Clinical Biochemistry 133–134 (2024) 110826

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

The changing landscape of autoantibody testing in myasthenia gravis in the setting of novel drug treatments

Ali Mousavi [a,b], Pankaj Kumar [a,b], Hans Frykman [a,b,c,*]

[a] Division of Neurology, Department of Medicine, University of British Columbia, Vancouver, British Columbia, Canada
[b] BC Neuroimmunology Lab. Inc., Vancouver, British Columbia, Canada
[c] Neurocode Lab. Inc. Bellingham, Washington, USA

ARTICLE INFO

Keywords:
Myasthenia Gravis
Acetylcholine receptor
Muscle-specific kinase (MuSK)
Lipoprotein-receptor-related protein 4 antibodies
RIPA
Fixed Cell-Based assay
Live Cell-Based assay

ABSTRACT

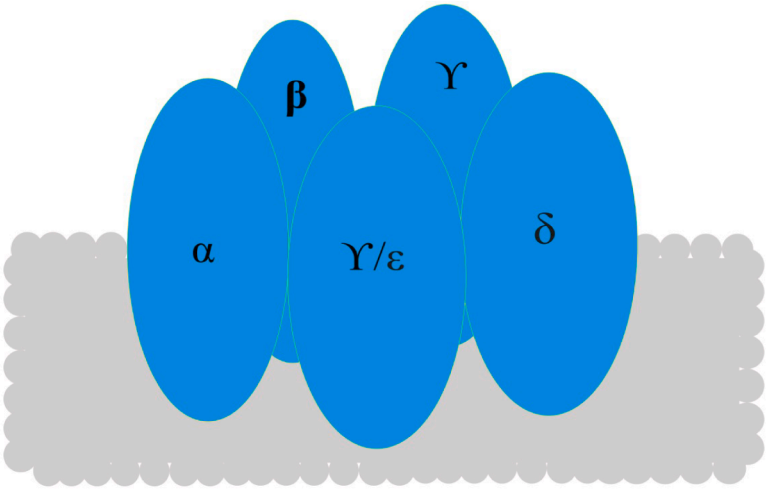
Acquired myasthenia gravis (MG) is an autoimmune disease targeting the specific proteins in the postsynaptic muscle membrane. 50% of ocular and 80% of generalized MG have acetylcholine receptor antibodies (AChR Abs). 1–10% of MG patients have antibodies against muscle-specific kinase (MuSK), and 2–50 % of seronegative MG cases have antibodies against lipoprotein-receptor-related protein4 antibodies (LRP4 Abs). Serological testing is crucial for diagnosing and determining the appropriate therapeutic approach for MG patients. The radioimmunoprecipitation assay (RIPA) method is a historical standard test for detecting the AChR Abs and MuSK Abs. While it has nearly 100% specificity in the AChR Abs detection, its sensitivity is between 50—92%. The sensitivity and specificity of RIPA for detecting MuSK Abs is much lower. The fixed and live Cell-Based assays (f-CBA and L- CBA) have higher sensitivity than RIPA. With advancements in the serological diagnosis and management of MG, we now recommend a complete reflex testing algorithm on the first pretreatment sample of a suspected MG patient, starting with the binding and blocking assays for AChR Abs by RIPA and/ or f-CBA. If AChR Ab is negative, then reflex to MuSK Abs by RIPA and/ or CBAs. If AChR and MuSK Abs are negative, then use clustered L-CBA by request.

1. Introduction

Acquired myasthenia gravis (MG) is a chronic autoimmune disease of the neuromuscular junction of skeletal muscles characterized clinically by fatigable weakness [1]. Despite MG being a rare disease and its mortality has reduced during the last two decades, it still has a remarkable burden including increased hospitalization and social and economic costs. The incidence of MG varies with ethnicity, gender, and age, and its worldwide annual rate is 0.41 to 3 per 100,000. The prevalence of MG has been increasing due to improvements in its management, so it has more than doubled in the last twenty years [2–5]. While different types of MG are recognized according to the clinical phenotype, the age at onset, the accompanying thymic abnormalities, and autoantibodies, it is clinically classified as an ocular MG (OMG), bulbar, and generalized (gMG). The OMG is defined as impairment of the movements of extraocular muscles and rapidly progressive asymmetrical bilateral ptosis and/or diplopia that usually intensifies in the evening and improves with rest and naps. In approximately 15 % of MG patients, the symptoms remain ocular, but in the majority, the disease progresses to limb and bulbar and respiratory muscles within the first two years, resulting in generalized gMG [1,6,7]. Another clinical presentation of MG is the bulbar phenotype with weakness and fatiguability in the jaw and oropharyngeal muscles that can also occur early in MG. This bulbar weakness presents with dysarthria, dysphagia, difficulty in handling secretions, and nasal regurgitation. This form of MG is more frequently seen in young female and elderly male patients with positive muscle-specific kinase antibodies (MuSK Ab). gMG is another phenotype of MG that usually starts with ocular symptoms and over a few weeks to months affects the proximal limb muscle [1,8]. However, in 20 to 30 % of cases of gMG, the initial presentation is the involvement of extremities and the trunk, and it leads to difficulties with daily living activities [1,8]. Moreover, any skeletal muscle group particularly palatal, oropharyngeal, upper esophageal muscles, and neck flexors may be affected in gMG [1,8]. Involvement of respiratory muscles, including intercostal muscles and the diaphragm with concomitant bulbar muscle involvement may cause the myasthenic crisis [1,8,9]. Other types of MG with distinct pathophysiology are neonatal MG, due to the transferring of antibodies from an affected mother, pediatric MG, a rare autoimmune

* Corresponding author.
E-mail address: hans.frykman@ubc.ca (H. Frykman).

https://doi.org/10.1016/j.clinbiochem.2024.110826
Received 22 January 2024; Received in revised form 16 September 2024; Accepted 17 September 2024
Available online 30 September 2024



B. Normal NMJ

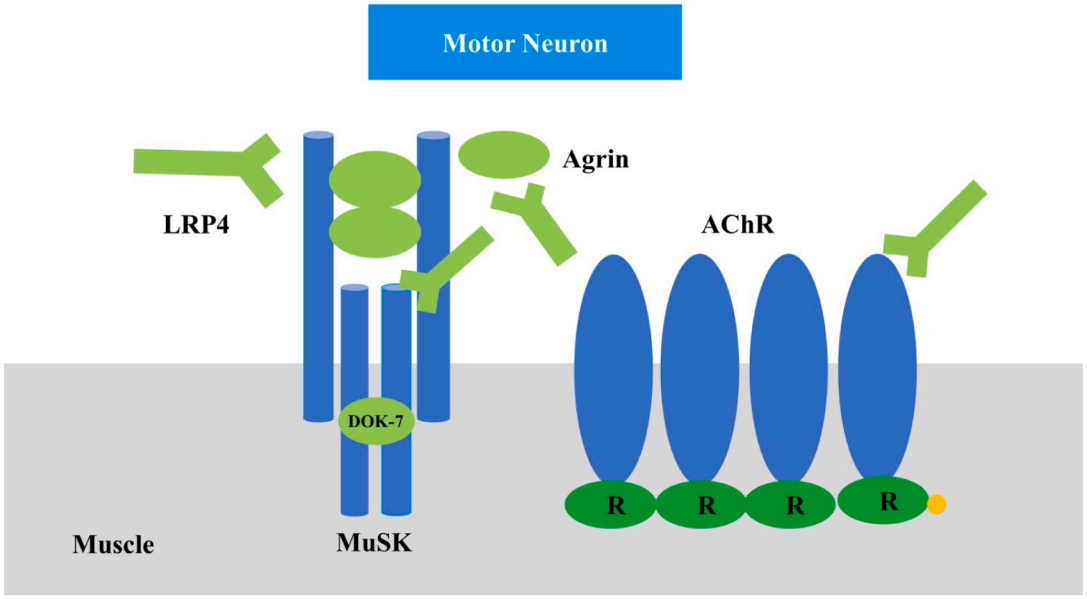


Fig. 1. The fetal AChR glycoprotein is made up of 2α, 1β, 1δ, and1γ subunits. In adult AChR, the γ-subunit is replaced by the ε-subunit. Each AChR subunit has an extracellular domain, four transmembrane domains, and an intracellular domain.

disease and difficult to diagnose and congenital MG. Congenital MG is a very uncommon and heterogeneous form of MG and is typically inherited as an autosomal recessive disease and may even occur in adulthood. [11] It should be noted that since congenital MG is not an autoimmune disease and it may even present in adulthood, it is the most important differential diagnosis of seronegative MG [1,10]. MG was originally recognized as a disease in young women, but its incidence has increased in both elderly females and males in the last decades [11–13]. Therefore, according to the age at onset, MG cases are classified into early-onset and late-onset when the onset is under or above the age of 50 [5,14]. Early-onset cases are more frequent among young females with higher levels of acetylcholine receptor (AChR) Abs and thymic hyperplasia. In comparison, late-onset MG is more common in elderly males with the presence of thymoma, AChR Abs, and more often ocular and bulbar phenotypes [15,16]. Acquired MG is a prototype of autoimmune disorders and is caused by antibodies against the AChR, MuSK, lipoprotein-receptor-related protein 4 antibodies (LRP4 Abs), or other AChR-related proteins in the postsynaptic muscle membrane [17–19] MG can be classified based on its autoantibody status which is useful in defining the clinical subsets as well as guiding its therapeutic strategies [7,17,18,20]. In this regard, MG with AChR antibodies (Abs) is the most common type of MG, followed by MG associated with MuSK and anti-LRP4 Abs. AChR Abs are detected in approximately 50 % of ocular and 80 % of generalized MG. Moreover, 1–10 % of MG cases have antibodies against MuSK and 2–50 % of seronegative MG cases have antibodies against LRP4 [21–24]. However, the remaining cases that do not have both anti-AChR and anti-MuSK Abs are double seronegative (dSN) MG [1,17,25–28]. Moreover, with the use of newer techniques such as the fixed and live cell-based assay (f-CBA and L-CBA) to detect antibodies against novel muscle antigens or low-affinity anti-AChR Abs (See Immunopathogenesis of MG, below), the true frequency of "seronegative MG" has gone down [1,16,29].

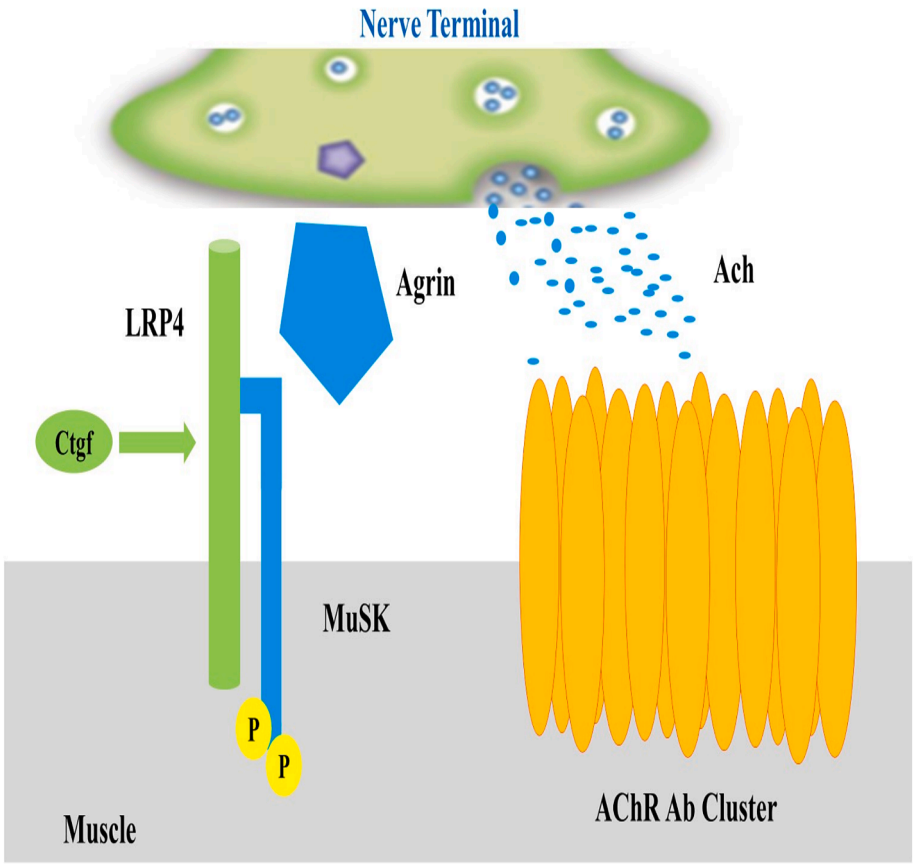


Fig. 2. AChR Ab Clustering. Schematic of the NMJ and molecules involved in AChR clustering by MuSK phosphorylation (p). Agrin binds to LRP4 to phosphorylate MuSK. Connective tissue growth factor (Ctgf) also binds to Lrp4 to enhance MuSK phosphorylation.

2. Immunopathology of autoimmune MG

The synaptic connection between the motor nerve terminal and skeletal muscle membrane (neuromuscular junction) is responsible for conducting action potentials from nerve to muscle fibers. The autoantibodies targeted proteins in MG are located throughout the post-connecting area of the neuromuscular junction (NMJ) and can be classified into transmembrane or extracellular antigens, and cytoplasmic or intracellular antigens (Fig. 1A) [1,29–31]. The nicotinic muscle AChR with its transmembrane pentameric structure is the most common target for Abs attack in MG patients. While the fetal AChR glycoprotein is made up of 2α, 1β, 1δ, and1γ subunits, in the developed adult AChR, the γ-subunit is replaced by the ε-subunit [1,29,30,32]. Each AChR subunit has an extracellular domain, four transmembrane domains, and an intracellular domain (Fig. 1A, B) [1,29,31,32]. The autoantibodies can target extracellular domains of all subunits of AChR including the γ subunit, but the α-subunit is its main immunogenic region (MIR), and Abs targeting it are more pathogenic [1,29,32]. AChR Abs primarily belong to IgG1 and IgG3 subclasses and can be detected in up to 80 % of gMG patients and 50 % of OMG [32,33]. The anti-AChR Abs possibly can affect neuromuscular function by the complement cascade activation, cross-linking of AChR, and directly blocking the acetylcholine binding to the AChR site (Fig. 1B) [31,34,35]. The deposition of C3, C9, and the membrane attack complex (MAC) at the muscle endplate and its consequences including postsynaptic folds destruction and impairment of NMJ, have been observed in MG patients [36,37]. The MAC is also responsible for other pathological changes such as simplification of muscle endplates, widening of the synaptic cleft, and perhaps the permanent AChR loss [36,37]. Antigenic modulation (the ability of an Ab to cross-link two antigen molecules) is another possible mechanism of AChR damage and impairment of neuromuscular transmission. F(ab)2 fragments of anti-AChR antibodies can mediate this process and lead to endocytosis and degradation of junctional and extra-junctional receptors [42]. Anti- AChR blocking Abs were found in a variable number of MG sera, but their importance in the pathophysiology of MG is unclear [39]. Even though this is an uncommon pathogenic mechanism in MG, it may be clinically important due to enhancing complement-mediated damage and/or accelerating receptor degradation. Indeed, the AChR blocking process can cause acute clinical deterioration in MG patients, and it explains the rapid clinical improvement after plasmapheresis [39,40]. The second most common antigenic target for Ab attack in MG patients is the MuSK protein. It is an anchoring protein with three extracellular, transmembrane, and intracellular (with tyrosine kinase activity) domains [1,29, 38, 41]. The extracellular domain has three immunoglobulin-like regions (Ig1, Ig2, and Ig3) and a cysteine-rich frizzled-like region. MuSK protein is necessary for maintaining the NMJ structure and plays a crucial role in the AChR clustering process [1,29,30]. MuSK Ab primarily belongs to the IgG4 subclass, which is unable to activate the complement cascade, incapable of binding to Fc receptors, and ultimately unable to activate the feedback loop controlling IgG synthesis. It directly blocks the MuSK protein interaction with the LRP4-agrin complex and interferes with AChR clustering at NMJ (Fig. 2) [22,29,43]. Anti-MuSK Abs of the IgG1-3 subclasses, in a lower amount, exist in MG patients, and they might activate the complement cascade. They also block the LRP4-agrin signaling that leads to the inactivation of MuSK and inhibition of AChR clustering at the NMJ [44–46].

Another complexity of the immunopathology of MG is its thymic pathological features. Abnormality of the thymus gland exists in 80 to 90 % of MG patients. The majority of MG patients who are young and particularly AChR Ab positive have follicular hyperplasia with germinal center formation. They have a natural thymus architecture that is more

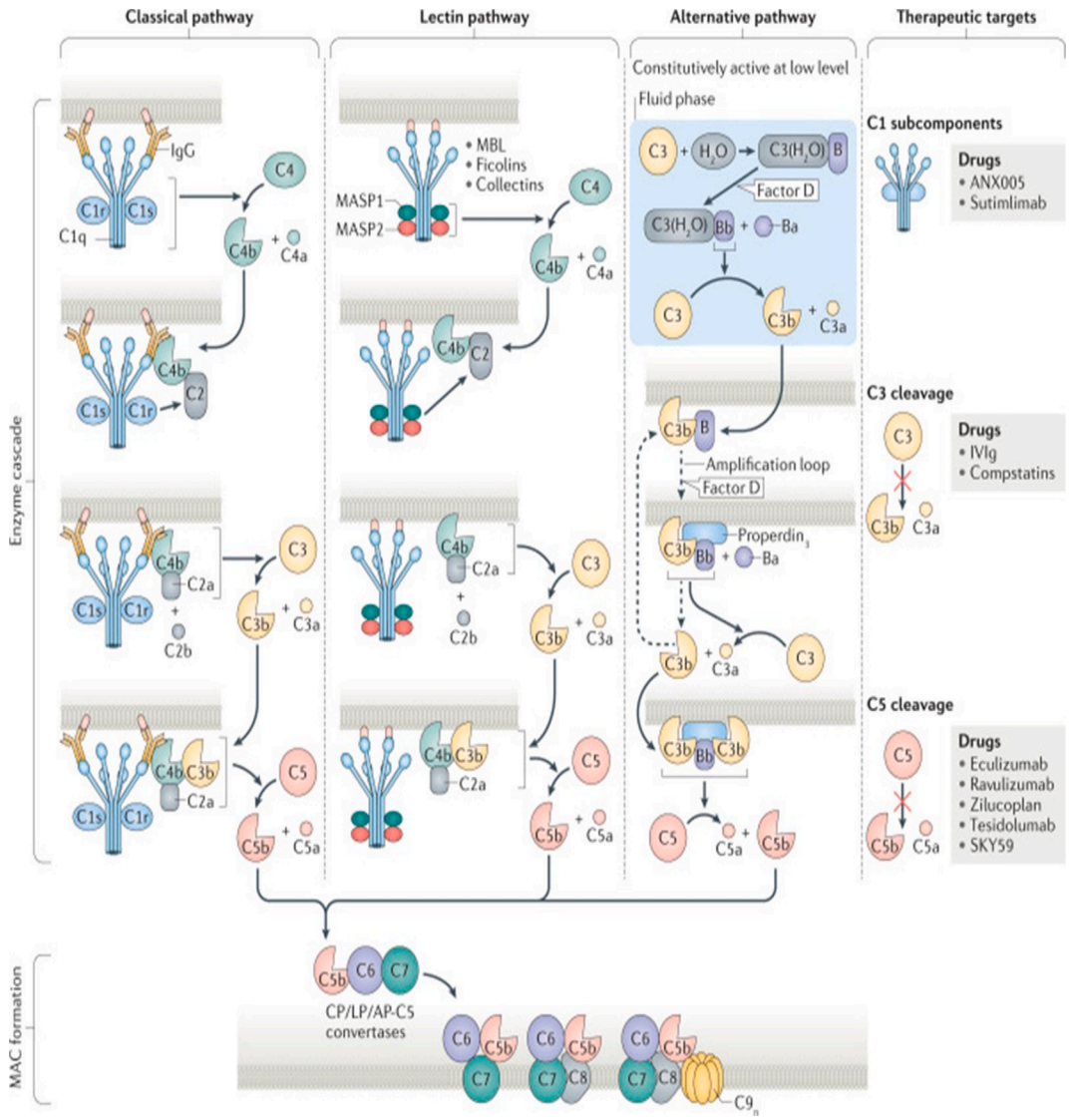


Fig. 3. The main proteins involved in the complement activation cascades (Adapted from *Nat Rev Neurol*). A schematic of complement cascades activation: Anti-AChR Abs essentially belongs to complement-fixing IgG 1 and IgG 3 subclass. The main immune mechanism in MG patients is the activation of the classical complement pathway via the binding of C1q to the Fc domain of the anti-AChR Ab. The critical steps in this cascade are the cleavage of C3 into C3a and C3b, and of C5 into C5a and C5b while the latter cause MAC (C5b-9) formation.

like a lymph node with maturing B and T cells with the ability of AChR Abs secretion [1,47]. However, thymomas are found in 10 % and up to 30 % of MG patients, and it is more frequent in elderly males [48–50]. The thymus of MG patients, particularly with follicular hyperplasia, contains all the essential elements for the build-out of the AChR Abs and thymus cells can express various subunits of AChR. Additionally, immune response-inducing factors such as chemokines with the attraction of immigrant CD4 + T and B cells, AChR-reactive B and CD4 + T cells, anti-AChR Ab secreting plasma cells, depression of CD4 + CD25 + regulatory T cell function have been recognized in the hyperplastic thymus [29,30,51]. Moreover, the expression of MuSK on the human thymic cell suggests that they may play a role in the development of MuSK MG [1,51,52]. As there is still ambiguity about whether or how the thymus acts in the production of anti-AChR Abs, understanding its mysterious role may lead to the determination of MG immunopathogenesis [29,51–53].

3. Complement activation profile in MG

MG is a classic form of a type II hypersensitivity reaction that means IgG class autoantibodies target synaptic proteins at the cell surface or extracellular region and cause NM damage. Interestingly, different subclasses of IgG are involved in the immunopathogenesis of MG. For example, AChR was targeted by IgG1 and IgG3 antibodies, LRP4 protein was targeted by IgG 1 and IgG 2, and MuSK protein was targeted by IgG4 [20]. Anti-AChR Abs essentially belongs to complement-fixing IgG 1 and IgG 3 subclass and their pathogenicity is by activation of the complement system and formation of membrane attack complex (MAC) [54]. Indeed, the main immune mechanism in MG patients is the activation of the classical complement pathway via the binding of C1q to the Fc domain of the anti-AChR Ab. The critical steps in this cascade are the cleavage of C3 into C3a and C3b, and of C5 into C5a and C5b while the latter cause MAC (C5b-9) formation. Fig. 3 [55,56]. Since the process of complement activation is a multiphasic cascade of events, multiple possible targets for immunotherapeutic intervention are available in MG patients.

4. Diagnosis of autoimmune MG

The diagnosis of acquired MG in highly clinical suspicion (the

presence of fluctuating weakness and fatigability that appears on exercise and relief with rest) is confirmed by bedside tests, pharmacologic testing, electromyography (EMG) studies, and serum Ab assay.

Rest test, sustained upgaze test, heat, and ice-pack tests are the bedside tests that assess muscle fatigability in MG patients [57,58]. The heat or ice-pack tests are helpful in the diagnosis of ocular MG in patients who present with ptosis. A latex party balloon filled with ice or hot water (up to 45 °C) and applied on the eyelid for 2 min. After taking pictures, the palpebral aperture before and immediately after each test will be measured by a ruler. The mean improvements in ptosis are 2.3 (±1.5) and 0.33 (±1.4) mm. The sensitivity and specificity of the ice-pack test for diagnosing MG are 28 % and 100 %, and they increase to 73 % and 97 % when combined with sustained upgaze [58,59]. The rationale behind the use of pharmacological agents as a diagnostic probe in MG, relates to the inhibition of acetylcholinesterase (AChE) which is responsible for the breakdown of the ACh, resulting in increased amounts of ACh to bind and activate AChR. In patients with MG, inhibition of AChE results in improvement in strength, which can then be demonstrated on the neurological examination or, electrophysiological testing [60,61].

The specific electromyographic tests for MG are repetitive nerve stimulation (RNS) and single fiber electromyography (SFEMG). In MG, muscle fibers cannot consistently respond to supramaximal ACh release that is recognized as the blocking. This phenomenon presents as reduced CMAP amplitudes during slow-rate RNS and is determined as a decremental response. A 10 % or more decrease between the first and fourth CMAP waveforms is abnormal [62,63].

The SFEMG uses a specifically constructed single-fiber needle or facial concentric needle electrode to study the action potentials of adjacent muscle fibers from the same motor unit. In MG, the jitter (the variation of the inter potential interval of consecutive discharges of two single muscle fibers related to a similar motor unit) increase [63–65]. SFEMG can also detect blocking, which reflects the intermittent loss of action potential within a muscle fiber of a motor unit [66,67]. The reported sensitivity and specificity of RNS and SFEMG alter widely among different studies. The sensitivity of RNS is between 14 and 94 %, and its specificity varies between 73 and 100 %, the sensitivity of SFEMG is between 64 and 100 %, and its specificity ranges from 22 to 100 % [58,63,68–70]. The main purpose of diagnosing autoimmune MG is to identify its subtypes, which certainly rely on autoantibodies detection. Therefore, serological testing is the pivotal step in the MG patients' assessment, and it plays a crucial role in its diagnostic confirmation and therapeutic direction [1,30,71]. With the introduction of new, highly effective, antigen-specific treatment options (see below), detecting the specific antibody responsible for pathogenesis in individual patients is important [29,30,72]. The AChR Abs are specific for the MG diagnosis if measured by radioimmunoprecipitation assay (RIPA) or Cell-Based assays (CBA) [1,3,30]. RIPA is the historical gold standard method of detection of AChR Abs, with nearly 100 % specificity. In RIPA, AChR usually is obtained from human muscles or AChR-expressing cell lines, such as the TE671 cell line (express fetal AChR) or CN21 cell line (express both fetal and adult AChR) [20,29,73]. RIPA technique is mainly based on the labeling of human AChR antigens with 125I-α-bungarotoxin(α-BT) and then precipitating the labeled complex of labeled AChR-with patient's AChR binding Abs using a secondary anti-human IgG antibody. The precipitated is counted and compared with the healthy control serum. When the test result is positive blocking with cold α-BT (unlabeled) is performed as control [1,20,29,71,74,75]. While the RIPA can detect binding Ab in up to 80 % of generalized MG, detection of modulating and blocking AChR-Ab using commercially available RIPA has limited diagnostic value, though their significance has been reported in a recent publication [71,76–78]. In addition, by using a mixture of the adult and fetal forms of the receptors the accuracy of RIPA improves. On the other hand, in the high-volume service laboratories, approximately 0.05 % false positivity of AChR Ab by RIPA has been reported that can be attributed to an immunoprecipitation artifact of the radioligand or the valid immunobiological seropositivity of other neurological diseases [79,80]. Hence, to eliminate the technical causes of invalid results, the positive RIPA with high radiation values (CPM; count per minute) should be repeated. Moreover, if patients have received immunomodulators such as intravenous immunoglobulin (IVIG) or plasma exchange (PLEX) within six weeks of their test or monoclonal antibodies (mAbs; Rituximab, Eculizumab) within 24 weeks of their test, confounded test results and false negatives are possible [1,29]. Therefore, any unexpected RIPA findings or negative results in the highly clinical suspicion should always be confirmed with independent confirmatory methods, for example, a highly- specific Live Cell-Based assay (L-CBA), to provide a definitive diagnosis. In addition, all clinically relevant antibodies do not bind well to 125I-α-bungarotoxin labeled AChR antigens in standard RIPA. In contrast, the AChR Abs with lower affinity, in standard RIPA, bind better to clustered AChR in its native form in the L-CBA [1,29,30,81–83]. Indeed, in L-CBA, HEK293 cells are transfected with fetal or adult AChR subunits at a density similar to its expression in the NMJ and co-transfected with rapsyn as well to promote AChR clustering on the cell surface, (clustered AChR). Ultimately, the binding of the patient's serum is detected with a fluorescently labeled secondary antibody on a fluorescent microscope. Commercial (Fixed) CBAs with the synthetic expression of different AChR subunits (adult and fetal type) and rapsyn, the same as L-CBA are also available. However, anti-AChR Abs can be detected by other techniques such as enzyme-linked immunosorbent assay (ELISA), fluorescence immunoprecipitation assay (FIPA), and dot-blot methods. But they have lower sensitivity and specificity than the RIPA or the CBAs, making it difficult to rely on them for clinical diagnosis [1,29,30].

The power of CBA in improving the detection of AChR Abs compared to RIPA has been reported in several studies [29,30]. In routine diagnostic settings, clustered AChR Abs are detected in around 20 % of SNMG patients [1,20,29,30,81]. In a recent study comparing the performance of a commercial CBA with RIA, the f-CBA showed a 4 % increase in sensitivity with 99.6 % specificity [84]. Recently, the performance of fixed and live CBA for AChR Ab detection were compared, showing L-CBA was more sensitive than the fixed CBA in detecting AChR Ab [85]. In another prospective multicenter double blind study, Li and colleagues enrolled 2043 clinically diagnosed MG cases and 229 non-MG and healthy controls from 9 centers across China to compare fixed CBA, RIPA, and ELISA performance in AChR Ab detection [86]. The sensitivity of fixed CBA was 72.3 % compared to 64.1 % (RIPA) and 62.7 % (ELISA), and specificity of fixed CBA and RIPA were similarly high at 97.8 % while ELISA had lower specificity (94.8 %) [86]. Although this study in continuity with two previous studies, [84,85] indicated high sensitivity of fixed CBA, it also confirmed that RIPA had reasonable sensitivity and high specificity for MG, as a traditional gold standard test, for AChR Ab detection [86]. In addition, 6 % of those who were negative for AChR Ab by fixed CBA were positive by RIPA, and by combining the two methods, sensitivity increased, suggesting the benefit of a testing algorithm in suspected MG. Moreover, in this study live CBA, which has higher accuracy than fixed CBA and RIPA was not compared with two other methodologies. Moreover, in MG, the affinity maturation of autoantibodies could potentially contribute to intensified tissue destruction, heightened clinical severity and paradoxically reduction in antibody titers [87–89]. This is particularly true in children and pediatric MG, patients who do not have detectable AChR Ab or muscle-specific tyrosine kinase antibodies by radioimmunoprecipitation (RIPA) [90]. As CBAs are a semiquantitative method, AChR Ab detection by quantitative flow cytometry can offer a viable alternative to current CBAs, but its clinical application needs further evaluation [91].

The MuSK Abs can be detected by RIPA, which is a highly specific assay [1,16,20,29,92]. False-positive results of MuSK Abs are uncommon among healthy individuals; therefore, the MuSK Abs detection in patient serum with highly clinical suspicions supports the MG diagnosis [1,29]. MuSK cell-based assay (MuSK-CBAs) with transfecting of

HEK293 cells with MuSK recombinant antigen has been developed with increased sensitivity by 6–10 % due to additional detection of MuSK Abs [1,29,30,93–95]. The commercial test kits for MuSK CBA are also available [86,96]. The detection of MuSK Abs by quantitative flow cytometry is also being further evaluated for clinical application [97,98]. ELISA and FIPA methods can also detect MuSK Abs, but precise evaluations are required before their usage in routine practice [1,17,29,30]. In a study the diagnostic accuracy of ELISA for detection of MuSK Ab compared with MuSK CBA and RIPA in 89 patients with a clinical suspicion of AChR Ab-negative gMG. MuSK Ab was positive in 22 (24.7 %), 25 (28.1 %), and 14 of 51 patients (27.5 %) by ELISA, CBA, and RIPA respectively [96]. In another study comparing fixed and Live CBA for MuSK Ab detection, among 86 RIA-dSN patients, f-CBA detected MuSK Abs in 1 case and L-CBA in 11 cases [85]. In this study the sensitivity and specificity of f-CBA and L-CBA was calculated by detection of MuSK Ab in 40 samples of RIA positive and 40 samples of healthy control that for both assays, the sensitivity and specificity for MuSK Ab was 100 % [85]. In a recent prospective multicenter double blind study MuSK Ab were found in 59, 50, and 54 out of 2043 MG patients' serum by CBA, RIPA and ELISA, respectively. The f- CBA increased the detection of MuSK antibody in MG patients by 0.3 %–0.5 % compared to the RIPA and ELISA with the same specificity as RIPA (100 %) and higher specificity than ELISA (99.1 %) [86].

Among various methods for the detection of MuSK Abs, each technique had a particular advantage and drawback, but RIPA is still a common method, for MuSK Ab measurement. As in MuSK associated MG intraindividual alterations in antibody titers has a strong correlation with variations in disease severity, RIPA is helpful to follow antibody titer and evaluate treatment response [99,100].

LRP4 has been recognized as a third autoimmune target in MG patients. The data about the clinical findings and response to therapy of MG patients with LRP4 is more limited [101]. However, the detection of these antibodies may lead to prospective, randomized therapeutic trials and future therapeutic choices. Moreover, LRP4 Abs are also present in around 8 % of AChR Abs positive patients, 15–20 % of MuSK positive patients, and 3.6 % of patients with other neurological conditions [102–104]. The LRP4 Abs in SNMG patient sera can be detected by CBAs (HEK293 cells transfected with LRP4 recombinant protein) or ELISA [105]. Although the expression of the LRP4 transmembrane protein in CBA is difficult, co-expression with the chaperone Mesdc2 enhances its transport to the cell surface with limited efficacy. Alternatively, transfected cells can be fixed and permeabilized, but this procedure should be initially optimized [106]. Despite raising some questions about the LRP4-IgG service line testing, [54] the detection techniques, including quantitative methods or mixed techniques, as well as patient demographics, may influence the results of LRP4 Ab testing [107,108]. Therefore, future clinical validation and research studies must focus on establishing a consensus testing platform with optimal accuracy and its application in diagnosing MG cases with LRP-4 IgG positive. However, we recommend LRP4 testing in seronegative MG cases as research-use-only (RUO) testing at this time.

5. Therapeutic strategies in MG patients

The current conventional treatments in MG are symptomatic therapy and immunotherapy including immunomodulators and immunosuppressive. The clinical pattern, autoantibody detection, and disease severity guide the therapeutic approach in MG. The first line of treatment in AChR Ab seropositive MG is the cholinesterase inhibitors (ChEIs). With blocking of ACh hydrolysis, ChEIs increase ACh quanta at the postsynaptic membrane and cause more activation of postsynaptic AChRs and consequently increased NM transmission. They are the best option for maintenance therapy to minimize fatigue and improve muscle power. However, ChEIs have restricted or no effect on controlling the symptoms in patients with MuSK Ab-associated MG. Moreover, the ChEIs cannot modify the underlying immune process in MG patients [1,109]. While ChEIs can improve symptoms in mild cases and are usually used as the initial symptomatic therapy, most MG patients need immunotherapy to suppress autoantibody production. Oral corticosteroids are used as a first-line immunosuppressant to induce remission, and they are continued with a low dose alongside or without non-steroidal immunosuppressants for maintenance therapy. Corticosteroid side effects include increased appetite and weight gain, diabetes, acne, rapid mood changes, muscle weakness, and delayed wound healing, leading to its intolerability and discontinuation. Azathioprine, mycophenolate mofetil, cyclosporine, tacrolimus, and methotrexate have been used as non-steroidal agents for the first or second line of immunosuppressive therapy in the management and long-term treatment of MG patients. Cyclophosphamide is also used in MG management, but it is usually reserved for patients with poor responsiveness to other immunosuppressant agents [2,102,110]. Plasmapheresis (PLEX) and Intravenous immune globulin (IVIG) are two immunomodulators with rapid and short-lasting effects that are used for controlling acute attacks, crises, or refractory cases. IVIG may also be used for maintenance therapy in refractory cases or when there are contraindications to immunosuppressive treatment [111]. While, thymectomy is beneficial in cases with thymoma-associated MG and a subset of early onset non-thymomatous AChR Ab seropositive gMG patients, its usefulness in older cases and seronegative MG has not been determined [112]. Patients with MuSK-MG have limited response to IVIg. Thymectomy is also not beneficial in MUSK-MG, because there are no consistent thymic abnormalities in these patients. In addition, as MuSK Abs does not activate the complement pathway, the complement-blocking agents are ineffective in MuSK-MG. Several biological drugs have been developed and utilized in MG patients to control their symptoms, particularly in refractory cases. These drugs influence the underlying immunopathogenesis of the disease. Rituximab was the first biological drug used in MuSK and AChR Abs seropositive MG. It is a monoclonal antibody with fragmented antigen-binding (Fab) region domains. It targets CD20-expressing B cells which cause the depletion of circulating B lymphocytes and, subsequently, the autoantibodies. However, it does not target B cells in the bone marrow and lymph nodes, as well as stem cells, pro-B cells, long-lived plasma cells, and plasma blasts [26,111]. During the last few years, some novel biological drugs for preventing disease progression via complement inhibition, such as Eculizumab and Ravulizumab, or Fc receptor (FcRn) antagonists, such as Efgartigimod alfa and Rozanolixizumab have got FDA approval. Table 1[113,114]. Eculizumab, a humanized monoclonal antibody, inhibits the cleavage of the terminal complement protein C5 into C5a and C5b and prevents MAC formation. Fig. 3 Its intravenous induction therapy with 900 mg weekly for 4 weeks followed by 1200 mg maintenance every 2 weeks for AChR Ab positive generalized MG has been approved by the FDA. It carries a risk of meningococcal infection and the vaccination against Neisseria meningitidis is mandatory at least 2 weeks before the first infusion [115]. Ravulizumab is another complement inhibitor agent with an action mechanism similar to eculizumab but with long-acting C5 inhibition. It needs fewer injections for maintenance due to its long half-life [2,113].

Table 1
Targets of novel therapeutic agents in myasthenia gravis.

Therapeutic Class	Drug Name
B Cell Depletion	Rituximab
Target CD20	Inebilizumab
Target CD19	
Complement inhibition	Eculizumab
C5 inhibitors	Ravulizumab
C5 and C5b inhibitor	Pozelimab
	Zilucoplan
FcRn blockers	Efgartigimod, Rozanolixizumab, Nipocalimab, Batoclimab
Plasma cell targeting	Bortezomib
Cytokine and Chemokine pathways targeting	Iscalimab, Belimumab, Telitacicept, Tocilizumab, Satralizumab

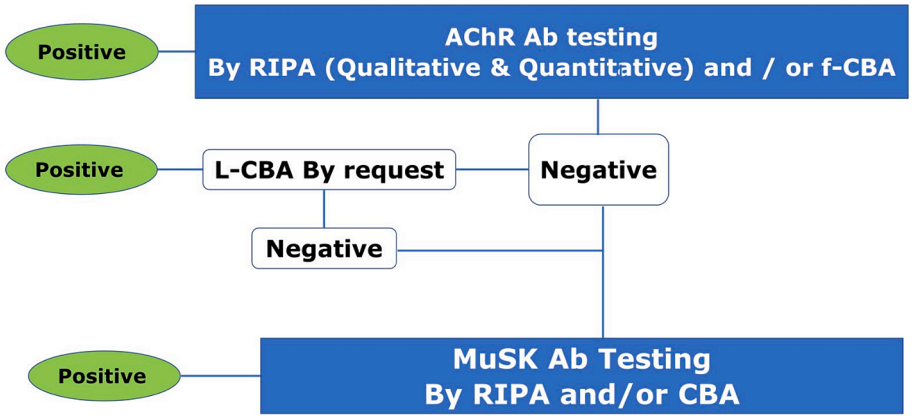


Fig. 4. MG Serological Testing Algorithm. A complete reflex testing algorithm on the first pretreatment sample of clinically suspected MG, starting with the binding and blocking assays for AChR Abs by RIPA and/or f- CBA. If AChR Ab is negative, then reflex to MuSK Abs by RIPA and/or CBA. If AChR Ab negative by RIPA and/or f-CBA and MuSK negative by RIPA and/or CBA, then AChR Ab by L-CBA (in high index of suspicion for MG) by request.

Zilucoplan is another novel biological agent that prevents the terminal activation of the complement cascades:1) by binding to the C5 component and preventing its cleavage and 2) by binding to the C5b and avoiding its attachment to C6. It has a small molecule, and it is not an antibody, therefore it can be administrated subcutaneously and concomitant with IVIg therapy [2,113]. Pozelimab with Cemdisiran is another complement inhibitor agent including a human monoclonal Ab against C5 complement (Pozelimab) and a small synthetic interfering ribonucleic acid (Cemdisiran) with the ability to suppress the hepatic production of C. Both of them are safe and well tolerated when injected subcutaneously. A highly effective approach for the selective removal of IgG antibodies in MG patients is the use of therapeutic agents targeting the FcRn [116,117]. Each immunoglobulin molecule has a variable antigen-binding region (Fab fragments) and a constant crystallizable complement binding region (Fc fragments). The bond of the Fc fragments to Fc receptors is essential for their interaction with the cellular immune process and enhancement of the IgG levels in the serum. This latter action is regulated by FcRn, which are in endothelial and myeloid cells. The binding of FcRn occurs only in an acidic Ph. When the IgG is transported to the acidic environment of the cell endosomes, FcRn binds to IgG and protects it from degradation and transports it back to the surface and releases it in the neutral physiological Ph [113,118]. As all the antibodies of seropositive MG belong to the IgG subclasses, FcRn blocking can interfere with their degradation process and reduce their concentrations [119]. This technique may be considered the same as a plasma exchange leading to the rapid reduction in IgG levels. Interestingly, while FcRn blocking agents reduce the pathological autoantibodies, they preserve the concentrations of the other antibody subtypes (IgM and IgA) [120]. Another advantage of FcRn blocking agents is their potential to allow the production of an effective immune response during therapy, accompanied by a reduced risk of infection. In addition, by decreasing the pathogenic autoantibodies, these agents prevent all the pathological effects of the autoantibodies, including complement activation, apoptosis by internalization, and blocking in MG patients. Their various products are engineered Fc fragments (Efgartigimod), monoclonal antibodies against FcRn (Rozanolixizumab, Nipocalimab, Batoclimab, and Orilanolimab), and peptide fragments. While the Efgartigimod has been approved for the therapy of gMG, the others are in different phases of development. A mild to moderate reduction of serum albumin levels has been reported as a side effect of these agents in healthy volunteers and patients, and it can affect the blood levels of highly albumin-bound medications. Autologous hematopoietic stem cell transplantation (HSCT) as a technique to weaken or even completely halt disease activity in many autoimmune neurological diseases including MG has been developing during the last few years [121]. The processes in HSCT include the stimulation of hematopoietic cell production, collection of cells from circulation, immune system ablation and restoration, and re-infusion of treated cells into patients. The evidence for HSCT in MG is very limited to a few patients. The early and late severe side effects including infections, secondary autoimmune diseases, and neoplasms have brought HSCT to a lower rank among the treatment options.

6. Conclusion

The crucial diagnostic evaluation of clinical MG is performed by serologic testing. RIPA has been used for the last four decades as a gold standard assay for the detection of AChR and MuSK Abs. Cell-Based assays (CBA) are an improved technique for detecting AChR Abs, MuSk, and LRP4 Abs. Other techniques such as ELISA, FIPA, and dot-blot methods, have lower sensitivity and specificity and it is difficult to rely on them for the diagnosis of MG. Moreover, while the presence of AChR Abs has a very high positive predictive value (PPV), its presence does not predict the course of the disease, and the titer of AChR Ab does not correlate with clinical severity on a group level. However, on an individual level, deterioration or improvement in clinical activity often accompanies an increase or decrease in AChR Abs. The poor correlation of Abs titer with disease severity may be related to the current assays used for AChR Abs detection, which primarily measure the binding circulating antibodies. Further, despite MG is one of the best-known immune diseases, there are still essential needs in its therapeutic planning. While conventional therapies can induce remission in 70 – 80 % of MG patients, very few of them attain permanent stable remission [122]. The inconsistency in therapeutic response and the risk of long-term exposure to immunosuppressive agents imposes adverse effects on patients' quality including corticosteroid side effects, opportunistic infections, malignancies, and systemic organ dysfunction. The main reason for such toxicities is the non-selective targets of the immune system among the current MG standard medications. In addition, the necessity of long-term corticosteroids to delay the need for non-steroidal agents complicates the management of MG patients with their multiple metabolic and immunological side effects. Moreover, patients with severe comorbidities are intolerable to steroids and other immunotherapeutic agents. In addition, approximately 10–15 % of MG patients may be refractory to standard therapies that cause recurrent exacerbations with the need for hospitalizations and respiratory support and susceptibility to end-organ dysfunction and failures [123,124]. Indeed, measurement of a combination of Abs, including those that bind to complement or modulate the AChR is essential for obtaining more accurate Abs titers for having a better correlation with disease severity. Considering the heterogeneity of MG, the live Cell-Based assay helps to detect the clustered antibodies that contribute to the increased circulation of the AChR. With

advancements in the diagnosis and management of MG, there has been a recently proposed change to MG testing algorithms. Because immunotherapeutic and biological drugs confound the test results (e.g., false-negative in patients who have received IVIG, plasma exchange, rituximab or eculizumab) and the necessity of early immune-targeted therapy, we now recommend a complete reflex testing algorithm on the first pretreatment sample from a patient with suspected MG, starting with the binding and blocking assays for AChR Abs by RIPA and/ or f-CBA (Fig. 4). If AChR Ab is negative, then reflex to MuSK Abs by RIPA and /or CBAs. If AChR and anti- Abs are negative, then use AChR Abs L-CBA (in high index of suspicion for MG) by request.

CRediT authorship contribution statement

Ali Mousavi: Writing – review & editing, Writing – original draft, Resources, Conceptualization. **Pankaj Kumar:** . **Hans Frykman:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

Hans Frykman is a full owner and Medical Director of BC Neuroimmunology Lab. Inc. (BCNI). Ali Mousavi is an employee of BCNI. Pankaj Kumar is CEO and labartary director of BCNI. This study did not receive any external funding.

References

[1] J. Oger, H. Frykman, An update on laboratory diagnosis in myasthenia gravis ☆, Published Online (2015) https://doi.org/10.1016/j.cca.2015.01.042.

[2] D. Menon, V. Bril, Pharmacotherapy of generalized myasthenia gravis with special emphasis on newer biologicals, Drugs 82 (8) (2022) 865–887, https://doi.org/10.1007/s40265-022-01726-y.

[3] L. Dresser, R. Wlodarski, K. Rezania, B. Soliven, Myasthenia gravis: Epidemiology, pathophysiology and clinical manifestations, J. Clin. Med. 10 (11) (2021), https://doi.org/10.3390/jcm10112235.

[4] J.R. Parr, M.J. Andrew, M. Finnis, D. Beeson, A. Vincent, S. Jayawant, How common is childhood myasthenia? The UK incidence and prevalence of autoimmune and congenital myasthenia, Arch. Dis. Child. 99 (6) (2014) 539–542, https://doi.org/10.1136/archdischild-2013-304788.

[5] Z. Pakzad, T. Aziz, J. Oger, Increasing incidence of myasthenia gravis among elderly in British Columbia, Canada. *Neurology*. 76 (17) (2011) 1526–1528, https://doi.org/10.1212/WNL.0b013e318217e735.

[6] C.T. Bever, A.V. Aquino, A.S. Penn, R.E. Lovelace, L.P. Rowland, Prognosis of ocular myasthenia, Ann. Neurol. 14 (5) (1983) 516–519, https://doi.org/10.1002/ana.410140504.

[7] N.E. Gilhus, S. Tzartos, A. Evoli, J. Palace, T.M. Burns, J.J.G.M. Verschuuren, Myasthenia gravis, Nat. Rev. Dis. Primers. 5 (1) (2019) 30, https://doi.org/10.1038/s41572-019-0079-y.

[8] Kuks JBM. 2009 Clinical Presentation and Epidemiology of Myasthenia Gravis. In: *Myasthenia Gravis and Related Disorders*. Humana Press:79-94. doi:10.1007/978-1-59745-156-7_5.

[9] A. Chaudhuri, P.O. Behan, Myasthenic crisis, QJM 102 (2) (2008) 97–107, https://doi.org/10.1093/qjmed/hcn152.

[10] Y. Zhang, X. Cheng, C. Luo, et al., Congenital myasthenic syndrome caused by a novel hemizygous CHAT mutation, Front. Pediatr. 8 (2020), https://doi.org/10.3389/fped.2020.00185.

[11] L. Dresser, R. Wlodarski, K. Rezania, B. Soliven, myasthenia gravis: epidemiology, pathophysiology and clinical manifestations, J. Clin. Med. 10 (11) (2021) 2235, https://doi.org/10.3390/jcm10112235.

[12] E. Westerberg, A.R. Punga, Epidemiology of myasthenia gravis in Sweden 2006–2016, Brain Behav. 10 (11) (2020), https://doi.org/10.1002/brb3.1819.

[13] E. Rodrigues, E. Umeh, N.N. Aishwarya, A. Cole, K. Moy, Incidence and prevalence of myasthenia gravis in the <scp>United States</scp> : a claims-based analysis, Muscle Nerve 69 (2) (2024) 166–171, https://doi.org/10.1002/mus.28006.

[14] E.G. Pedersen, J. Hallas, K. Hansen, P.E.H. Jensen, D. Gaist, Late-onset myasthenia not on the increase: a nationwide register study in <scp>D</scp> enmark, 1996–2009, Eur. J. Neurol. 20 (2) (2013) 309–314, https://doi.org/10.1111/j.1468-1331.2012.03850.x.

[15] D.H. Donaldson, M. Ansher, S. Horan, R.B. Rutherford, S.P. Ringel, The relationship of age to outcome in myasthenia gravis, Neurology 40 (5) (1990) 786, https://doi.org/10.1212/WNL.40.5.786.

[16] S.A. Živković, P.R. Clemens, D. Lacomis, Characteristics of late-onset myasthenia gravis, J. Neurol. 259 (10) (2012) 2167–2171, https://doi.org/10.1007/s00415-012-6478-6.

[17] K. Lazaridis, S.J. Tzartos, Autoantibody specificities in myasthenia gravis; implications for improved diagnostics and therapeutics, Front. Immunol. 11 (2020), https://doi.org/10.3389/fimmu.2020.00212.

[18] H. Frykman, P. Kumar, J. Oger, Immunopathology of autoimmune myasthenia gravis: implications for improved testing algorithms and treatment strategies, Front. Neurol. 11 (2020) 596621.

[19] A. Vincent, Unravelling the pathogenesis of myasthenia gravis, Nat. Rev. Immunol. 2 (10) (2002) 797–804, https://doi.org/10.1038/nri916.

[20] A. Vincent, S. Huda, M. Cao, et al., Serological and experimental studies in different forms of myasthenia gravis, Ann. N. Y. Acad. Sci. 1413 (1) (2018) 143–153, https://doi.org/10.1111/nyas.13592.

[21] Zisimopoulou P EPTJLKZVMR et al. A comprehensive analysis of the epidemiology and clinical characteristics of anti-LRP4 in myasthenia gravis. J Autoimmun.

[22] O. Higuchi, J. Hamuro, M. Motomura, Y. Yamanashi, Autoantibodies to low-density lipoprotein receptor-related protein 4 in myasthenia gravis, Ann. Neurol. 69 (2) (2011) 418–422, https://doi.org/10.1002/ana.22312.

[23] M.H. Rivner, B.M. Quarles, J.X. Pan, et al., Clinical features of LRP4/agrin-antibody–positive myasthenia gravis: a multicenter study, Muscle Nerve 62 (3) (2020) 333–343, https://doi.org/10.1002/mus.26985.

[24] C.J. Klein, G. Beecher, C. Lamb, et al., LRP4-IgG service line testing in seronegative myasthenia gravis and controls, J. Neuroimmunol. 368 (2022), https://doi.org/10.1016/j.jneuroim.2022.577895.

[25] A. Vincent, D. Beeson, B. Lang, Molecular targets for autoimmune and genetic disorders of neuromuscular transmission, Eur. J. Biochem. 267 (23) (2000) 6717–6728, https://doi.org/10.1046/j.1432-1033.2000.01785.x.

[26] N.E. Gilhus, J.J. Verschuuren, Myasthenia gravis: subgroup classification and therapeutic strategies, Lancet Neurol. 14 (10) (2015) 1023–1036, https://doi.org/10.1016/S1474-4422(15)00145-3.

[27] A. Vincent, Z. Li, A. Hart, et al., Seronegative myasthenia gravis: evidence for plasma factor(s) interfering with acetylcholine receptor functiona, Ann. N. Y. Acad. Sci. 681 (1) (1993) 529–538, https://doi.org/10.1111/j.1749-6632.1993.tb22936.x.

[28] T. Yamamoto, A. Vincent, T.A. Ciulla, B. Lang, I. Johnston, J. Newsom-Davis, Seronegative myasthenia gravis: a plasma factor inhibiting agonist-induced acetylcholine receptor function copurifies with IgM, Ann. Neurol. 30 (4) (1991) 550–557, https://doi.org/10.1002/ana.410300407.

[29] H. Frykman, P. Kumar, J. Oger, Immunopathology of autoimmune myasthenia gravis: implications for improved testing algorithms and treatment strategies, Front. Neurol. 11 (2020), https://doi.org/10.3389/fneur.2020.596621.

[30] H. Frykman, P. Kumar, Laboratory testing of Myasthenia Gravis: New Treatments Drive Change, J. Appl. Lab. Med. 6 (4) (2021) 1087–1089, https://doi.org/10.1093/jalm/jfaa199.

[31] H. Cetin, D. Beeson, A. Vincent, R. Webster, The structure, function, and physiology of the fetal and adult acetylcholine receptor in muscle, Front. Mol. Neurosci. 13 (2020), https://doi.org/10.3389/fnmol.2020.581097.

[32] J. Luo, J. Lindstrom, Antigenic structure of the human muscle nicotinic acetylcholine receptor main immunogenic region, J. Mol. Neurosci. 40 (1–2) (2010) 217–220, https://doi.org/10.1007/s12031-009-9271-y.

[33] D. Kalamida, K. Poulas, V. Avramopoulou, et al., Muscle and neuronal nicotinic acetylcholine receptors, FEBS J. 274 (15) (2007) 3799–3845, https://doi.org/10.1111/j.1742-4658.2007.05935.x.

[34] M.N. Meriggioli, D.B. Sanders, Muscle autoantibodies in myasthenia gravis: beyond diagnosis? Expert Rev. Clin. Immunol. 8 (5) (2012) 427–438, https://doi.org/10.1586/eci.12.34.

[35] A.G. Engel, K. Arahata, The membrane attack complex of complement at the endplate in myasthenia gravis, Ann. N. Y. Acad. Sci. 505 (1987) 326–332, https://doi.org/10.1111/j.1749-6632.1987.tb51301.x.

[36] J.F. Howard, Myasthenia gravis: the role of complement at the neuromuscular junction, Ann. N. Y. Acad. Sci. 1412 (1) (2018) 113–128, https://doi.org/10.1111/nyas.13522.

[37] A.G. Engel, K. Sahashi, G. Fumagalli, The immunopathology of acquired my asthenia gravis, Ann. N. Y. Acad. Sci. 377 (1981) 158–174, https://doi.org/10.1111/j.1749-6632.1981.tb33730.x.

[38] D.B. Drachman, C.W. Angus, R.N. Adams, J.D. Michelson, G.J. Hoffman, Myasthenic antibodies cross-link acetylcholine receptors to accelerate degradation, N. Engl. J. Med. 298 (20) (1978) 1116–1122, https://doi.org/10.1056/NEJM197805182982004.

[39] D.P. Richman, R.L. Wollmann, R.A. Maselli, et al., Effector mechanisms of myasthenic antibodies, Ann. N. Y. Acad. Sci. 681 (1993) 264–273, https://doi.org/10.1111/j.1749-6632.1993.tb22891.x.

[40] L.L. Kusner, N. Satija, G. Cheng, H.J. Kaminski, Targeting therapy to the neuromuscular junction: Proof of concept, Muscle Nerve 49 (5) (2014) 749–756, https://doi.org/10.1002/mus.24057.

[41] W. Hoch, J. McConville, S. Helms, J. Newsom-Davis, A. Melms, A. Vincent, Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies, Nat. Med. 7 (3) (2001) 365–368, https://doi.org/10.1038/85520.

[42] I. Koneczny, J.A.A. Stevens, A. De Rosa, et al., IgG4 autoantibodies against muscle-specific kinase undergo Fab-arm exchange in myasthenia gravis patients, J. Autoimmun. 77 (2017) 104–115, https://doi.org/10.1016/j.jaut.2016.11.005.

[43] B. Ohkawara, M. Ito, K. Ohno, Secreted signaling molecules at the neuromuscular junction in physiology and pathology, Int. J. Mol. Sci. 22 (5) (2021) 2455, https://doi.org/10.3390/ijms22052455.

[44] K. Otsuka, M. Ito, B. Ohkawara, et al., Collagen Q and anti-MuSK autoantibody competitively suppress agrin/LRP4/MuSK signaling, Sci. Rep. 5 (1) (2015) 13928, https://doi.org/10.1038/srep13928.

[45] J.S. Tzartos, Autoantibodies to lipoprotein-related protein 4 in patients with double-seronegative myasthenia gravis, Arch. Neurol. 69 (4) (2012) 445, https://doi.org/10.1001/archneurol.2011.2393.

[46] M. Cao, W.W. Liu, S. Maxwell, et al., IgG1-3 MuSK antibodies inhibit AChR cluster formation, restored by SHP2 inhibitor, despite normal MuSK, DOK7, or AChR subunit phosphorylation, Neurol Neuroimmunol Neuroinflamm. 10 (6) (2023), https://doi.org/10.1212/NXI.0000000000200147.

[47] Hohlfeld R, WH. Reflections on the “intrathymic pathogenesis” of myasthenia gravis.

[48] C.R. Thomas, C.D. Wright, P.J. Loehrer, Thymoma: state of the art, J. Clin. Oncol. 17 (7) (1999) 2280, https://doi.org/10.1200/JCO.1999.17.7.2280.

[49] Z.F. Mao, X.A. Mo, C. Qin, Y.R. Lai, M.L. Hackett, Incidence of thymoma in myasthenia gravis: a systematic review, Journal of Clinical Neurology. 8 (3) (2012) 161, https://doi.org/10.3988/jcn.2012.8.3.161.

[50] C. Potagas, G. Dellatolas, A. Tavernarakis, et al., Myasthenia gravis: changes observed in a 30-years retrospective clinical study of a hospital-based population, J. Neurol. 251 (1) (2004) 116–117, https://doi.org/10.1007/s00415-004-0278-6.

[51] R. Hohlfeld, H. Wekerle, Reflections on the “intrathymic pathogenesis” of myasthenia gravis, J. Neuroimmunol. 201–202 (2008) 21–27, https://doi.org/10.1016/j.jneuroim.2008.05.020.

[52] A.I. Levinson, Modeling the intrathymic pathogenesis of myasthenia gravis, J. Neurol. Sci. 333 (1–2) (2013) 60–67, https://doi.org/10.1016/j.jns.2012.12.025.

[53] L. Mesnard-Rouiller, J. Bismuth, A. Wakkach, S. Poëa-Guyon, S. Berrih-Aknin, Thymic myoid cells express high levels of muscle genes, J. Neuroimmunol. 148 (1–2) (2004) 97–105, https://doi.org/10.1016/j.jneuroim.2003.11.013.

[54] I. Koneczny, R. Herbst, Myasthenia gravis: pathogenic effects of autoantibodies on neuromuscular architecture, Cells. 8 (7) (2019) 671, https://doi.org/10.3390/cells8070671.

[55] E. Tüzün, B.G. Scott, E. Goluszko, S. Higgs, P. Christadoss, Genetic evidence for involvement of classical complement pathway in induction of experimental autoimmune myasthenia gravis, J. Immunol. 171 (7) (2003) 3847–3854, https://doi.org/10.4049/jimmunol.171.7.3847.

[56] M.C. Dalakas, H. Alexopoulos, P.J. Spaeth, Complement in neurological disorders and emerging complement-targeted therapeutics, Nat. Rev. Neurol. 16 (11) (2020) 601–617, https://doi.org/10.1038/s41582-020-0400-0.

[57] G. Saluja, A. Samdani, P. Bhatia, Importance of clinical tests in ocular myasthenia, BMJ Case Rep. 12 (12) (2019) e231296, https://doi.org/10.1136/bcr-2019-231296.

[58] K. Yoganathan, A. Stevenson, A. Tahir, R. Sadler, A. Radunovic, N. Malek, Bedside and laboratory diagnostic testing in myasthenia, J. Neurol. 269 (6) (2022) 3372–3384, https://doi.org/10.1007/s00415-022-10986-3.

[59] E. Marinos, K. Buzzard, C.L. Fraser, S. Reddel, Evaluating the temperature effects of ice and heat tests on ptosis due to Myasthenia Gravis, Eye 32 (8) (2018) 1387–1391, https://doi.org/10.1038/s41433-018-0101-8.

[60] Ropper A. H. SMAKJP, SPS. 2019 *Disorders of the Neuromuscular Junction, Myotonias, and Persistent Muscle Fiber Activity.* . Vol 11th ed. (Adams and Victor’s, ed.). McGraw Hill / Medical. USA.

[61] A. Evoli, G. Antonini, C. Antozzi, et al., Italian recommendations for the diagnosis and treatment of myasthenia gravis, Neurol. Sci. 40 (6) (2019) 1111–1124, https://doi.org/10.1007/s10072-019-03746-1.

[62] S.J. Oh, N. Eslami, T. Nishihira, et al., Electrophysiological and clinical correlation in myasthenia gravis, Ann. Neurol. 12 (4) (1982) 348–354, https://doi.org/10.1002/ana.410120406.

[63] N.G. Sirin, E. Kocasoy Orhan, H. Durmus, F. Deymeer, M.B. Baslo, Repetitive nerve stimulation and jitter measurement with disposable concentric needle electrode in newly diagnosed myasthenia gravis patients, Neurophysiol. Clin. 48 (5) (2018) 261–267, https://doi.org/10.1016/j.neucli.2018.01.003.

[64] J.A. Kouyoumdjian, E.V. Stålberg, Concentric needle single fiber electromyography: comparative jitter on voluntary-activated and stimulated Extensor Digitorum Communis, Clin. Neurophysiol. 119 (7) (2008) 1614–1618, https://doi.org/10.1016/j.clinph.2008.03.008.

[65] E.V. Stålberg, D.B. Sanders, Jitter recordings with concentric needle electrodes, Muscle Nerve 40 (3) (2009) 331–339, https://doi.org/10.1002/mus.21424.

[66] Crum BA, Harper CM. Single-Fiber Electromyography. In: Rubin DI, ed. *Clinical Neurophysiology*. Oxford University PressNew York; 2021:525-548. doi:10.1093/med/9780190067854.003.0027.

[67] E. Stålberg, D.B. Sanders, S. Ali, et al., Reference values for jitter recorded by concentric needle electrodes in healthy controls: a multicenter study, Muscle Nerve 53 (3) (2016) 351–362, https://doi.org/10.1002/mus.24750.

[68] A. Abraham, M. Alabdali, A. Alsulaiman, et al., Repetitive nerve stimulation cutoff values for the diagnosis of myasthenia gravis, Muscle Nerve 55 (2) (2017) 166–170, https://doi.org/10.1002/mus.25214.

[69] Vahabi Z, Nazari F, Fatehi F, et al. 2021 Electrophysiological studies in patients with seropositive/seronegative myasthenia gravis. *Curr J Neurol*. Published online November 7. doi:10.18502/cjn.v20i3.7686.

[70] H.D. Katzberg, V. Bril, A comparison of electrodiagnostic tests in ocular myasthenia gravis, J. Clin. Neuromuscul. Dis. 6 (3) (2005) 109–113, https://doi.org/10.1097/01.cnd.0000155026.66153.f0.

[71] R.T. Rousseff, Diagnosis of myasthenia gravis, J. Clin. Med. 10 (8) (2021) 1736, https://doi.org/10.3390/jcm10081736.

[72] S. Suzuki, New clinical entity of myasthenia gravis with autoimmune targets of heart and skeletal muscles, Rinsho Shinkeigaku 52 (11) (2012) 1312–1314, https://doi.org/10.5692/clinicalneurol.52.1312.

[73] Beeson D JLNDJVA. A transfected human muscle cell line expressing the adult subtype of the human muscle acetylcholine receptor for diagnostic assays in myasthenia gravis.

[74] J. Patrick, J. Lindstrom, B. Culp, J. Mcmillan, Studies on purified eel acetylcholine receptor and anti-acetylcholine receptor antibody, Proc. Natl. Acad. Sci. 70 (12) (1973) 3334–3338, https://doi.org/10.1073/pnas.70.12.3334.

[75] K. Ohta, A. Fujinami, T. Saida, M. Nishimura, S. Kuno, M. Ohta, Frequency of anti-AChR ∈ subunit-specific antibodies in MG, Autoimmunity 36 (3) (2003) 151–154, https://doi.org/10.1080/0891693031000094935.

[76] S. Shelly, P. Paul, H. Bi, et al., Improving accuracy of myasthenia gravis autoantibody testing by reflex algorithm, Neurology 95 (22) (2020), https://doi.org/10.1212/WNL.0000000000010910.

[77] F.M. Howard, V.A. Lennon, J. Finley, J. Matsumoto, L.R. Elveback, Clinical correlations of antibodies that bind, block, or modulate human acetylcholine receptors in myasthenia gravis, Ann. N. Y. Acad. Sci. 505 (1) (1987) 526–538, https://doi.org/10.1111/j.1749-6632.1987.tb51321.x.

[78] B.K. Lozier, T.R. Haven, M.E. Astill, H.R. Hill, Detection of acetylcholine receptor modulating antibodies by flow cytometry, Am. J. Clin. Pathol. 143 (2) (2015) 186–192, https://doi.org/10.1309/AJCPYEOR6SGE8ZLU.

[79] M. Apiwattanakul, A. McKeon, S.J. Pittock, T.J. Kryzer, V.A. Lennon, Eliminating false-positive results in serum tests for neuromuscular autoimmunity, Muscle Nerve 41 (5) (2010) 702–704, https://doi.org/10.1002/mus.21653.

[80] R. Rajput, A. Sachdev, N. Din, E.M. Damato, A. Murray, False positive acetylcholine receptor antibodies in a case of unilateral chronic progressive external ophthalmoplegia: case report and review of literature, Orbit (London). 37 (5) (2018) 385–388, https://doi.org/10.1080/01676830.2017.1423350.

[81] Y. Hong, P. Zisimopoulou, N. Trakas, et al., Multiple antibody detection in ‘seronegative’ myasthenia gravis patients, Eur. J. Neurol. 24 (6) (2017) 844–850, https://doi.org/10.1111/ene.13300.

[82] M.I. Leite, S. Jacob, S. Viegas, et al., IgG1 antibodies to acetylcholine receptors in ‘seronegative’ myasthenia gravis†, Brain 131 (7) (2008) 1940–1952, https://doi.org/10.1093/brain/awn092.

[83] J. Cossins, K. Belaya, K. Zoltowska, et al., The search for new antigenic targets in myasthenia gravis, Ann. N. Y. Acad. Sci. 1275 (1) (2012) 123–128, https://doi.org/10.1111/j.1749-6632.2012.06833.x.

[84] A. Mirian, M.W. Nicolle, P. Edmond, A. Budhram, Comparison of fixed cell-based assay to radioimmunoprecipitation assay for acetylcholine receptor antibody detection in myasthenia gravis, J. Neurol. Sci. 432 (2022) 120084.

[85] G. Spagni, M. Gastaldi, P. Businaro, et al., Comparison of fixed and live cell-based assay for the detection of AChR and MuSK antibodies in myasthenia gravis, Neurology - Neuroimmunology Neuroinflammation. 10 (1) (2023) e200038, https://doi.org/10.1212/NXI.0000000000200038.

[86] Z. Li, C. Zhang, T. Chang, et al., A multicentre, prospective, double-blind study comparing the accuracy of autoantibody diagnostic assays in myasthenia gravis: the SCREAM study, Lancet Reg Health West Pac. (2023) 38, https://doi.org/10.1016/j.lanwpc.2023.100846.

[87] E. Gomez-Bañuelos, Y. Yu, J. Li, et al., Affinity maturation generates pathogenic antibodies with dual reactivity to DNase1L3 and dsDNA in systemic lupus erythematosus, Nat. Commun. 14 (1) (2023) 1388, https://doi.org/10.1038/s41467-023-37083-x.

[88] K. Elkon, P. Casali, Nature and functions of autoantibodies, Nat. Clin. Pract. Rheumatol. 4 (9) (2008) 491–498, https://doi.org/10.1038/ncprheum0895.

[89] R.J. Ludwig, K. Vanhoorelbeke, F. Leypoldt, et al., Mechanisms of autoantibody-induced pathology, Front. Immunol. 8 (2017), https://doi.org/10.3389/fimmu.2017.00603.

[90] H. Frykman, A. Fronda, Validation of live clustered cell based acethylcholine antibody assay and utility in children with myasthenia gravis (5239), Neurology 94 (15_supplement) (2020), https://doi.org/10.1212/WNL.94.15_supplement.5239.

[91] D. Keefe, D. Hess, J. Bosco, et al., A rapid, fluorescence-based assay for detecting antigenic modulation of the acetylcholine receptor on human cell lines, Cytometry B Clin. Cytom. 76B (3) (2009) 206–212, https://doi.org/10.1002/cyto.b.20454.

[92] A. Evoli, A.P. Batocchi, M.T. Palmisani, M. Lo Monaco, P. Tonali, Long-term results of corticosteroid therapy in patients with myasthenia gravis, Eur. Neurol. 32 (1) (1992) 37–43, https://doi.org/10.1159/000116785.

[93] A. Evoli, P.E. Alboini, V. Damato, et al., Myasthenia gravis with antibodies to MuSK: an update, Ann. N. Y. Acad. Sci. 1412 (1) (2018) 82–89, https://doi.org/10.1111/nyas.13518.

[94] D. Beeson, L. Jacobson, J. Newsom-Davis, A. Vincent, A transfected human muscle cell line expressing the adult subtype of the human muscle acetylcholine receptor for diagnostic assays in myasthenia gravis, Neurology 47 (6) (1996) 1552–1555, https://doi.org/10.1212/WNL.47.6.1552.

[95] Evoli A APDVIRPCBEMM. Myasthenia gravis with antibodies to MuSK: an update. .

[96] Y.N. Kwon, M. Woodhall, J.J. Sung, et al., Clinical pitfalls and serological diagnostics of MuSK myasthenia gravis, J. Neurol. 270 (3) (2023) 1478–1486, https://doi.org/10.1007/s00415-022-11458-4.

[97] M.L. Fichtner, R. Jiang, A. Bourke, R.J. Nowak, K.C. O’Connor, Autoimmune pathology in myasthenia gravis disease subtypes is governed by divergent mechanisms of immunopathology, Front. Immunol. 11 (2020), https://doi.org/10.3389/fimmu.2020.00776.

[98] E. Bartoccioni, F. Scuderi, G.M. Minicuci, M. Marino, F. Ciaraffa, A. Evoli, Anti-MuSK antibodies: correlation with myasthenia gravis severity, Neurology 67 (3) (2006) 505–507, https://doi.org/10.1212/01.wnl.0000228225.23349.5d.
[99] E.H. Niks, Y. van Leeuwen, M.I. Leite, et al., Clinical fluctuations in MuSK myasthenia gravis are related to antigen-specific IgG4 instead of IgG1, J. Neuroimmunol. 195 (1–2) (2008) 151–156, https://doi.org/10.1016/j.jneuroim.2008.01.013.
[100] A.G. Vakrakou, E. Karachaliou, E. Chroni, et al., Immunotherapies in MuSK-positive myasthenia gravis; an IgG4 antibody-mediated disease, Front. Immunol. 14 (2023), https://doi.org/10.3389/fimmu.2023.1212757.
[101] P. Zisimopoulou, P. Evangelakou, J. Tzartos, et al., A comprehensive analysis of the epidemiology and clinical characteristics of anti-LRP4 in myasthenia gravis, J. Autoimmun. 52 (2014) 139–145, https://doi.org/10.1016/j.jaut.2013.12.004.
[102] M.H. Rivner, S. Liu, B. Quarles, et al., Agrin and low-density lipoprotein-related receptor protein 4 antibodies in amyotrophic lateral sclerosis patients, Muscle Nerve 55 (3) (2017) 430–432, https://doi.org/10.1002/mus.25438.
[103] I. Cordts, N. Bodart, K. Hartmann, et al., Screening for lipoprotein receptor-related protein 4-, agrin-, and titin-antibodies and exploring the autoimmune spectrum in myasthenia gravis, J. Neurol. 264 (6) (2017) 1193–1203, https://doi.org/10.1007/s00415-017-8514-z.
[104] A.I. Tsonis, P. Zisimopoulou, K. Lazaridis, et al., MuSK autoantibodies in myasthenia gravis detected by cell based assay — a multinational study, J. Neuroimmunol. 284 (2015) 10–17, https://doi.org/10.1016/j.jneuroim.2015.04.015.
[105] Z. Yu, M. Zhang, H. Jing, et al., Characterization of LRP4/agrin antibodies from a patient with myasthenia gravis, Neurology 97 (10) (2021), https://doi.org/10.1212/WNL.0000000000012463.
[106] O. Chuquisana, F. Stascheit, C.W. Keller, et al., Functional signature of LRP4 antibodies in myasthenia gravis, Neurol Neuroimmunol Neuroinflamm. 11 (3) (2024), https://doi.org/10.1212/NXI.0000000000200220.
[107] T. Hoshi, T. Tezuka, K. Yokoyama, S. ichiro Iemura, T. Natsume, Y. Yamanashi, Mesdc2 plays a key role in cell-surface expression of Lrp4 and postsynaptic specialization in myotubes, FEBS Lett. 587 (23) (2013) 3749–3754, https://doi.org/10.1016/j.febslet.2013.10.001.
[108] C.J. Klein, G. Beecher, C. Lamb, et al., LRP4-IgG service line testing in seronegative myasthenia gravis and controls, J. Neuroimmunol. 368 (2022) 577895, https://doi.org/10.1016/j.jneuroim.2022.577895.
[109] J.S. Tzartos, P. Zisimopoulou, M. Rentzos, et al., <scp>LRP</scp> 4 antibodies in serum and <scp>CSF</scp> from amyotrophic lateral sclerosis patients, Ann. Clin. Transl. Neurol. 1 (2) (2014) 80–87, https://doi.org/10.1002/acn3.26.
[110] Mehndiratta MM, Pandey S, Kuntzer T. 2014 Acetylcholinesterase inhibitor treatment for myasthenia gravis. *Cochrane Database of Systematic Reviews*. Published online October 13. doi:10.1002/14651858.CD006986.pub3.
[111] C. Schneider-Gold, P. Gajdos, K.V. Toyka, R.R. Hohlfeld, Corticosteroids for myasthenia gravis, Cochrane Database Syst. Rev. 2011 (6) (2005), https://doi.org/10.1002/14651858.CD002828.pub2.
[112] M.K. Hehir, T.M. Burns, J. Alpers, M.R. Conaway, M. Sawa, D.B. Sanders, Mycophenolate mofetil in AChR-antibody-positive myasthenia gravis: outcomes in 102 patients, Muscle Nerve 41 (5) (2010) 593–598, https://doi.org/10.1002/mus.21640.
[113] P. Narayanaswami, D.B. Sanders, G. Wolfe, et al., International consensus guidance for management of myasthenia gravis, Neurology 96 (3) (2021) 114–122, https://doi.org/10.1212/WNL.0000000000011124.
[114] D. Sánchez-Tejerina, J. Sotoca, A. Llaurado, V. López-Diego, R. Juntas-Morales, M. Salvado, New targeted agents in myasthenia gravis and future therapeutic strategies, J. Clin. Med. 11 (21) (2022) 6394, https://doi.org/10.3390/jcm11216394.
[115] S.S. Nair, S. Jacob, Novel immunotherapies for myasthenia gravis, Immunotargets Ther. 12 (2023) 25–45, https://doi.org/10.2147/ITT.S377056.
[116] M. DeHart-McCoyle, S. Patel, X. Du, New and emerging treatments for myasthenia gravis, BMJ Medicine. 2 (1) (2023) e000241.
[117] J.F. Howard, K. Utsugisawa, M. Benatar, et al., Safety and efficacy of eculizumab in anti-acetylcholine receptor antibody-positive refractory generalised myasthenia gravis (REGAIN): a phase 3, randomised, double-blind, placebo-controlled, multicentre study, Lancet Neurol. 16 (12) (2017) 976–986, https://doi.org/10.1016/S1474-4422(17)30369-1.
[118] L.J. Blumberg, J.E. Humphries, S.D. Jones, et al., Blocking FcRn in humans reduces circulating IgG levels and inhibits IgG immune complex–mediated immune responses, Sci. Adv. 5 (12) (2019), https://doi.org/10.1126/sciadv.aax9586.
[119] P. Mina-Osorio, M.H. Tran, A.A. Habib, Therapeutic plasma exchange versus FcRn inhibition in autoimmune disease, Transfus. Med. Rev. 38 (1) (2024), https://doi.org/10.1016/j.tmrv.2023.150767.
[120] J.D. Lünemann, Getting specific: targeting Fc receptors in myasthenia gravis, Nat. Rev. Neurol. 17 (10) (2021) 597–598, https://doi.org/10.1038/s41582-021-00547-z.
[121] G.I. Wolfe, E.S. Ward, H. de Haard, et al., IgG regulation through FcRn blocking: a novel mechanism for the treatment of myasthenia gravis, J. Neurol. Sci. 430 (2021) 118074, https://doi.org/10.1016/j.jns.2021.118074.
[122] P. Ulrichts, A. Guglietta, T. Dreier, et al., Neonatal Fc receptor antagonist efgartigimod safely and sustainably reduces IgGs in humans, J. Clin. Investig. 128 (10) (2018) 4372–4386, https://doi.org/10.1172/JCI97911.
[123] A. Bryant, H. Atkins, C.E. Pringle, et al., Myasthenia gravis treated with autologous hematopoietic stem cell transplantation, JAMA Neurol. 73 (6) (2016) 652, https://doi.org/10.1001/jamaneurol.2016.0113.
[124] D. Grob, N. Brunner, T. Namba, M. Pagala, Lifetime course of myasthenia gravis, Muscle Nerve 37 (2) (2008) 141–149, https://doi.org/10.1002/mus.20950.