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MuSK myasthenia gravis in mice

The mouse passive-transfer model of MuSK myasthenia gravis: disrupted MuSK signaling causes synapse failure

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While the majority of myasthenia gravis patients express antibodies targeting the acetylcholine receptor, the second most common cohort instead displays autoantibodies against muscle-specific kinase (MuSK). MuSK is a transmembrane tyrosine kinase found in the postsynaptic membrane of the neuromuscular junction. During development, MuSK serves as a signaling hub, coordinating the alignment of the pre- and postsynaptic components of the synapse. Adult mice that received repeated daily injections of IgG from anti-MuSK⁺ myasthenia gravis patients developed muscle weakness, associated with neuromuscular transmission failure. MuSK autoantibodies are predominantly of the IgG4 type. They suppress the kinase activity of MuSK and the phosphorylation of target proteins in the postsynaptic membrane. Loss of postsynaptic acetylcholine receptors is the primary cause of

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neuromuscular transmission failure. MuSK autoantibodies also disrupt the capacity of the motor nerve terminal to adaptively increase acetylcholine release in response to the reduced postsynaptic responsiveness to acetylcholine. The passive IgG transfer model of MuSK myasthenia gravis has been used to test candidate treatments. Pyridostigmine, a first-line cholinesterase inhibitor drug, exacerbated the disease process, while 3,4-diaminopyridine and albuterol were found to be beneficial in this mouse model.

Keywords: myasthenia gravis; neuromuscular junction; neuromuscular disease; muscle-specific kinase; mouse models

Introduction

Antibodies targeting the acetylcholine receptor (AChR) were first identified as the cause of myasthenia gravis (MG) in the 1970s.^{1–3} This left a minority of generalized MG patients who were seronegative for anti-AChR. Mice that were repeatedly injected with immunoglobulin from these anti-AChR⁺ patients displayed impaired neuromuscular transmission, suggesting that pathogenic autoantibodies were indeed present.⁴ Many of these patients were subsequently shown to harbor antibodies targeting muscle-specific kinase (MuSK).^{5,6} MuSK was a good autoantigen candidate at the time because it is expressed in the postsynaptic membrane of the neuromuscular junction (NMJ) and is essential for NMJ development.^{7,8} Every step in the investigation of MuSK MG has involved a dialogue between clinical observations and the evolving story of the role played by MuSK in development and adaptation of the NMJ.

The MuSK–rapsyn system at the healthy NMJ

Loss of AChR from the endplate is a hallmark of MG, so it is important to consider the role of MuSK in clustering AChRs within the postsynaptic membrane. During embryonic development of muscle, MuSK is expressed at the highest levels in the sarcolemma near the center of each developing muscle fiber. This fosters the formation of AChR clusters in the central region, even before the arrival of the embryonic motor axons.⁹ Neural agrin, a proteoglycan secreted by the motor axon, can bind to a coreceptor called LRP4 to form an agrin–LRP4–MuSK protein complex.^{10,11} The active tyrosine kinase complex causes phosphorylation/activation of Abl and Src-family tyrosine kinases, leading to phosphorylation of the AChR.^{12–15} This recruits a cytoplasmic protein called rapsyn to help stabilize the AChRs¹⁶ (Fig. 1, prosynaptic pathway). Activation of MuSK also slows the ongoing proteolytic degradation of rapsyn, thereby increasing the amount of rapsyn in the cytoplasm.^{17,18}

Rapsyn stabilizes the matrix of AChRs within the plane of the postsynaptic membrane (the AChR cluster) via several complementary mechanisms. First, rapsyn cross-links adjacent AChRs.¹⁹ Through its multiple interaction domains, rapsyn also recruits protein kinase A²⁰ and suppresses the activity of calcium-activated proteases in the vicinity of the AChR cluster.^{21,22} In addition, rapsyn functions as an E3 ligase, covalently tagging AChRs with Nedd8, a small ubiquitin-like protein.²³ This combination of actions slows the metabolic turnover of the clustered AChRs and helps recruit and sustain the very high density of AChRs within the functioning postsynaptic membrane.

The activity of the MuSK–rapsyn system is probably regulated by several factors in addition to neural agrin. Different muscles of the mouse express differing amounts of MuSK mRNA, and such differences may affect NMJ formation/stability in different parts of the body.^{9,24} DOK7 binds to MuSK protein dimers and stabilizes the kinase-active complex.²⁵ Experimentally elevating the level of DOK7 in a muscle fiber can increase the tyrosine kinase activity of MuSK, resulting in the growth of giant NMJs.²⁶ Biglycan and some members of the Wnt family of proteins bind to MuSK. Each of these extracellular ligands can influence MuSK signaling.^{27,28} Neuregulins can indirectly modulate MuSK function via intracellular signaling pathways.^{29,30} Thus, the prosynaptic function of MuSK can be modulated by multiple extracellular ligands and intracellular signaling networks.

Passive IgG transfer model of MuSK MG

Our passive IgG transfer mouse model of MuSK MG was based on a protocol introduced by Klaus Toyka and colleagues in the 1970s and later adapted to investigate immunoglobulins of seronegative cases.^{1,4} Young adult female C57BL6J mice were given daily intraperitoneal injections of IgG sourced from anti-MuSK⁺ MG patients undergoing therapeutic plasmapheresis.³¹ To suppress a potential active immune response to the human protein, the mouse also received a single immunosuppressive dose of cyclophosphamide (300 mg/kg) 24 h after the first IgG injection. Each day, the mouse was weighed and assessed for weakness. Grade 1 weakness was scored if the mouse was immobile, lying prone with its head, trunk, and tail all resting on the bench, after a standard exercise regime (12 times backward walking across a cage grill). Grade 2 was scored if the mouse displayed this behavior before any exercise.^{32,33} Mice receiving daily injections of IgG (30–45 mg) from severely affected MuSK MG patients (MGFA grades 2B–4B) started to lose weight after 10–12 days and typically displayed grade 2 weakness by day 14.³¹ Control mice injected with pooled IgG from nonmyasthenic patients (and including the cyclophosphamide injection) continued to gain weight and showed no weakness.

The weakness was the result of impaired neuromuscular transmission. Compound muscle action potential recordings revealed a decrement in amplitude during repetitive nerve stimulation (3 impulses/s). Decrement was detectable as early as 6 days after the start of anti-MuSK IgG injections.

Ex vivo endplate potential recordings from fibers of the diaphragm muscle revealed a progressive reduction in the amplitude of the endplate potentials and miniature endplate potentials as the anti-MuSK injection series proceeded.³⁴ These changes could, in turn, be explained as a progressive decline in the number and density of postsynaptic AChRs.³⁴ Other groups likewise found reductions in endplate potential amplitudes and postsynaptic AChR numbers after mice received repeated daily injections of IgG from anti-MuSK⁺ patients.^{35,36} Studies involving active immunization of animals with MuSK protein reported a variety of structural and/or functional abnormalities at the NMJ. However, the broad conclusion from both passive IgG transfer and active immunization models of MuSK MG was that the loss of postsynaptic AChRs was the primary cause of neuromuscular transmission failure and weakness (reviewed in Ref. 33 and elsewhere).

Anti-MuSK IgG causes AChR loss by suppressing MuSK function

The pathological effects of MuSK autoantibodies do not depend on classical immunopathology. Lines of mice with genetic deficiencies in the antibody-mediated complement pathway developed severe MG when actively immunized with MuSK.³⁷ More importantly, MuSK antibodies from MG patients are predominantly of the IgG4 subtype.⁶ IgG4 is considered functionally monovalent and cannot activate complement.^{38,39} The monovalent binding of IgG4 is caused by the dynamic Fab-arm exchange reaction, which has been confirmed to affect MuSK IgG4 autoantibodies.⁴⁰ Thus, anti-MuSK IgG4 would not be expected to cross-link MuSK or initiate complement attack on the endplate. Klooster and colleagues fractionated the IgG4 and IgG1–3 components from plasma from anti-MuSK MG patients, injecting each separately into mice. They found that daily injections of as little as 0.5–4 mg of the IgG4 fraction was sufficient to cause body weight loss and weakness, suggesting that anti-MuSK IgG4 was the main pathogenic component in patient plasma, at least for the patients tested.³⁶

Anti-MuSK IgG4 causes disassembly of the NMJ by blocking the physiological function of MuSK. In 2008, LRP4 was identified as the missing essential coreceptor for neural agrin.^{10,11} This led to the key finding that anti-MuSK IgG4 blocked the assembly of the agrin–LRP4–MuSK complex,^{41–43} explaining why, in the presence of anti-MuSK IgG4, neural agrin could not induce AChR cluster formation on cultured muscle cells. In our mouse passive-transfer model, injection of total IgG from MuSK MG patients caused reductions in tyrosine phosphorylation of target proteins at the endplate (Src kinase and AChR). There were also reductions in the endplate density of rapsyn. All of this is consistent with suppressed MuSK kinase activity within the postsynaptic membrane.⁴⁴ Endplate AChR clusters became fragmented, and the AChRs were degraded at an accelerated rate.⁴⁴ Together, these studies support the idea that MuSK autoantibodies cause neuromuscular transmission failure

primarily by impairing the capacity of MuSK to maintain AChR clustering in the postsynaptic membrane of the NMJ.

Non-IgG4 MuSK autoantibodies may affect MuSK function in a different way. Bivalent anti-MuSK IgG raised in animals can cross-link MuSK, thereby inducing autophosphorylation and activation of MuSK.^{45,46} Total IgG from one MuSK MG patient similarly activated MuSK.⁴⁷ When added to cultured C2C12 myotubes, the IgG1–3 fraction from MuSK MG patients disrupted preexisting large AChR clusters, even when the clusters had been generated in the absence of agrin (by overexpressing DOK7⁴²). Using the nonmuscle cell line HEK293, the latter study found no evidence that anti-MuSK IgG1–3 caused internalization of MuSK. However, when neural agrin was applied to C2C12 muscle cells, activated MuSK became internalized and was degraded in the lysosome.^{48,49} It remains possible that, when the NMJ is exposed to a subset of autoantibodies that persistently activate MuSK, the lysosomal degradation of MuSK might be accelerated in a similar way.

At the mouse motor endplate, we found a marked reduction in the intensity of MuSK immunolabeling after a series of daily injections of total IgG from anti-MuSK⁺ patients.^{44,47} We then used an adeno-associated viral vector to elevate the expression of MuSK within the mouse tibialis anterior muscle. When the mice were subsequently challenged with a series of injections of IgG from an anti-MuSK⁺ patient, muscles expressing the additional (recombinant) MuSK retained more MuSK and more AChR at their endplates. They also showed less impairment of neuromuscular transmission compared with contralateral (empty vector) muscles.⁵⁰ Thus, an autoantibody-induced loss of MuSK protein from the motor endplate might help accelerate synapse disassembly. The mechanism by which MuSK becomes displaced from the motor endplate remains uncertain. It may involve activation/degradation, as discussed above. On the other hand, binding of autoantibody is known to disrupt the tethering of MuSK to collagen Q in the basement membrane.^{51,52} This might allow MuSK to diffuse laterally in the membrane, escaping into the extrasynaptic sarcolemma. The full impact of autoantibodies upon the molecular interactions of MuSK remains to be defined.

Presynaptic effects of anti-MuSK

The precise effects of MuSK autoantibodies upon the presynaptic motor nerve terminal also remain uncertain. In rodents actively immunized with MuSK, immunofluorescence revealed shrinkage of

nerve terminals at NMJs.^{53,54} In mice that became weak after repeated daily injections of anti-MuSK⁺ patient IgG, we initially reported that nerve terminals became misaligned with the postsynaptic AChRs.³¹ In subsequent studies, using IgG samples from additional patients and different analytical methods, misalignment could be largely accounted for by patchy loss of AChRs from beneath nerve terminals.^{34,55} Additional, high-resolution quantitative studies are needed to clarify the precise changes to the molecular structure of the nerve terminal in mouse models of MuSK MG.

Intracellular electrophysiology provides consistent evidence of impaired presynaptic function in rodent models of MuSK MG. The number of quanta of acetylcholine released per nerve impulse (quantal content) was consistently reduced in diaphragm muscles of mice that became weak after active immunization with MuSK.^{35,37,56} In one such study, reduced quantal content was accompanied by intermittent failure of the presynaptic action potential.⁵⁶ In rodent models of anti-AChR⁺ MG, the reduction in postsynaptic acetylcholine sensitivity triggers a robust increase in quantal release from the presynaptic nerve terminal.^{57–61} This form of presynaptic adaptation failed completely in mice that became weak after repeated injections of anti-MuSK⁺ patient IgG.^{34–36,55} The failure of presynaptic adaptation likely exacerbates the neuromuscular transmission failure. These findings also suggest that MuSK may be involved in the retrograde feedback system that adjusts presynaptic quantal release when there are changes in postsynaptic quantal amplitude.

Initial testing of therapeutics in the passive IgG transfer model

Cholinesterase inhibitors, such as pyridostigmine, are a first-line treatment for anti-AChR⁺ MG patients. They prolong the survival of acetylcholine after it is released into the synaptic cleft, allowing it to activate more AChR cation channels for longer. However, for anti-MuSK⁺ MG patients, cholinesterase inhibitors have often been found to confer little benefit or even to cause harm. Indeed, patients and features typical of cholinergic excess can be seen even in the absence of cholinesterase inhibitors.^{62–65} Excessive, prolonged use of cholinesterase inhibitors in experimental rats has long been known to cause damage to the NMJ.⁶⁶ MuSK autoantibodies can interfere with the binding of collagen Q to MuSK, and this might cause a reduction in synaptic acetylcholinesterase.^{37,51,67} Our evolving understanding of NMJ development provides a mechanism to explain how even a moderate reduction in synaptic cholinesterase function might exacerbate the loss of postsynaptic AChR in MuSK⁺ MG. The MuSK–rapsyn pathway is now thought to protect postsynaptic AChRs from an opposing the synapse disassembly pathway driven by acetylcholine.^{68,69} The opening of AChR cation channels allows entry of tiny amounts of Ca²⁺. Inositol trisphosphate receptors are concentrated directly beneath the postsynaptic AChRs, and these appear to amplify the local calcium influx. When

subsynaptic calcium fluxes are prolonged and excessive, they can then activate calpain and caspase-3.⁷⁰ Once activated, these proteases can then cleave the MuSK binding-partner protein, Disheveled, and result in internalization and loss of AChRs.²² Postsynaptic AChRs would normally be protected from this destructive pathway by rapsyn, which acts downstream of agrin–LRP4–MuSK^{21,22} (Fig. 1).

We tested the effects of pyridostigmine in our passive IgG transfer model of MuSK MG. Mice were given injections of IgG from anti-MuSK⁺ MG patients every day for 2 weeks. Halfway through the IgG injection series, a mini-pump was implanted subcutaneously to deliver pyridostigmine systemically. The concentration of pyridostigmine in the pump was adjusted so as to inhibit blood cholinesterase activity to about 60% of its normal level, comparable to activity levels found in effectively treated myasthenia patients.⁵⁵ Pyridostigmine exacerbated the disease process. Pyridostigmine-treated mice lost more AChRs from their motor endplates and displayed lower endplate potential amplitudes compared with myasthenic mice that received only vehicle from their implanted mini-pumps. Another cohort of mice received smaller injections of myasthenic IgG, insufficient to cause body weight loss or weakness. Treatment with pyridostigmine for 7 days pushed these subclinical mice into overt weakness and weight loss.⁵⁵ Together, these experiments show that in mice (at least) pyridostigmine can potentiate anti-MuSK–induced loss of postsynaptic AChRs, neuromuscular transmission failure, and weakness.

In the same study, we also tested the effects of 3,4-diamino-pyridine (3,4-DAP) in mice receiving daily injections of anti-MuSK⁺ patient IgG. As expected, 3,4-DAP acted on the motor nerve terminal to increase the quantal content. Unlike pyridostigmine, 7 days of treatment with 3,4-DAP resulted in increased endplate potential amplitude compared with vehicle-treated myasthenic mice.⁵⁵ In the mouse active immunization model of MuSK MG, acute treatment of myasthenic muscles with 3,4-DAP produced a similar improvement in endplate potential amplitudes.⁷¹ An initial case study reported that 3,4-DAP may indeed be useful for treating MuSK MG patients.⁷²

Pyridostigmine and 3,4-DAP both increase the endplate potential amplitude, but they do so via different mechanisms. Cholinesterase inhibitors prolong the opening of AChR cation channels, and this can cause activation of caspase-3.⁷⁰ In contrast, 3,4-DAP increases the number of quanta of acetylcholine released by the nerve terminal. The NMJ is a very large synapse, composed of hundreds of transmitter release sites. Each quantum of acetylcholine is thought to act on a spatially discrete (nonoverlapping) patch of AChRs (< 1 μm diameter critical area), causing just a brief opening of the local AChR cation channels.⁷³ The postjunctional membrane infoldings would tend to keep each

quantal calcium influx compartmentalized beneath this small critical area. Thus, while quantal Na^+ currents sum together to produce an endplate potential, the (much smaller) quantal Ca^{2+} influx signal would normally be both brief and discrete. Cholinesterase inhibitors extend the actions of each quantum of acetylcholine in time and space, thereby potentiating AChR loss via the synapse disassembly pathway. In contrast, 3,4-DAP increases the number of brief, discrete quantal currents that contribute to the endplate potential without prolonging the local Ca^{2+} signal.

Sympathomimetics, including albuterol, have been useful for treating congenital myasthenia involving *DOK7* mutations.⁷⁴ This raised the possibility that such drugs might also be helpful for anti-MuSK⁺ MG. In our IgG passive-transfer mouse model, albuterol staved off weakness and weight loss, but the mechanism through which it conferred this benefit remains unclear. In the muscles examined, we found no substantial improvement in the endplate AChR area, endplate potential amplitude, or muscle fiber diameters that might explain the sparing of weakness in our mice.⁷⁵ Sympathomimetics act at many sites in the body that might affect muscle strength over different time frames. Further studies involving longer term treatment in active immunization models of MuSK MG might help elucidate the therapeutic potential of albuterol for MuSK MG.

Competing interests

The authors declare no competing interests.

References

1. Toyka, K.V., D.B. Brachman, A. Pestronk, *et al.* 1975. Myasthenia gravis: passive transfer from man to mouse. *Science* **190**: 397–399.
2. Lennon, V.A., J.M. Lindstrom & M.E. Seybold. 1975. Experimental autoimmune myasthenia: a model of myasthenia gravis in rats and guinea pigs. *J. Exp. Med.* **141**: 1365–1375.
3. Aharonov, A., O. Abramsky, R. Tarrab-Hazdai, *et al.* 1975. Humoral antibodies to acetylcholine receptor in patients with myasthenia gravis. *Lancet* **2**: 340–342.
4. Mossman, S., J. Newsom-Davis & A. Vincent. 1986. Myasthenia gravis without acetylcholine receptor antibody: a distinct disease entity. *Lancet* **327**: 116–119.
5. Hoch, W., J. McConville, S. Helms, *et al.* 2001. Autoantibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. *Nat. Med.* **7**: 365–368.

6. McConville, J., M.E. Farrugia, D. Beeson, *et al.* 2004. Detection and characterization of MuSK antibodies in seronegative myasthenia gravis. *Ann. Neurol.* **55**: 580-584.
7. Valenzuela, D.M., T.N. Stitt, P.S. DiStefano, *et al.* 1995. Receptor tyrosine kinase specific for the skeletal muscle lineage: expression in embryonic muscle, at the neuromuscular junction, and after injury. *Neuron* **15**: 573-584.
8. DeChiara, T.M., D.C. Bowen, D.M. Valenzuela, *et al.* 1996. The receptor tyrosine kinase MuSK is required for neuromuscular junction formation *in vivo*. *Cell* **85**: 501-512.
9. Kim, N. & S.J. Burden. 2008. MuSK controls where motor axons grow and form synapses. *Nat. Neurosci.* **11**: 19-27.
10. Kim, N., A.L. Stiegler, T.O. Cameron, *et al.* 2008. Lrp4 is a receptor for agrin and forms a complex with MuSK. *Cell* **135**: 334-342.
11. Zhang, B., S. Luo, Q. Wang, *et al.* 2008. LRP4 serves as a coreceptor of agrin. *Neuron* **60**: 285-297.
12. Mitaud, P., P.A. Marangi, S. Erb-Vogtli, *et al.* 2001. Agrin-activation of acetylcholine receptor-bound Src family kinases requires rapsyn and correlates with acetylcholine receptor clustering. *J. Biol. Chem.* **276**: 14505-14513.
13. Mohamed, A.S., K.A. Rivas-Plata, J.R. Kraas, *et al.* 2001. Src-class kinases act within the agrin/MuSK pathway to regulate acetylcholine receptor phosphorylation, cytoskeletal anchoring, and clustering. *J. Neurosci.* **21**: 3806-3818.
14. Smith, C.L., P. Mitaud, E.D. Prescott, *et al.* 2001. Src, Fyn, and Yes are not required for neuromuscular synapse formation but are necessary for stabilization of agrin-induced clusters of acetylcholine receptors. *J. Neurosci.* **21**: 3151-3160.
15. Finn, A.J., G. Feng & A.M. Pendergast. 2003. Postsynaptic requirements for Abl kinases in assembly of the neuromuscular junction. *Nat. Neurosci.* **6**: 717-723.
16. Borges, L., S. Yechikov, Y.I. Lee, *et al.* 2008. Identification of a motif in the acetylcholine receptor β -subunit whose phosphorylation regulates rapsyn association and postsynaptic receptor localization. *J. Neurosci.* **28**: 11468-11476.
17. Brockhausen, J., R.N. Cole, O.L. Gervásio, *et al.* 2008. Neural agrin increases postsynaptic ACh receptor packing by elevating rapsyn protein at the mouse neuromuscular synapse. *Dev. Neurobiol.* **68**: 1153-1169.
18. Luo, S., B. Zhang, X. Dong, *et al.* 2008. HSP90 β regulates rapsyn turnover and subsequent AchR cluster formation and maintenance. *Neuron* **60**: 97-110.
19. Zuber, B. & N. Unwin. 2013. Structure and superorganization of acetylcholine receptor-rapsyn complexes. *Proc. Natl. Acad. Sci. USA* **110**: 10622-10627.
20. Choi, K.R., M. Berrera, M. Reischl, *et al.* 2012. Rapsyn mediates subsynaptic anchoring of PKA type I and stabilisation of acetylcholine receptor *in vivo*. *J. Cell Sci.* **125**: 714-723.
21. Chen, F., L. Qian, Z.-H. Yang, *et al.* 2007. Rapsyn interaction with calpain stabilizes AChR clusters at the neuromuscular junction. *Neuron* **55**: 247-260.
22. Wang, J.Y., F. Chen, X.Q. Fu, *et al.* 2014. Caspase-3 cleavage of dishevelled induces elimination of postsynaptic structures. *Dev. Cell* **28**: 670-684. .
23. Li, L., Y. Cao, H. Wu, *et al.* 2016. Enzymatic activity of the scaffold protein rapsyn for synapse formation. *Neuron* **92**: 1-13.
24. Punga, A.R., M. Maj, S. Lin, *et al.* 2011. MuSK levels differ between adult skeletal muscles and influence postsynaptic plasticity. *Eur. J. Neurosci.* **33**: 890-898.

25. Bergamin, E., P.T. Hallock, S.J. Burden, *et al.* 2010. The cytoplasmic adaptor protein Dok7 activates the receptor tyrosine kinase MuSK via dimerization. *Mol. Cell* **39**: 100-109.
26. Arimura, S., T. Okada, T. Tezuka, *et al.* 2014. Neuromuscular disease. DOK7 gene therapy benefits mouse models of diseases characterized by defects in the neuromuscular junction. *Science* **345**: 1505-1508.
27. Amenta, A.R., H.E. Creely, M.L. Mercado, *et al.* 2012. Biglycan is an extracellular MuSK binding protein important for synapse stability. *J. Neurosci.* **32**: 2324-2334.
28. Barik, A., B. Zhang, G.S. Sohal, *et al.* 2014. Crosstalk between Agrin and Wnt signaling pathways in development of vertebrate neuromuscular junction. *Dev. Neurobiol.* **74**: 828-838.
29. Schmidt, N., M. Akaaboune, N. Gajendran, *et al.* 2011. Neuregulin/ErbB regulate neuromuscular junction development by phosphorylation of α -dystrobrevin. *J. Cell Biol.* **195**: 1171-1184.
30. Ngo, S.T., R.N. Cole, N. Sunn, *et al.* 2012. Neuregulin-1 potentiates agrin-induced acetylcholine receptor clustering via muscle specific kinase phosphorylation. *J. Cell Sci.* **125**: 1531-1543.
31. Cole, R.N., S.W. Reddel, O.L. Gervásio, *et al.* 2008. Anti-MuSK patient antibodies disrupt the mouse neuromuscular junction. *Ann. Neurol.* **63**: 782-789.
32. Stacy, S., B.E. Gelb, B.A. Koop, *et al.* 2002. Split tolerance in a novel transgenic model of autoimmune myasthenia gravis. *J. Immunol.* **169**: 6570-6579.
33. Phillips, W.D., P. Christadoss, M. Losen, *et al.* 2015. Guidelines for pre-clinical animal and cellular models of MuSK-myasthenia gravis. *Exp. Neurol.* **270**: 29-40.
34. Morsch, M., S.W. Reddel, N. Ghazanfari, *et al.* 2012. Muscle specific kinase autoantibodies cause synaptic failure through progressive wastage of postsynaptic acetylcholine receptors. *Exp. Neurol.* **237**: 286-295.
35. Viegas, S., L. Jacobson, P. Waters, *et al.* 2012. Passive and active immunization models of MuSK-Ab positive myasthenia: electrophysiological evidence for pre and postsynaptic defects. *Exp. Neurol.* **234**: 506-512.
36. Klooster, R., J.J. Plomp, M.G. Huijbers, *et al.* 2012. Muscle-specific kinase myasthenia gravis IgG4 autoantibodies cause severe neuromuscular junction dysfunction in mice. *Brain* **135**: 1081-1101.
37. Mori, S., S. Kubo, T. Akiyoshi, *et al.* 2012. Antibodies against muscle-specific kinase impair both presynaptic and postsynaptic functions in a murine model of myasthenia gravis. *Am. J. Pathol.* **180**: 798-810.
38. Tao, M., S.M. Canfield & S.L. Morrison. 1991. The differential ability of human IgG1 and IgG4 to activate complement is determined by the COOH-terminal sequence of the CH2 domain. *J. Exp. Med.* **173**: 1025-1028.
39. Aalberse, R.C., S.O. Stapel, J. Schuurman, *et al.* 2009. Immunoglobulin G4: an odd antibody. *Clin. Exp. Allergy* **39**: 469-477.
40. Koneczny, I., J.A. Stevens, A. De Rosa, *et al.* 2017. gG4 autoantibodies against muscle-specific kinase undergo Fab-arm exchange in myasthenia gravis patients. *J. Autoimmun.* **77**: 104-115.
41. Huijbers, M.G., W. Zhang, R. Klooster, *et al.* 2013. MuSK IgG4 auto-antibodies cause myasthenia gravis by inhibiting binding between MuSK and Lrp4. *Proc. Natl. Acad. Sci. USA* **110**: 20783-20788.

42. Koneczny, I., J. Cossins, P. Waters, *et al.* 2013. MuSK myasthenia gravis IgG4 disrupts the interaction of LRP4 with MuSK but both IgG4 and IgG1-3 can disperse preformed agrin-independent AChR clusters. *PLoS One* **11**: e80695.
43. Otsuka, K., M. Ito, B. Ohkawara, *et al.* 2015. Collagen Q and anti-MuSK autoantibody competitively suppress agrin/LRP4/MuSK signaling. *Sci. Rep.* **5**: 13928.
44. Ghazanfari, N., M. Morsch, S.W. Reddel, *et al.* 2014. Muscle specific kinase autoantibodies suppress the MuSK pathway and ACh receptor retention at the mouse neuromuscular junction. *J. Physiol.* **592**: 2881-2897.
45. Hopf, C. & W. Hoch. 1998. Dimerization of the muscle-specific kinase induces tyrosine phosphorylation of acetylcholine receptors and their aggregation on the surface of myotubes. *J. Biol. Chem.* **273**: 6467-6473.
46. Shigemoto, K., S. Kubo, N. Maruyama, *et al.* 2006. Induction of myasthenia by immunization against muscle-specific kinase. *J. Clin. Invest.* **116**: 1016-1024.
47. Cole, R.N., N. Ghazanfari, S.T. Ngo, *et al.* 2010. Patient autoantibodies deplete postsynaptic muscle specific kinase leading to disassembly of the ACh receptor scaffold and myasthenia gravis in mice. *J. Physiol.* **588**: 3217-3229.
48. Lu, Z., H.-S. Je, P. Young, *et al.* 2007. Regulation of synaptic growth and maturation by a synapse-associated E3 ubiquitin ligase at the neuromuscular junction. *J. Cell Biol.* **177**: 1077-1089.
49. Zhu, D., Z. Yang, Z. Luo, *et al.* 2008. Muscle-specific receptor tyrosine kinase endocytosis in acetylcholine receptor clustering in response to agrin. *J. Neurosci.* **28**: 1688-1696.
50. Ghazanfari, N., E.L.T.B. Linsao, S. Trajanovska, *et al.* 2015. Forced expression of muscle specific kinase slows postsynaptic acetylcholine receptor loss in a mouse model of MuSK myasthenia gravis. *Physiol. Rep.* **3**: 12658.
51. Kawakami, Y., M. Ito, M. Hirayama, *et al.* 2011. Anti-MuSK autoantibodies block binding of collagen Q to MuSK. *Neurology* **77**: 1-8.
52. Sigoillot, S.M., F. Bourgeois, M. Lambergeon, *et al.* 2010. ColQ controls postsynaptic differentiation at the neuromuscular junction. *J. Neurosci.* **30**: 13-23.
53. Xu, K., S. Jha, W. Hoch, *et al.* 2006. Delayed synapsing muscles are more severely affected in an experimental model of MuSK-induced myasthenia gravis. *Neuroscience* **143**: 655-659.
54. Punga, A.R., S. Lin, F. Oliveri, *et al.* 2011. Muscle-selective synaptic disassembly and reorganization in MuSK antibody-positive MG mice. *Exp. Neurol.* **230**: 207-217.
55. Morsch, M., S.W. Reddel, N. Ghazanfari, *et al.* 2013. Pyridostigmine but not 3,4-diaminopyridine exacerbates ACh receptor loss and myasthenia induced in mice by muscle specific kinase autoantibody. *J. Physiol.* **591**: 2747-2762.
56. Patel, V., O. Oh, A. Voit, *et al.* 2014. Altered active zones, vesicle pools, nerve terminal conductivity, and morphology during experimental MuSK myasthenia gravis. *PLoS One* **9**: e110571.
57. Plomp, J.J., G.T.H. van Kempen & P.C. Molenaar. 1992. Adaptation of quantal content to decreased postsynaptic sensitivity at single endplates in alpha-bungarotoxin-treated rats. *J. Physiol.* **458**: 487-499.
58. Plomp, J.J., G.T.H. van Kempen, M. DeBaets, *et al.* 1995. Acetylcholine release in myasthenia gravis: regulation at single end-plate level. *Ann. Neurol.* **37**: 627-636.

59. Wang, X., Q. Wang, K.L. Engisch, *et al.* 2010. Activity-dependent regulation of the binomial parameters p and n at the mouse neuromuscular junction *in vivo*. *J. Neurophysiol.* **104**: 2352-2358.
60. Wang, X., M.J. Pinter & M.M. Rich. 2016. Reversible recruitment of a homeostatic reserve pool of synaptic vesicles underlies rapid homeostatic plasticity of quantal content. *J. Neurosci.* **36**: 828-836.
61. Plomp, J.J., M. Morsch, W.D. Phillips, *et al.* 2015. Electrophysiological analysis of neuromuscular synaptic function in myasthenia gravis patients and animal models *Exp. Neurol.* **270**: 41-54.
62. Evoli, A., P.A. Tonali, L. Padua, *et al.* 2003. Clinical correlates with anti-MuSK antibodies in generalized seronegative myasthenia gravis. *Brain* **126**: 2304-2311.
63. Pasnoor, M., G.I. Wolfe, S. Nations, *et al.* 2010. Clinical findings in MuSK-antibody positive myasthenia gravis: a U.S. experience. *Muscle Nerve* **41**: 370-374.
64. Guptill, J.T., D.B. Sanders & A. Evoli. 2011. Anti-MuSK antibody myasthenia gravis: clinical findings and response to treatment in two large cohorts. *Muscle Nerve* **44**: 36-40.
65. Simon, N.G., S.W. Reddel, M.C. Kiernan, *et al.* 2013. Muscle-specific kinase autoantibodies a novel cause of peripheral nerve hyperexcitability? *Muscle Nerve* **48**: 819-823. .
66. Engel, A.G., E.H. Lambert & T. Santa. 1973. Study of long-term anticholinesterase therapy. *Neurology* **23**: 1273-1281.
67. Ohno, K., M. Ito, Y. Kawakami, *et al.* 2014. Collagen Q is a key player for developing rational therapy for congenital myasthenia and for dissecting the mechanisms of anti-MuSK myasthenia gravis. *J. Mol. Neurosci.* **53**: 359-361.
68. Misgeld, T., T.T. Kummer, J.W. Lichtman, *et al.* 2005. Agrin promotes synaptic differentiation by counteracting an inhibitory effect of neurotransmitter. *Proc. Natl. Acad. Sci. USA* **102**: 11088-11093.
69. Lin, W., B. Dominguez, J. Yang, *et al.* 2005. Neurotransmitter acetylcholine negatively regulates neuromuscular synapse formation by a Cdk5-dependent mechanism. *Neuron* **46**: 569-579.
70. Zhu, H., B.J. Bhattacharyya, H. Lin, *et al.* 2011. Skeletal muscle IP3R1 receptors amplify physiological and pathological synaptic calcium signals. *J. Neurosci.* **31**: 15269-15283.
71. Mori, S., M. Kishi, S. Kubo, *et al.* 2012. 3,4-Diaminopyridine improves neuromuscular transmission in a MuSK antibody-induced mouse model of myasthenia gravis. *J. Neuroimmunol.* **245**: 75-78.
72. Evoli, A., P.E. Alboini, V. Damato, *et al.* 2016. 3,4-Diaminopyridine may improve myasthenia gravis with MuSK antibodies. *Neurology* **86**: 1070-1071.
73. Matthews-Bellinger, J. & M.M. Salpeter. 1978. Distribution of acetylcholine receptors at frog neuromuscular junctions with a discussion of some physiological implications. *J. Physiol.* **279**: 197-213.
74. Liewluck, T., D. Selcen & A.G. Engel. 2011. Beneficial effects of albuterol in congenital endplate acetylcholinesterase deficiency and Dok-7 myasthenia. *Muscle Nerve* **44**: 789-794.
75. Ghazanfari, N., M. Morsch, N. Tse, *et al.* 2014. Effects of the β 2-adrenoceptor agonist, albuterol, in a mouse model of anti-MuSK myasthenia gravis. *PLoS One* **9**: e87840.

Figure 1. Opposing signaling systems sculpt the size of the developing NMJ. Neural agrin released by the motor nerve terminal binds LRP4, activating the MuSK complex. This recruits rapsyn, which stabilizes postsynaptic AChRs. DOK7 binds the cytoplasmic aspect of the MuSK dimer, favoring the growth of the NMJ. Subsynaptic Ca^{2+} fluxes initiated by prolonged opening of AChR cation channels can activate calpain and caspase-3. This can cause the pruning of AChRs from the AChR cluster (cartoon snake). The prosynaptic agrin–MuSK–rapsyn system usually suppresses this synapse-disassembly pathway. MuSK autoantibodies of the IgG4 type block the assembly of the active MuSK complex, thereby reducing the activity of the prosynaptic pathway. In this situation, cholinesterase inhibitors can drive the synapse-disassembly pathway, accelerating AChR loss and neuromuscular transmission failure.

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