotype (Kwiatkowski et al., 2009). Mouse models of ALS have been derived from the mutated genes found to cause ALS in patients. Here we describe the common mouse models and some new findings in ALS research.

Dominant mutations in the superoxide dismutase 1 (*SOD1*) gene have been recognized as one of the most prominent causes of inherited ALS. Transgenic mice carrying human *SOD1* mutations develop progressive weakness similar to ALS patients, and they are consequently used as models to understand the pathogenesis and develop new therapies (Fischer et al., 2004). The most commonly used models include the $SOD1^{G93A}$ mouse model and $SOD1^{G37R}$ mouse model. $SOD1^{G93A}$ mutant mice are widely used transgenic animal models (Chiu et al., 1995). They have proven useful for the examination of the cellular pathology of ALS and the acquirement of preclinical data for new drugs against ALS. The $SOD1^{G93A}$ mouse models used for research primarily originate from the Jackson Laboratory (Sacramento, CA). The background strain of a mouse model can significantly affect phenotypic expression, and B6SJL-TgN (SOD1-G93A)1GUR, a B6SJL mixed hybrid strain, is a common mouse background strain for studies of the human *SOD1* mutation. The $SOD1^{G93A}$ mutant from the Jackson Laboratory was used to validate that alterations of the distal axon and NMJ were already present prior to the onset of functional symptoms. Additionally, higher levels of LRP-4, rapsyn, and dystrophin found in post-symptomatic skeletal muscle may suggest ongoing neuronal repair attempts (Clark et al., 2016). The same mouse model of $SOD1^{G93A}$ was used to test the hypothesis that therapeutics that prevent the initial NMJ disassembly may offer optimal functional outcomes and delay ALS progression (Sengupta-Ghosh et al., 2019).

$SOD1^{G37R}$ mice (lox$SOD1^{G37R}$) are well known for slow disease progression, making them ideal models to follow the chronological changes of single motor units (Boillee et al., 2004; Lobsiger et al., 2009). An accumulation of G37R mutant proteins could lead to progressive and severe motor neuron diseases. Only lower motor neurons are affected when the mutant accumulation is relatively low, but, as the accumulated amount grows, more severe abnormalities emerge and a variety of other neuronal populations are affected as well (Wong et al., 1995). Mutant SOD1 damage causing ALS was found to develop within multiple cell types. Mutant damage within motor neurons is crucial to initiate the disease, and mutant synthesis within adjacent microglia or astrocytes accelerates disease progression. Lobsiger et al. (2009) reported a surprising finding that when the synthesis of a ful-

ly dismutase active ALS-linked mutant (SOD1G37R) drops by 70% in Schwann cells, disease progression would accelerate significantly, and the amount of insulin-like growth factor 1 (IGF-1) would also decline in nerves. Martineau et al. (2018) identified asynchronous dismantlement of single motor units in *SOD1^{G37R}* mice and revealed that, weeks prior to complete axonal degeneration, axonal branches were already dismantling and new axonal sprouting were already happening. Consequently, synapses were formed on nearby NMJs (Martineau et al. 2018).

Fused in sarcoma (FUS) is an RNA-binding protein that participates in ALS. Mutations in the *FUS* gene account for about 5% of familial ALS cases (Kwiatkowski et al., 2009). As the most aggressive ALS subtype, FUS-ALS is characterized by early-onset and rapid disease progression, and it is caused by heterozygous mutations in the *FUS* gene. FUS- ALS has been studied with knock-in *FUS* mouse models (Table 2) (Picchiarelli et al., 2019). Knock-in mice with mislocalized cytoplasmic FUS expression and mice with *FUS* completely knocked out were studied by Scekic-Zahirovic et al. (2016) to learn the impact of FUS on neurodegeneration. The models demonstrated similar perinatal lethality, respiratory insufficiency, reduced body length and body weight, and alterations in gene expression and mRNA splicing patterns, suggesting that mislocalized FUS cannot fully exert its normal functions (Scekic-Zahirovic et al., 2016). Picchiarelli and colleagues (2019) identified postsynaptic NMJ defects in newborn homozygous mutants, and these defects were attributed to mutant FUS toxicity in skeletal muscle. Smaller neuromuscular endplates were observed in adult heterozygous knock-in mice, and the animals were denervated prior to the loss of motor neurons. An enrichment of FUS was observed in the subsynaptic myonuclei, and such innervation-dependent enrichment is distorted in FUS-ALS. It demonstrated that FUS regulates the gene expression of AChRs in the subsynaptic muscle nucleus, and the inherent muscle toxicity of the ALS mutant strain FUS may cause sequelae motor neuropathy (Picchiarelli et al., 2019).

The GGGGCC (G4C2) hexanucleotide repeat expansion (HRE) in the first intron and noncoding region of the chromosome 9 open reading frame 72 (*C9orf72*) gene is the most common genetic mutation in sporadic and familial ALS from European populations (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Several models have been generated to help understand the molecular mechanism of C9orf72-ALS. Among them, *C9orf72*- knockout models failed to cause neurodegenerative changes, which means that loss of C9orf72 is not adequate to cause disease (Cooper-Knock et al., 2015). *C9orf72*-knockin models introduced by adeno-associated viral vectors could lead to mild neurodegeneration, but not the severe phenotypes observed in ALS (Chew et al., 2015). Novel transgenic mouse models produced with bacterial artificial chromosome (BAC) constructs were developed to show the clinical and neurodegenerative features of ALS. A sex-dependent disease phenotype was found in the C9-BAC mouse lines, which correlated severity with repeat length and expression. With a single copy of 37 short repeats, C9-37 caused

Table 2. Summary of common ALS-related mutant mouse models.

Mutated genes	Mouse models	Phenotype and notes	References
SOD1	*SOD1^{G93A}*	Useful for the examination of the cellular pathology of ALS, slow ALS course	Fischer et al. 2004
	SOD1^{G37R}	Slow disease progression	Boillee et al. 2004, Lobsiger et al. 2009
FUS	Knock-in *FUS*	Cytoplasmic FUS mislocalization	Scekic-Zahirovic et al. 2016
C9orf72	*C9orf72*-knockout	No disease phenotype	Koppers et al. 2015
	C9orf72-knockin	Mild neurodegeneration	Chew et al. 2015
	C9-37	No disease phenotype	Liu et al. 2016
	C9-500/32	Rapid and severe disease progression	Liu et al. 2016
	C9-500		
TARDBP	M337V	A mild overexpression of human mutant TDP43 display many ALS-like hallmarks	Wils et al., 2010
	G298S		Shan et al., 2010
	Q331K		Arnold et al., 2013

no disease phenotype, however, with transgenes expressing copies containing a mix of 500 and 32 repeats or 500 repeats alone, C9-500/32 and C9-500 showed a more rapid and severe disease progression (Liu et al., 2016) (Table 2). It was suggested that the repeat expansion in *C9orf72* not only contributes to neurodegeneration but also influences immune homeostasis (Lai and Ichida, 2019).

TAR DNA-binding protein-43 (TDP-43) is a nuclear DNA/RNA-binding protein that was found clustered in the cytoplasm in the majority of ALS patients. TDP-43 mutations are relatively rare, accounting for 4% of familial forms of ALS (Taylor et al., 2016). There are approximately 20 different mouse models of TDP-43 (Lutz, 2018). The earlier mouse models (A315T and M337V) used the prion protein (Prnp) gene promoter to generate TDP-43 cDNA. It was found that pathologic aggregates of ubiquitinated proteins existed in specific neurons of these transgenic mice, and their phenotype severity was correlated with the levels of mutant TDP-43 expression (Wegorzewska et al., 2009; Stallings et al., 2010; Xu et al., 2010; Xu et al., 2011). Similar results were shown in mouse models (A315T and G298S) which used the Thy1 promoter (Wils et al., 2010; Shan et al., 2010). Also using the Prnp promoter, the TDP43-Q331K and TDP43-M337V mouse model mildly overexpressed human mutant TDP43, which displayed many ALS-like hallmarks such as progressive motor dysfunction, muscle atrophy, reduced NMJ integrity, and motor neuron degeneration (Table 2).

TRVA242 was determined to be a potential compound that could significantly improve efficiency in rescuing locomotor, motor neuron, and NMJ synaptic deficits in a *C. elegans* TDP-43 model, multiple zebrafish genetic models (TDP-43, *SOD1*, and *C9ORF72*) and a *SOD1*^{G37R} mouse model of ALS (Bose et al., 2019). Thus, the effective compound was identified across different animal models of ALS.

Additionally, genetic models are often restricted by a specific phenotype characterization. A model of environmentally-induced motor neuron degeneration in zebrafish, based on exposure to bisphenol A, displays many hallmarks of ALS-like features. Thus, environmental factors may be more likely to be involved in ALS than single dominant gene mutations (Morrice et al., 2018).

Research on induced pluripotent stem cells (iPS cells) in ALS

iPS cells are advantageous because cells carrying the desired genetic profile can be cultured and induced from somatic cells of the patient to the specific cell type of interest. One study in 2008 used iPS cells differentiated from skin fibroblasts of an 82-year-old patient with familial ALS. These iPS cells were then used to produce patient-specific motor neurons and glia, two affected cell types in ALS (Dimos et al., 2004); the iPS cell lines were established using *SOD1*^{G93A} mice, and these were compared with mouse embryonic stem cells (E14) and normal iPS cells. Results showed that all three cells have similar potency for neuronal differentiation into the motoneuron-like phenotype (Yao et al., 2013). While motor neurons differentiated from iPS cells are often used to build drug screening systems for ALS, Yoshioka et al. (2002) developed an NMJ model comprised of myotubes and motor neurons differentiated from iPS cells and C2C12 myoblasts, respectively. With this model, the myotubes' contractile activity and force generation capability via the NMJ were measured in both two- and three-dimensional cell culture systems (Yoshioka et al. 2020). In addition, Osaki et al. (2018) developed an ALS-on-a-chip technology as a platform to test drug candidates by using 3D skeletal muscle bundles, as well as iPS cells and light-sensitive channelrhodopsin-2–induced motor neuron spheroids from a sporadic ALS patient. Light was used to activate muscle contractions, which were measured based on column deflection. Compared with the normal motor unit, the motor unit of ALS produced less muscle contraction, showed motor neuron degradation, and showed increased apoptosis in the muscle (Osaki et al., 2018).

NMJ dysfunction was found in the most common mouse models of ALS (Alhindi et al., 2021). It was demonstrated that alterations of the distal axon and NMJ occurred earlier than the onset of functional symptoms in the *SOD1*^{G93A} mouse models (Clark et al., 2016). Therefore, it is necessary to focus on the therapeutics that prevent initial NMJ disassembly, the distal axon and motor neurons. Furthermore, as iPS technology has become more developed, its application in ALS is increasing. However, the pathophysiological mechanisms of ALS remain unclear. We look forward to more results from the various experimental models in the future.

Congenital myasthenic syndromes

CMSs are a series of genetic disorders that affect neuromuscular transmission. These genetically-inherited syndromes generally result from defective synaptic transmission at the cholinergic NMJs, and 30 genes have been identified to cause CMS if mutated (Nicole et al., 2017). Specific CMS-related mutant animal models have been described by Webster (Webster 2018). Here, we will describe the most recent discoveries in CMS animal models.

DOK7 is a component of the AGRN-LRP4-MUSK-DOK7 signaling pathway, and it is located in the postsynaptic specialization of the muscle membrane. The AGRN-LRP4- MUSK-DOK7 signaling pathway is critical to building and maintaining the synaptic structure of an NMJ. Webster et al. (2020) studied the effect of salbutamol, a β2-adrenergic agonist, on synaptic function and structures by ex-vivo electrophysiological analyses and microscope observations. Their results showed that the DOK7-CMS mouse model displayed significantly reduced weight gain and much higher perinatal lethality. On the other hand, salbutamol treatment improved weight gain and survival for DOK7 myasthenic mice, and the animals exhibited more active and detectable NMJs compared to DOK7-CMS mice during endplate recording (Webster et al., 2020).

AGRN is a vital organizing factor derived from the nerves, and it is responsible for NMJ organization and localization. A mutation in AGRN-encoding genes is rarely reported as a cause for CMS. Wang et al. (2020) reported a pediatric proband exhibiting weakness in trunk and limb muscles combined with intellectual disability and skeletal malformation. The authors revealed a new compound heterozygous mutation in *AGRN*: c.125A>C (p. Glu42Ala) in the N-terminal AGRN domain (NtA) and c.4516G>A (p. Ala1506Thr) in the laminin G1 domain (LG1). According to bioinformatics analysis, this mutation was predicted to disrupt the known functions of AGRN and undermine the formation and maintenance of the NMJ via both neural and muscular AGRN pathways (Wang et al., 2020).

It has been verified that salbutamol can prolong survival and increase the number of NMJs in a model of severe DOK7-CMS. Mutations in AGRN-encoding genes can disrupt the function of AGRN, then influence the NMJ, leading to CMS. Moreover, there are many genes that have been identified to cause CMS. To understand the pathophysiological mechanism of CMS more clearly, additional research is needed in the future.

Spinal muscular atrophy

Mouse models in SMA

SMA is a motor neuron disease commonly seen in children, and it is caused by *SMN1* gene mutations (Lefebvre et al., 1995; Rodrigues et al., 1995). The severity of SMA can vary from minor motor impairment to infant mortality both in humans and mice. In 2012, Bebee et al. summarized the genetic characteristics of SMA mouse models, which express differing levels of SMN protein in both severe and mild SMA, manifested as neurological and physiological disorders. Here, we will focus on some new explorations in SMA. Boido et al. (2018) observed that AGRN expression dropped by 50% in quadriceps of P10 SMA mice compared to age-matched WT controls. The authors treated SMAΔ7 mice (an experimental model of SMA2) from birth with therapeutic AGRN biological NT-1654 to investigate the role of AGRN in the disease, and discovered a significant improvement in motor behavior and survival as a result of NT-1654 treatment. Additionally, NT-1654 treatment remarkably prevented the size of shrinkage of muscle fibers, and NMJ morphology analyses on whole-mount diaphragm preparations revealed that SMA mice exhibited more mature NMJs and reduced neurofilament accumulation when treated with NT-1654 (Boido et al., 2018). Their results are consistent with Kim et al.'s findings (2017).

DOK7 is an NMJ organizer and a substance active downstream of AGRN. Kaifer et al. (2020) administered AAV-DOK7 to an intermediate SMA mouse model to investigate the potential of DOK7 as a modifier of SMA. When treated with AAV9-DOK7, the mouse models exhibited improvements in their NMJ architectures and muscle fiber atrophy, as well as showing a subtle reduction of phenotypic severity, which was verified by improved grip strength and longer survival. Therefore, DOK7 was identified as a novel modifier of SMA (Kaifer et al., 2020). Courtney et al. (2019) used the *SMN2*$^{B/-}$ SMA mouse model and demonstrated that the onset of NMJ degeneration is a predictor for cell body loss of motor neurons. Additionally, they found that once presynaptic swelling at the NMJ occurs, it coincided with an increase in the amounts of P53-associated transcripts in the spinal cord. To find out whether a causal relationship between the two phenomena existed, the authors crossed the *SMN2*$^{B/-}$ mouse model with a mouse carrying a *P53* knockout induced by tamoxifen and found that NMJ loss is a result of P53 pathway activation (Courtney et al., 2019).

Research on induced pluripotent stem cells in SMA

To treat and even cure SMA, we must understand the NMJs formed by these patients' motor neurons to identify and study drugs that can restore the NMJs to normal function. In particular, Yoshida et al. (2015) built NMJ-like structures from motor neurons differentiated from iPS cells cultured from SMA patients. In these structures, AChR clustering was remarkably impaired, and such impairment could be alleviated by treatments with valproic acid and antisense oligonucleotides, leading to increased synthesis of full-length SMN transcripts (Yoshida et al., 2015). Lin et al. (2019) presented a novel two-step self-organiz-

ing approach to construct *in vitro* human NMJs from human iPS cells. The *in vitro* NMJs they constructed modeled the pathological features of SMA effectively (Lin et al., 2019).

SMA mouse models based on genetic characteristics have been widely studied. It was found that the activation of P53 pathway was responsible for the loss of NMJ using the *SMN2*$^{B/-}$ SMA mouse model. Meanwhile, NT-1654 and AAV-DOK7 might be considered as potential cures for SMA. Certainly, the *in vitro* model of NMJs derived from iPS cells would be useful to investigate the pathophysiological mechanisms of SMA.

CONCLUSIONS

The diseases related to NMJ disorders result from many factors including structural changes in the NMJ, mutations of genes that code essential proteins, and the degeneration of the NMJ. LEMS, MG, ALS, CMS, and SMA are the primary identified types of disease resulting from NMJ dysfunction. Many animal models have been used to investigate the pathophysiological mechanisms of these diseases. Among them, a series of mutant mouse models were used extensively to uncover underlying mechanisms of NMJ disorders. However, every model has its pros and cons. Models are often restricted by specific phenotype characterizations, and it's possible that environmental factors may be more likely to be involved in the disease than single dominant gene mutations. Thus, more animal model systems are needed to construct additional models based on mutated genes, as well as environment-induced models.

The development of iPS-NMJ to study the diseases related to NMJ disorders has opened a new approach allowing for deeper investigation into the cell physiology of neurons derived from actual mutation carriers and diagnosed patients. The iPS cells generated from individual patients may enable large-scale production of affected cell types for disease modeling, drug testing, drug discovery, and, eventually, autologous cell replacement therapies. More iPS-NMJ models are expected to reveal the mechanisms and pathophysiology of diseases related to NMJ disorders as they represent an infinite source of patient-specific neuronal cells that are relevant to the disease.

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