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Abtract

Our understanding of transmission at the neuromuscular junction has increased greatly in recent years. We now recognise a wide variety of autoimmune and genetic diseases that affect this specialised synapse, causing muscle weakness and fatigue. These disorders greatly affect quality of life and rarely can be fatal. Myasthenia Gravis is the most common disorder and is most commonly caused by auto-antibodies targeting postsynaptic acetylcholine receptors (AChRs). Antibodies to muscle-specific kinase (MuSK) are detected in a variable proportion of the remainder. Treatment is symptomatic and immunomodulatory. Lambert-Eaton Myasthenic Syndrome is caused by antibodies to presynaptic calcium channels, and approximately 50% of cases are paraneoplastic, most often related to small cell carcinoma of the lung. Botulism is an acquired disorder caused by neurotoxins produced by clostridium botulinum, impairing acetylcholine release into the synaptic cleft. In addition several rare congenital myasthenic syndromes have been identified, caused by inherited defects in presynaptic, synaptic basal lamina, and postsynaptic proteins necessary for neuromuscular transmission. This review focuses on recent advances in the diagnosis and treatment of these disorders.

Introduction

Abnormalities of synaptic function contribute to many neurological and psychiatric diseases. The archetypical "synaptopathies" are a group of relatively rare disorders affecting the neuromuscular junction (NMJ). Both inherited and acquired disorders of the NMJ cause weakness and fatigability. Much progress has been made in understanding the pathogenesis and diagnosis of these disorders in recent years. This review highlights some recent advances in the understanding of this group of diseases and also considers the emerging evidence regarding their optimal management.

Neuromuscular transmission

The fundamental principles of synaptic transmission were first identified at the NMJ (Fig. 1). Many of the individual steps are directly implicated in neuromuscular disease. Voltage-gated calcium channels open in the presynaptic end-plate in response to the nerve action potential. The subsequent influx of calcium ions triggers exocytosis of vesicles containing acetylcholine (ACh) into the synaptic cleft, in a mechanism that depends on co-ordinated assembly of proteins known as the SNAREs (Soluble NSF Attachment protein Receptors). The SNARE proteins include syntaxin 1, SNAP 25 and synaptobrevin, and are capable of forming a tight complex that brings the vesicle and the plasma membranes into close apposition, in preparation for exoctytosis. Binding of ACh to the postsynaptic receptors opens a cation-permeable pore, which depolarises the muscle fibre. This depolarization can be measured experimentally as an end-plate potential (EPP). Voltage-gated sodium channels open in response to the EPP, triggering a muscle action potential, which leads to release of calcium ions from the sarcoplasmic reticulum. Calcium ions bind to troponin C, which leads to muscle contraction mediated by actin and myosin filaments.

Under normal conditions, neuromuscular transmission has a high safety factor; that is, the EPP is more than sufficient to trigger the action potential (1,2). However, under conditions of impaired transmission, due to insufficient presynaptic ACh release or alternatively because of a defect of postsynaptic receptors, the EPP can become sub-threshold, leading to muscle weakness. The NMJ is a target of several autoimmune diseases, caused by antibodies to pre- or postsynaptic proteins. It is also affected in botulism, where SNARE proteins are cleaved. And several congenital myasthenic syndromes (CMS) result from inherited defects of proteins at all stages of neuromuscular transmission.

Myasthenia gravis (MG)

Epidemiology

MG is the commonest disorder of the NMJ, with a prevalence of around 15 per 100,000, although it is probably under-diagnosed in the elderly population. The incidence is bimodal, with a female to male ratio of approximately 2:1 in young adults, and a reversed sex ratio in the older group that accounts for 60% of cases (3-6).

Immunopathogenesis

MG is firmly established as an autoimmune disease (1,2). In the commonest form of MG, antibodies that bind to ACh receptors are detected in the synaptic cleft (7-9). These antibodies are of the IgG1 or IgG3 subtypes and activate complement, resulting in focal destruction of the postsynaptic membrane by the membrane attack complex (10). In addition, they cross-link AChRs, leading to an increase in the rate of destruction, and may also inhibit receptor function directly (7).

A distinct form of MG is caused by antibodies targeting muscle-specific kinase (MuSK) and will be discussed separately.

The thymus has an important role in the pathogenesis of early onset MG associated with anti-AChR antibodies, and is frequently enlarged with lymphocytic infiltrates, germinal centres and myoid cells, which express AChRs. (7) The thymus gland in patients developing MG later in life is however typically atrophic, and its immunogenic role is less clear.

A thymoma is present in 10% of patients with MG, with a peak incidence in the 4th to 6th decades and an equal frequency in males and females. Patients with thymoma often have antibodies to various components of striated muscle: titin, myosin, actin and ryanodine receptors which are associated with a more severe clinical MG phenotype (11,12) .

Clinical Manifestations

In common with other acquired and inherited disorders of the NMJ, MG manifests as painless fatigable muscle weakness. The pattern of muscle involvement varies, but extraocular muscles are most commonly affected, causing diplopia and ptosis. Approximately 80% of patients presenting with ocular symptoms will eventually develop generalised MG(13) . Up to 20% of patients with MG have prominent bulbar symptoms early in their disease (14). Limb weakness can affect any muscle group, although proximal muscles are most often affected. Symptoms typically worsen towards the end of the day and with exercise. Extreme heat, emotional stress, infection, pregnancy and menstruation can all exacerbate myasthenia, as can many drugs, including aminoglycoside antibiotics and penicillamine.

Prognosis/ Natural History

MG has a highly variable course. If symptoms remain purely ocular for more than 2 years, the risk of progression to generalised MG is less than 10% (13). However, approximately 39% of patients have severe MG and half of these will require invasive ventilation during the course of their disease (13). The forced vital capacity should be measured in all patients to look out for

this complication. The mortality of MG has nevertheless dropped significantly from over 30% in the 1950s to less than 5%. This is most likely due to improved ventilatory care and increased availability of immunomodulatory treatments(15,3). Assessment of outcome has been helped by the establishment of a task force by the Myasthenia Gravis Foundation of America in 1997, which led to recommendations for clinical research standards, including uniformity in the reporting of clinical trials (16,17) .

Diagnosis

Relevant questions to the patient include pattern of muscle weakness, fatigability and exacerbating factors. Sensory deficits and absent/increased reflexes are not features of MG. Fatigability can be elicited by observing for the development of ptosis and diplopia during prolonged upgaze or by testing neck flexion/extension, and shoulder abduction before and after unilateral repetitive movement. A relatively sensitive and specific bedside test that distinguishes myasthenia from other causes of ptosis involves application of crushed ice in a latex glove to the eye. This leads to improvement of ptosis in MG and has been reported to have a sensitivity of 89% (18).

Edrophonium test

Although this test is uncommonly performed, it can be useful if there are delays in obtaining other investigations, and has a high sensitivity for generalised MG. The acetylcholinesterase inhibitor is administered intravenously, observing for transient improvement in muscle strength (such as resolution of ptosis) (19). Oral acetylcholinesterase inhibitors should be withheld for at least 24 hours before testing, and atropine and resuscitation facilities should be available.

Neurophysiological tests

Routine nerve conduction tests and EMG are not usually informative, although the compound muscle action potential (CMAP) can be reduced in severe cases. However, repetitive nerve

stimulation (RNS) typically elicits a decremental response at 3-10 Hz, with a decrement >10% considered abnormal (20). The sensitivity of RNS for diagnosing MG ranges from 53% to 100%. It is however frequently negative in ocular MG (21).

Single fibre EMG (SFEMG) is more sensitive, and especially useful in ocular MG, although it is less specific Jitter, the trial to trial variation in the latency from stimulus to response, is increased, and there may also be intermittent failure of excitation of muscle fibres ('block').

Immunology

Not all patients with MG will have demonstrable antibodies but in those that do, these tests are highly specific. Approximately 85% of patients with generalised MG, and almost all patients with an associated thymoma, have detectable circulating autoantibodies to AChRs (22) A variable proportion of patients who do not have anti-AChR antibodies instead have antibodies to MuSK, and the remaining patients are described as 'seronegative', although a seroconversion rate of 15% has been reported. Immunosuppression itself can occasionally lead to disappearance of antibodies (23).

MuSK

MuSk (Muscle-Specific tyrosine Kinase) antibodies occur in up to 70% of patients who test negative for AChR antibodies, and are more prevalent in countries close to the equator (24). MuSK is essential for AChR clustering at the NMJ (25). MuSK positive patients are more likely to have early bulbar and respiratory symptoms with less severe limb involvement and women are more commonly affected (26). The clinical neurophysiology in MuSK usually shows abnormal SFEMG of facial muscles but RNS of limb muscles can be normal. Thymic pathology in MuSK patients does not show lymphocytic infiltrates or complement deposition In addition muscle biopsy does not show reduced AChR density as it does in AChR antibody positive patients (27). The pathogenicity of anti-MuSK antibodies, although confirmed by passive transfer experiments is incompletely understood. However, it has been shown that interfering with MuSk synthesis can cause declustering of AChRs (28).

Seronegative MG

A small proportion of patients remain consistently negative for both AchR and MuSK antibodies. Clinical features and thymic pathology imply that seronegative MG (SNMG) is similar to MG caused by AChR antibodies. This has led to the hypothesis that the patients have antibodies to AChR which are undetectable with conventional assays. Indeed, many such patients can be shown to have antibodies that bind AChRs expressed in a cell line together with rapsyn, which contributes to clustering AChRs (29). It remains to be determined whether other antigens are recognized by circulating antibodies in the remaining 5% of so of patients with MG in whom this improved assay is negative.

Treatment of MG

Treatment of MG is symptomatic (AChE inhibition) or immunomodulatory, although there is a paucity of controlled randomised controlled trials to provide definitive guidance.

Acetylcholinesterase (AChE) inhibitors are usually first line therapy, providing symptomatic relief in ocular and generalised myasthenia. There are no placebo-controlled randomized studies but clinical experience and case reports support their effectiveness (30). Patients with anti MuSK antibodies may not respond as well as those with anti-AChR MG. Side-effects include hyperhidrosis, salivation and lacrimation. Cholinergic crisis can occur with high doses.

AChE inhibitors have a short half life and thus require regular dosing. A controlled-release form of pyridostigmine is available in some countries, but not in the UK. Most patients will require immunomodulatory treatment but given the adverse effects associated with these drugs, it may be reasonable to treat with an AChE alone initially.

Immunosuppression

Corticosteroids are generally the first line immunosuppressive treatment. A Cochrane review found only limited evidence from randomised controlled trials; nonetheless several observational trials support their efficacy (31). Early use of steroids in ocular MG may prevent generalisation (32,33). High initial doses of prednisolone (prednisone) may transiently exacerbate weakness and even trigger a crisis, and it may be prudent to admit patients to hospital for escalation of treatment. Bone protection strategies and alternate day dosing are often used to counteract steroid-related side-effects.

Azathioprine is the most widely used second-line immunosuppressant. Although a Cochrane review did not demonstrate any significant improvement in quantitative MG (QMG) score at six month, its use does allow a lower steroid dose (34-37). Simultaneously commencing azathioprine and prednisolone may allow more rapid steroid tapering. Adverse effects include hepatotoxicity, cytopenia, and lymhoproliferative disease. One case of progressive multifocal leukoencephalopathy (PML) has been reported (38). Thiopurine methyltransferase (TPMT) activity should be measured before commencement as patients with reduced activity of this enzyme will become toxic at normal doses. Regular monitoring of full blood count (FBC) and liver and renal function is warranted.

Cyclosporine has been shown to be effective in improving muscle strength and reducing steroid dose and indeed has the strongest evidence base of the immunosupressants used in MG (39,40). Despite this, concerns about side-effects, mainly nephrotoxicity, have limited its widespread use. *Tacrolimus* has also been shown to be efficacious in initial treatment of MG (41). Use of *cyclophosphamide* improves muscle weakness but because of severe side-effects it is generally reserved for patients who are intolerant of other immunosuppressants (42). Despite a lack of randomised controlled trials, *methotrexate* is increasingly used in the management of MG. Its use has been recommended as a second line agent by the European Federation of Neurological Societies (43), although it is relatively contra-indicated in women of child-bearing age.

Mycophenolate mofetil has been used in transplant medicine and in other autoimmune diseases without producing major organ toxicity (44). It has been adopted widely in MG (45-47). However, two recent randomized studies have failed to demonstrate additional benefit of mycophenolate with prednisolone compared to prednisolone alone (48,49). The negative outcome of these studies has not deterred many clinicians from using it in MG, mainly because of its favourable side-effect profile, and further evidence is needed to determine its role.

Intravenous Immunoglobulin (IVIG) and *plasma exchange* are both effective in acute exacerbations of MG, although their long term effectiveness has not been demonstrated in clinical trials (50-52). Their efficacy is similar although IVIG is better tolerated. Their main role is in bringing about a rapid improvement in patients with worsening myasthenic symptoms (53). A small minority of patients remain refractory to oral medication require frequent courses of IVIG or plasma exchange.

Rituximab is a monoclonal antibody to the B cell marker CD20. Its use in MG has been described in case reports (54-57) It has been used both in AChR- and MuSK-antibody positive patients, and in patients with thymoma, although there is no randomised evidence. Side-effects include neutropenia and increased susceptibility to infections, Other B cell directed approaches include a humanized anti CD20 antibody (*ofatumumab*) and *atacicept* which sequesters B cell survival factors (58). Use of *etanercept*, soluble recombinant TNF receptor FC protein, in MG has been disappointing (59). A novel complement inhibitor REV576 was recently used in an experimental rodent model of MG rats with promising results, and a trial of *eculuzimab*, which has a similar mechanism of action, is under way (60) .

Surgery

Surgery with or without *radiotherapy* is indicated in the treatment of thymoma. It is sometimes necessary to stabilise myasthenic symptoms preoperatively, and IVIG and plasma exchange have been used in this situation.

The value of *thymectomy* in patients with non-thymomatous MG is less clear. Retrospective follow up studies suggest benefit but clinical effects may not be seen for 6-12 months (61-63).

Despite its use since the 1930s no randomized trials have been published; one is currently underway (64).

The outcomes of different surgical approaches appear equivalent but the routine use of minimally invasive procedures is controversial since thymoma or thymic carcinoma may only be identified intraoperatively (65). Consensus is lacking regarding the age limits for thymectomy, its use in ocular myasthenia or the stage of disease that thymectomy should be performed (58). In general thymectomy is offered to young AChR antibody-positive patients with generalised MG. There is no evidence that patients with MuSK antibodies benefit from thymectomy

Ocular MG

Extraocular muscles are affected in at least 85% of patients with MG but it is unclear why they are so vulnerable. They have a high blood flow, mitochondrial content and metabolic rate. They also have small motor units that fire at high firing frequencies, and possibly a smaller safety factor, making them prone to fatigue. There may also be differences in intrinsic regulators of the complement cascade, making them more susceptible to complement-mediated injury(66)

AChE inhibitors are first line therapy in ocular myasthenia and in very mild disease may be all that is required. However, ocular symptoms can be disabling and 60-80% of patients will require additional therapy. Prednisolone therapy has been shown to reduce ocular symptoms of ptosis and diplopia and observational studies suggest benefit (67-70). Steroids have been reported in retrospective studies to delay the onset of generalised MG but there is no definitive evidence regarding this.

Myasthenic Crisis

Myasthenic crisis is a neurological emergency requiring intensive care unit admission for ventilatory support, and removal of circulating autoantibodies with IVIG or plasma exchange

can be highly effective (71). Cholinesterase inhibitors have a limited role in the treatment of myasthenic crisis, and indeed, a cholinergic crisis an occasionally mimic it. Case-control studies have suggested the long term beneficial effect of immunosuppressive therapy in preventing myasthenic crisis (72).

Lambert-Eaton Myasthenic Syndrome

Lambert-Eaton Myasthenic Syndrome (LEMS) is much less common than MG (73). It usually begins in mid to late life and affects males and females equally ((74). It is associated with a malignancy, most often small cell carcinoma of the lung (SCLC), in approximately 50% of cases. Conversely, 1-3% of SCLC patients have LEMS. In the vast majority of cases, the tumour is detected within a year of diagnosis of LEMS (75). In patients who do not have an associated tumour, there a female preponderance and a strong association with HLA-B8. Around 25% of non tumour LEMS patients have a past history of organ specific autoimmune disease (ref*).

Most patients develop proximal limb weakness, especially of the legs. Areflexia, dry mouth and signs of autonomic failure also occur (74). Some patients, most often those with small cell carcinoma of the lung, may have associated paraneoplastic cerebellar degeneration.

Antibodies in LEMS are directed against presynaptic calcium channels, most likely of the P/Q-type (CaV2.1), and interfere with the release of ACh. They are detectable in 85% of patients and are very specific (2). In a recent study antibodies to SOX1, a DNA binding transcription factor were found in paraneoplastic LEMS patients but not in those who did not have a tumour (76).

In contrast to MG, the CMAP amplitude increases in LEMS in response to high frequency stimulation (77). This is thought to be due to presynaptic accumulation of calcium, which slowly recruits additional vesicles for exocytosis. The same phenomenon can be demonstrated clinically by the return of previously absent deep tendon reflexes after muscle contraction.

The diagnosis of LEMS should spark an intensive search for malignancy, including CT thorax, bronchoscopy and PET. Patients with a history of smoking, cerebellar dysfunction and older patients with rapid progression of symptoms are more likely to have an underlying malignancy(78). The prognosis in patients with both LEMS and SCLC is poor, with a 2 year survival of less than 10%, albeit better than in patients without LEMS, suggesting a possible anti-tumour effect of the antibodies.

Treatment of muscle weakness in LEMS is symptomatic or immune-based. Aminopyridines such as *3,4-diaminopyridine* block potassium channels, and prolong the duration of the nerve action potential increasing neurotransmitter release at the synapse. Their efficacy has been summarized in a Cochrane review of two randomized trial with up to 79% of patients demonstrating significant symptomatic improvement (79-81). AChE inhibitors provide a moderate response at best and are generally not used as monotherapy. Guanidine is an alternative treatment that increases free intracellular calcium and ACh release. It has not been subjected to randomized controlled trials. Side-effects include gastrointestinal disturbance, bone marrow suppression and renal failure. It is generally less well tolerated than the aminopyridines.

IVIG has been shown to be useful in LEMS, leading to clinical improvement and a decrease in antibody titre. It is generally used as an adjuvant therapy in treatment-resistant patients (82) Oral prednisolone has been used alone and in combination with azathioprine, and case reports have suggested benefit(79). More recently a single case report has highlighted a beneficial response to rituximab in a non-paraneoplastic LEMS patients who had failed conservative therapy (83)

Botulism

Botulism is a rare disease caused by exposure to the neurotoxins produced by the anaerobe clostridium botulinum that is found in soil and aquatic sediment. The three toxin subtypes A, B

and E all act by cleaving SNARE proteins, although they recognize different sequences. Their action prevents the close apposition of vesicles to the presynaptic membrane, leading to a failure of ACh release (84,85) (Fig. 1). There are four types of botulism, classified according to the mode of exposure.

Food-borne botulism is caused by consumption of toxin formed under anaerobic conditions, such as in inadequately sterilized home-made preserves (86). *Wound* botulism is particularly seen in drug users who use 'skin popping' to self-administer heroin. The organism does not normally survive ingestion but can do so in neonates, where it causes *infant botulism* (84). Rarely intestinal colonization occurs in adults with functional bowel abnormality. Finally, *iatrogenic* botulism has been reported in patients treated with toxin for movement disorders or cosmetic purposes (87,88)

Botulism typically presents with a descending paralysis, causing symmetrical ptosis, opthalmoplegia, dysarthia and dysphagia. This progresses to proximal limb weakness and respiratory compromise. Autonomic involvement, including anhidrosis and postural hypotension also occur, and gastrointestinal symptoms may be the first sign of food-borne botulism. Reflexes are typically lost but sensation is normal.

Neurophysiological tests reveal changes similar to those in LEMS. An enzyme-linked immunosorbent assay, detecting toxin in serum, stool or in a food sample, has now displaced a mouse bioassay.

Supportive intensive care management has decreased mortality from 79% to 3-5%. It is essential to administer antitoxin early because the toxin is internalized and continues to cleave SNARE proteins after it has been cleared from the circulation. Public health authorities must be informed if a botulism outbreak is suspected.

Congenital Myasthenic Syndromes

A heterogenous group of genetic disorders can affect the NMJ. These are typically inherited in an autosomal recessive fashion. Early studies on patients with inherited myasthenic disorders used electrophysiology, electron microscopy, histochemistry and biochemical techniques to show that variable synaptic proteins were likely to be affected in different families. The congenital myasthenic syndromes (CMSs) were first classified on the basis of the site of the defective neuromuscular transmission – presynaptic, synaptic and postsynaptic. Within these headings different genes may be defective and the clinical picture varies. Currently, definitive diagnosis depends upon electrophysiological tests, morphological studies of the end-plate region in muscle biopsy specimens, and increasingly on identification of the specific genetic defect. To date 13 genes have been identified (Table 1). However, mutations have yet to be identified in approximately 30% of patients referred to the Oxford CMS diagnostic service whose clinical features strongly suggest a genetic myasthenic syndrome. As new CMS-associated genes are identified a revised classification is evolving, based on the gene defects and the underlying molecular mechanisms of the disorder.

AChR subunits

Initial studies identified mutations in the genes encoding the AChR subunits that impair ion channel gating, reduce the number of endplate receptors, or a combination of the two, leading to “slow channel”, “fast channel” or AChR deficiency syndromes (89). Slow channel syndrome is the only dominantly inherited CMS.

Presynaptic and synaptic CMS

Mutations were subsequently identified in the acetylcholinesterase collagen-like tail subunit gene (COLQ) and also in the enzyme choline acetyltransferase (ChAT) (90-92).

AChR clustering disorders

More recently, mutations have been found in the genes encoding key molecules involved in clustering of the AChR at the endplate and in maintaining synaptic structure (Fig 1). Agrin released from the nerve terminal activates MuSK on the postsynaptic membrane through low density lipoprotein receptor-related protein 4 (LRP4). This, in turn, leads to aggregation of AChRs through an interaction with rapsyn (93,94). The activation of MuSK is enhanced by the cytoplasmic protein Dok-7 (95). With the exception of LRP4, mutations in each of these components have been reported in CMS, emphasising their critical roles in this pathway (Table 1). To date there have been only isolated case reports of AGRIN or MUSK mutations (96). However, RAPSN and DOK 7 mutations are well recognized as major causes of CMS (97-103).

Dok-7 (gene DOK7) binds to MuSK and plays a role in amplifying the MuSK signaling to downstream pathways that control clustering, partially through phosphorylation of the AChR β subunit, and maintenance of the NMJ. The syndrome associated with DOK7 mutations is characterized by a limb girdle pattern of weakness. Children typically have normal initial motor milestones followed by an abnormal waddling or lordotic gait and frequent falls soon after learning to walk. Ptosis, stridor and respiratory problems may occur but eye movements are usually spared. Bulbar problems often develop later. Fluctuation in symptoms is common and patients may have been previously diagnosed with an unspecified congenital myopathy (102). There is a remarkable variation in disease severity with symptom onset ranging from birth to adolescence or even in adulthood.

Rapsyn is an AChR-clustering protein and mutations in the RAPSN gene cause a deficiency of AChR at the motor endplate. Both early and late onset cases have been reported. (98) Early onset cases are associated with hypotonia and bulbar dysfunction, which may require assisted feeding and ventilation. Mild facial malformations and contractures of hands and ankles are common. Patients may suffer severe exacerbations with life threatening respiratory failure; however, the condition does tend to improve over time with minimal

disability in adulthood. Interestingly, weakness of ankle dorsiflexion tends to remain into adult life and may provide a diagnostic clue. The overwhelming majority of patients harbour the missense mutation N88K, suggesting an original founder mutation (104). Other mutations have been identified in the promoter and coding regions of the RAPSN gene. In addition, a missense mutation in the AChR δ subunit gene δE381K has been identified that does not affect the function of the receptor but rather impairs rapsyn-induced AChR clustering. These patients bear, phenotypically, the hallmarks of rapsyn deficiency rather than AChR subunit deficiency and this mutation may prove useful for studying the AChR-rapysn interaction.

The facial malformations and joint contractures seen in rapsyn mutations are thought to result from lack of movement in utero. Neuromuscular transmission is generally mediated by the adult AChR, which has the stoichiometry $(\alpha_1)2\beta_1\delta\varepsilon$. There is however a fetal form of the AChR, $(\alpha_1)2\beta_1\gamma\delta$, which is crucial in certain periods of fetal development. Mutations in the fetal γ subunit can lead to severe developmental abnormalities or death. Surprisingly, some patients with γ subunit null alleles survive, suggesting that early expression of the adult ε subunit may compensate. It is likely that loss of function mutations in other subunits of the fetal AChR or other components of the developing NMJ are likely to be fatal. Indeed null mutations have now been found in *CHRNA1*, *CHRND*, *RAPSN* and *DOK7* (105,106).

Mutations are not identified in approximately 30% of patients with a clinical diagnosis of CMS. A form of limb girdle CMS that does not have a demonstrable DOK7 mutation and differs from this condition in its response to anticholinesterase and in the presence of tubular aggregates on muscle biopsy exists. Studies are currently underway to define the affected gene for this characteristic phenotype.

A mutation was recently described in the LAMB2 gene that codes the laminin β2 subunit. Mutations in Lamb2 have previously been shown to be involved in Pierson syndrome which causes ocular abnormalities and nephrosis. Mice lacking laminin β2 do not develop normal neuromuscular synapses (107).

Treatment

With increased understanding of the molecular mechanisms that underlie individual CMSs, therapy can be tailored to the individual. Most CMSs such as AChR deficiency, fast channel syndrome and rapsyn deficiency result from "loss of function" mutations that reduce signal transmission. These patients tend to respond to therapies that enhance neurotransmitter release such as AChE inhibitors and 3,4 diaminopyridine (3,4-DAP), although theoretically ChAT CMS could worsen in response to 3,4 DAP due to depletion of the presynaptic ACh.

By contrast in patients with slow channel syndrome or COLQ mutations, there is overstimulation of endplate receptors, calling for quite different therapy. The prolonged opening time of the AChR seen in slow channel syndrome can be curtailed by drugs that block the channel in its open state, so called "open channel blockers". Fluoxetine and quinidine sulphate have both been used for this purpse (108,109). In patients with COLQ mutations, prolonged exposure to ACh casues receptor desensitization and an endplate myopathy. This has been found to respond to ephedrine(110,111).

Dok-7 mutations may respond well to appropriate treatment. Cholinesterase inhibitors often elicit a brief initial response but subsequently cause worsening of symptoms. Response to 3,4-diaminopyridine is mixed, with some patients reporting benefits and others worsening.(80) However, patients usually respond well to ephedrine (101), although the basis for this is unknown. Patients may also respond to salbutamol (112) suggesting that

stimulation of β2-adrenergic receptors may be able to partially stabilize the end plate region.

Summary

Increased understanding of the fundamental mechanisms of synaptic transmission and of the development and maintenance of the end-plate has greatly enhanced the ability to diagnose patients with acquired or inherited diseases of the neuromuscular junction. Nevertheless, there remains a lack of robust evidence to guide the treatment of even the most common of the neuromuscular junction diseases, myasthenia gravis. Further research in the form of randomized controlled trials of currently used treatments is indicated. As we understand more about the pathogenic mechanisms that interrupt individual proteins at the neuromuscular junction, we will be able to develop more specific treatments for both the acquired and congenital disorders of this highly specialized synapse.

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Competing Interest: None declared.

Presynaptic CMS	***Gene***
CMS with episodic apnea	*CHAT*
Synaptic CMS	
Congenital endplate acetylcholinesterase deficiency	*COLQ*
CMS due to mutations in Agrin	*AGRN*
CMS due to mutations in the laminin β2 chain	*LAMB2*
Postsynaptic CMS	
AChR deficiency syndromes	*CHRNA, CHRNB, CHRND, CHRNE*
AChR deficiency syndromes due to mutations in rapsyn	*RAPSN*
Slow-channel CMS	*CHRNA, CHRNB, CHRND, CHRNE*
Fast-channel CMS	*CHRNA, CHRND, CHRNE*
CMS due to voltage-gated sodium channel mutations	*SCN4A*
CMS due to mutations in MuSK	*MUSK*
CMS due to mutations in Dok-7	*DOK7*
Multiple pterygium/Escobar syndromes due to AChR γ-subunit mutations	*CHRNG*
Fatal fetal akinesia deformation sequence due to NMJ protein mutations	*CHRNA, CHRND, RAPSN, DOK7*

Table 1. Classification of congenital myasthenic syndromes (CMS). The genetic causes of some cases of presynaptic and postsynaptic CMS remain to be defined.

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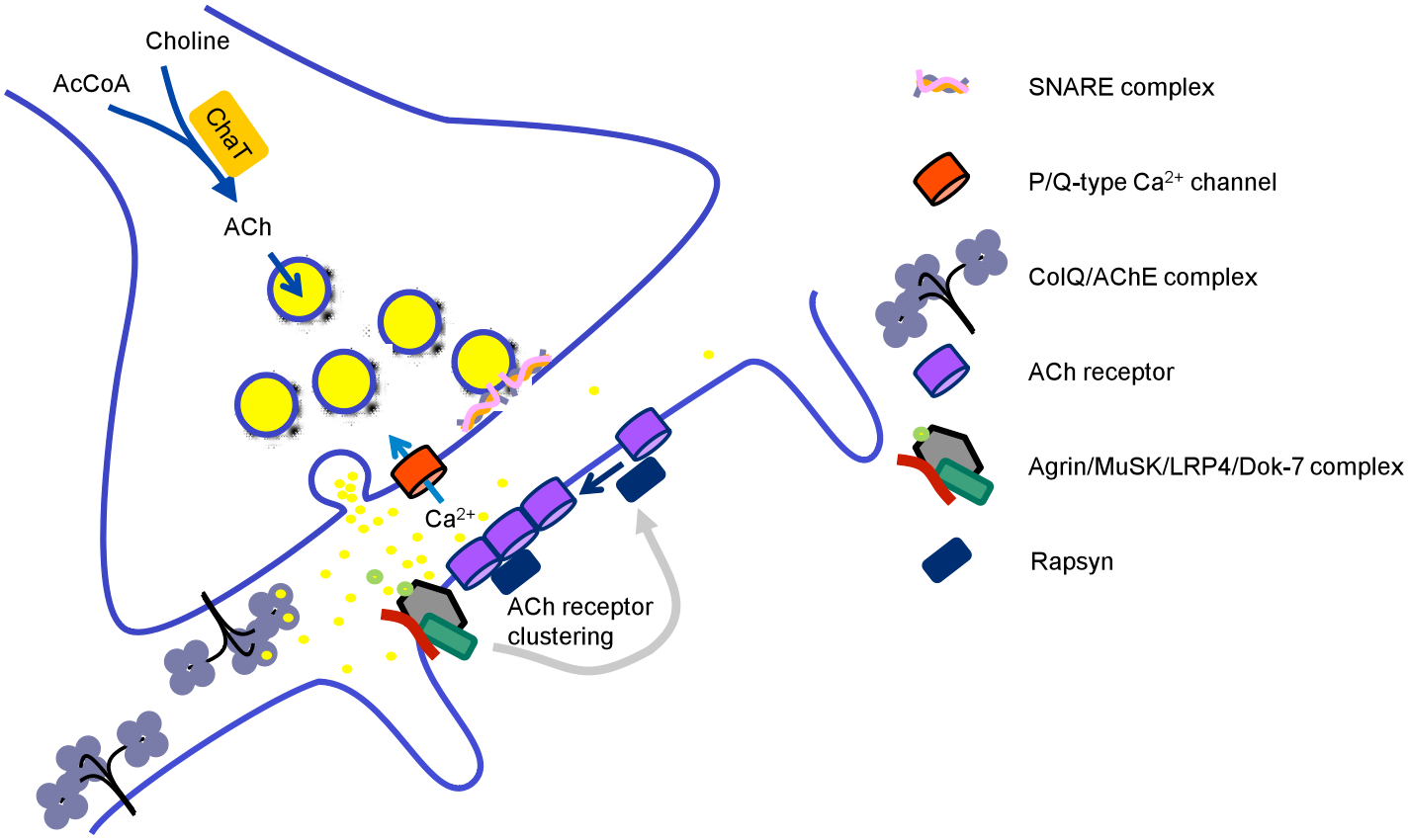
Figure legend

Figure 1.

Key steps in ACh synthesis and release at the neuromuscular junction and the core pathway responsible for ACh receptor clustering. AcCoA, Acetyl Coenzyme A; AChE, Acetylcholinesterase; ChAT, Choline Acetyltransferase; Dok-7, Downstream of tyrosine kinase 7; ColQ, AChE collagen-like tail subunit; LRP4, low density lipoprotein receptor-related protein 4; MuSK, Muscle-Specific Kinase; SNARE, Soluble NSF Attachment protein Receptor.


Choline
AcCoA
ChaT
ACh
Ca2+
ACh receptor
clustering
SNARE complex
P/Q-type Ca2+ channel
ColQ/AChE complex
ACh receptor
Agrin/MuSK/LRP4/Dok-7 complex
Rapsyn