Myasthenia gravis

Nils Erik Gilhus[1,2], Socrates Tzartos[3], Amelia Evoli[4,5], Jacqueline Palace[6], Ted M. Burns[7] and Jan J. G. M. Verschuuren[8]*

Abstract | Myasthenia gravis (MG) is an autoimmune disease caused by antibodies against the acetylcholine receptor (AChR), muscle-specific kinase (MuSK) or other AChR-related proteins in the postsynaptic muscle membrane. Localized or general muscle weakness is the predominant symptom and is induced by the antibodies. Patients are grouped according to the presence of antibodies, symptoms, age at onset and thymus pathology. Diagnosis is straightforward in most patients with typical symptoms and a positive antibody test, although a detailed clinical and neurophysiological examination is important in antibody-negative patients. MG therapy should be ambitious and aim for clinical remission or only mild symptoms with near-normal function and quality of life. Treatment should be based on MG subgroup and includes symptomatic treatment using acetylcholinesterase inhibitors, thymectomy and immunotherapy. Intravenous immunoglobulin and plasma exchange are fast-acting treatments used for disease exacerbations, and intensive care is necessary during exacerbations with respiratory failure. Comorbidity is frequent, particularly in elderly patients. Active physical training should be encouraged.

[1]*Department of Clinical Medicine, University of Bergen, Bergen, Norway.*

[2]*Department of Neurology, Haukeland University Hospital, Bergen, Norway.*

[3]*Department of Neurobiology, Hellenic Pasteur Institute and Tzartos NeuroDiagnostics, Athens, Greece.*

[4]*Istituto di Neurologia, Fondazione Policlinico A. Gemelli, IRCCS, Rome, Italy.*

[5]*Università Cattolica del Sacro Cuore, Rome, Italy.*

[6]*Nuffield Department of Clinical Neurosciences, University of Oxford, Hospitals Trust, Oxford, UK.*

[7]*Department of Neurology, University of Virginia, Charlottesville, VA, USA.*

[8]*Department of Neurology, Leiden University Medical Centre, Leiden, Netherlands.*

**e-mail: nils.gilhus@uib.no*

https://doi.org/10.1038/s41572-019-0079-y

Myasthenia gravis (MG) is an autoimmune disease that affects the postsynaptic membrane at the neuromuscular junction[1,2] (FIG. 1). The predominant manifestation is muscle weakness, which typically worsens with repeated muscle work such that function is usually the best in the morning, with more pronounced weakness at the end of the day. Permanent damage of muscles rarely occurs, and maximal muscle strength is often good. Muscle weakness differs between individual muscles and muscle groups. Extraocular muscles are frequently affected, usually asymmetrically, with typical symptoms being intermittent drooping of the upper eyelid (ptosis) and double vision (diplopia). Muscles innervated by the cranial nerves are often involved in MG, leading to reduced facial expression and speech and swallowing weakness. Oculobulbar muscle weakness is most common, but patients can develop more generalized MG, whereby proximal muscles of the extremities and the trunk — including the neck — are affected. Fifteen per cent of patients have ocular symptoms only, whereas 85% have more generalized MG including non-ocular muscle weakness[3]. Respiratory muscle weakness can occur infrequently, leading to a life-threatening condition that requires intensive care and respiratory support[4]. However, with adequate treatment, most patients with MG are in a stable condition with only mild muscle weakness and are fully capable of their daily functions[5]. Symptoms can fluctuate over time, but continuous disease progression does not occur in MG.

MG is caused by autoantibodies that bind to functionally important molecules at the postsynaptic membrane at the neuromuscular junction. Eighty per cent of patients with MG have detectable antibodies against the acetylcholine (ACh) receptor (AChR), whereas a small minority instead have antibodies against muscle-specific kinase (MuSK) or lipoprotein-receptor-related protein 4 (LRP4)[2]. The anti-LRP4 antibodies may be less MG-specific than those against AChR and MuSK, and this MG subgroup is less well established than the others. Antibodies are not detected in 10–15% of patients with generalized MG, usually because the sensitivity of the assay used is too low[6]. MG is classified into subgroups according to clinical manifestations, age at onset, the presence of autoantibody pattern and thymus pathology[1,2] (TABLE 1). These subgroups reflect differences in epidemiology, disease mechanisms, severity and therapeutic response and help guide personalized treatment. Ocular MG and MG with anti-LRP4 antibodies tend to be milder, whereas MuSK MG and probably also thymoma MG tend to be more severe[5].

The thymus has a key role in AChR-mediated MG[7], and thymectomy is a treatment option for patients with this subtype. MG is induced by a thymoma in 10% of patients, and thymectomy is a treatment option for patients with thymoma or thymic hyperplasia[2].

A major challenge in MG is to find therapies that prevent or cure the disease. Current treatments are either symptomatic or cause nonspecific immunosuppression. Although the pathogenesis of MG is well characterized and directly pathogenetic autoantibodies have been identified, treatments do not target the specific antibodies and usually do not induce a full remission without the need for further therapy.

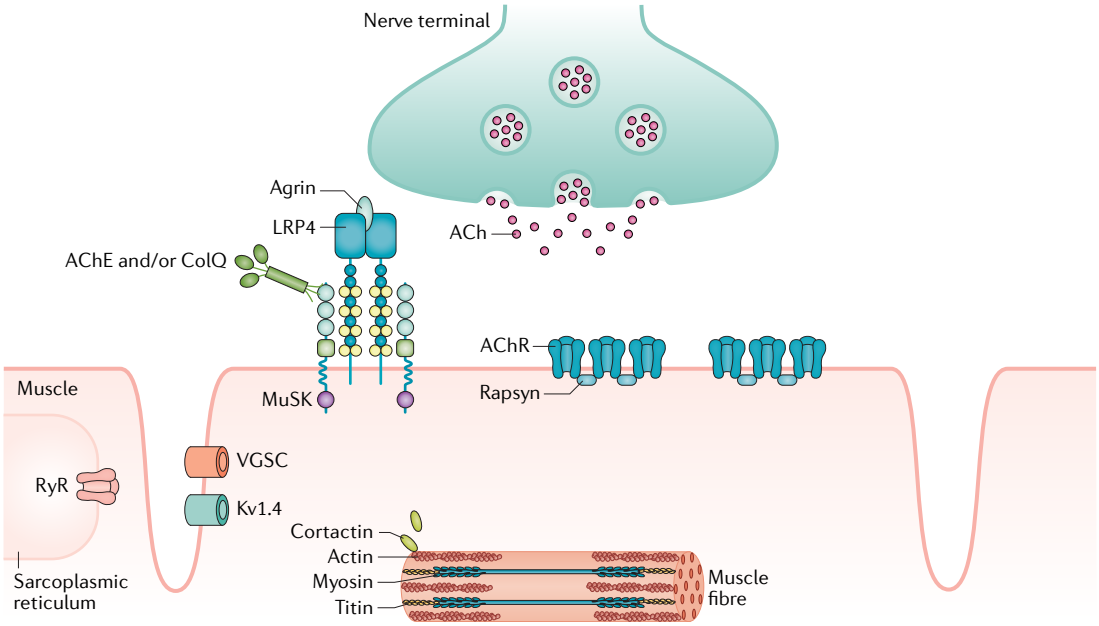

Fig. 1 | **Structure of the neuromuscular junction.** The neuromuscular junction comprises the presynaptic nerve terminal and the postsynaptic muscle cell. Agrin released from the nerve terminal binds to lipoprotein-receptor-related protein 4 (LRP4) and muscle-specific kinase (MuSK), leading to the activation of MuSK, which in turn causes clustering of the acetylcholine (ACh) receptors (AChRs), which is necessary for the maintenance of the postsynaptic structures. AChE, acetylcholinesterase; ColQ, collagen Q; Kv1.4, voltage-gated potassium channel; RyR, ryanodine receptor; VGSC, voltage-gated sodium channel.

This Primer describes the updated diagnostic and management guidelines of MG and discusses what to expect in the near future on the basis of new insights in disease mechanisms and the individual variability among patients.

Epidemiology

The overall prevalence of MG is 150–250 cases per million individuals, with an estimated annual incidence of 8–10 cases per million person-years[8]. These figures are similar in most examined populations[9–11]; however, the prevalence and incidence of each subgroup of MG vary markedly, partly owing to variation in demographics between countries.

In most populations, the age at onset of AChR MG has a bimodal pattern, with a lower peak at 30 years of age and a higher peak at 70–80 years of age[12]. In Europe, relatively more patients with MG have an onset after 50 years of age (and thus belong to the late-onset MG subgroup) than in Asia, Africa and South America[8]. In Japan, China and possibly in other countries in East Asia, juvenile MG with onset in early childhood is relatively more common. Indeed, a large proportion of Japanese and Chinese patients with MG have symptom onset before 8 years of age, with this age representing a third peak for onset age[13–15]. Juvenile MG tends to be of mild to moderate severity[16] and in China often has exclusively ocular manifestations[13]. Biomarkers do not differ between juvenile MG and early-onset MG; in general, the vast majority of patients have anti-AChR antibodies[13]. In Japan, but not in China, human leukocyte antigen (HLA) associations have been reported to differ in those with juvenile MG compared with early-onset MG, with onset at a higher age[15].

MG with MuSK antibodies (that is, MuSK MG) has a geographically distinct epidemiology. In Europe, MuSK MG appears to follow a south–north gradient and is most common in Mediterranean countries but is very rare in Scandinavia[2,17]. However, in China, MuSK MG is more common in the north[18]. A latitude-related factor, such as climate, is therefore unlikely to be causative for MuSK MG. Several HLA alleles, such as *HLADQB1*05*, *HLADRB1*14* and *HLADRB1*16*, are associated with an increased risk of MuSK MG[19]. Indeed, the geographical distribution of the predisposing HLA genes for MuSK MG parallels the prevalence of this disease[19]. Individuals with African genetic ancestry and severe, anti-AChR-antibody-negative MG are likely to have MuSK MG[20].

MG with LRP4 antibodies (LRP4 MG) detected using a sensitive assay was found in 7–33% of patients who did not have anti-AChR or anti-MuSK antibodies[21]. In this study, the proportion of patients with MG and anti-LRP4 antibodies was high in Poland (33%), Greece (27%) and the Netherlands (26%), with a low proportion in Turkey (7%) and Norway (7%)[21]. Thus, there was no distinct geographical pattern within Europe, with no similarity to the pattern for AChR MG or MuSK MG. The proportion of patients with LRP4 MG is low in China (7%), Japan (3%)[6,22] and the United States (10%)[23]. Variation between studies is probably in part due to variation in test sensitivity.

It has not been possible to identify MG clusters in location and time that could have helped in identifying causative factors. Migration studies show similar MG prevalence in the examined populations and no marked change in risk due to emigration[24]. Such studies have therefore failed to identify potential causative agents.

Table 1 | **Classification of MG subgroups**

Subgroup	Autoantibody	Age at onset	Thymus abnormalities
Early-onset MG[a]	AChR	<50 years of age	Hyperplasia common
Late-onset MG	AChR	>50 years of age	Atrophy common
Thymoma MG	AChR	Any	Type AB and B thymoma
MuSK MG	MuSK	Any	Normal
LRP4 MG	LRP4	Any	Normal
Seronegative MG	None detected	Any	Variable
Ocular MG[b]	AChR, MuSK, LRP4 or none	Any	Variable

AChR, acetylcholine receptor; LRP4, lipoprotein-receptor-related protein 4; MG, myasthenia gravis; MuSK, muscle-specific kinase. [a]Juvenile MG is not considered a separate subgroup and is part of early-onset MG. All patients at one time point can belong only to one subgroup. [b]Ocular MG includes the patients with ocular symptoms only and no clinical weakness in other muscles.

The prevalence of MG seems to be higher today than decades ago; however, it has not been proved that the true age-adjusted incidence of MG has changed. Several factors might have contributed to this increase in prevalence. For example, the treatment of MG has improved over time, such that life expectancy is now near normal in developed countries, whereas MG led to markedly increased mortality until a few decades ago[25,26]. A relative mortality of 1.41 was demonstrated for AChR MG in individuals diagnosed between 1985 and 2005 in Denmark[26]. A study in Norway did not find any increased mortality in patients with MG compared with controls after 1995 (REF.[25]). MG-associated thymomas increase the mortality, as does severe autoimmune comorbidity[27]. In addition, MG case-finding has improved owing to the widespread use of sensitive tests for MG-specific autoantibodies. Some studies have suggested a true increase in incidence, particularly for late-onset MG[11], including a recent study from Japan[28]. However, no factors have been suggested that might explain such an increase in incidence. Conversely, a nationwide registry-based study from Denmark found no variation in the incidence of MG in 1996–2009 (REF.[29]). In this study, the annual incidence rate for late-onset MG was 18.9 per million person-years and was 4.2 per million person-years for early-onset MG. Similar results were observed in Norway combining multiple disease registries[12] and in a Dutch–Norwegian study[30]. The prevalence of MG in Chile has been reported to be low but within the range described worldwide[31].

Risk factors

Both predisposing genetic factors and environmental factors play crucial roles in the induction of MG. Indeed, MG has a concordance of 35% in monozygotic twins and 5% in heterozygous twins[32], illustrating the role of both genetic and environmental factors as major contributors to MG risk. Many genes contribute to the MG risk, including *HLA* genes, *PTPN22*, *CTLA4*, *IL1B*, *IL10*, *TNF*, *IFNG*, *CD86*, *AKAP12*, *VAV1*, *TNFSF13B* (also known as *BAFF*) and *TNIP1* (REF.[2]). Some of these genes are linked to autoimmunity in general, but others have a more specific association to MG and MG subgroups (such as *HLADRB1*1501, HLADQ5* and *CTLA4* polymorphisms[2,33]). Genetic variation in the promoter region of *CHRNA1* (encoding the AChR α-subunit) can increase the risk of MG[34].

MicroRNAs mediate post-transcriptional gene silencing and are dysregulated in several autoimmune diseases. A reduction in microRNAs in peripheral blood lymphocytes from patients with MG correlated with an increase in pro-inflammatory cytokines[35]. Examples of dysregulated microRNAs in MG include miR-150-5p, miR-21-5p and let-7, which depend on both MG subgroup and ongoing immunosuppression. AChR antibody MG has elevated levels of miR-150-5p and miR-21-5p, whereas the let-7 family is upregulated in MuSK MG[35].

Sex hormones seem to play a role in MG predisposition, and the involvement of these hormones could explain the different sex ratio in early-onset and late-onset MG and the higher frequency of MG among young females and postpartum[2,8,36]. Indeed, early-onset MG is three times as common in females than in males, whereas late-onset MG is slightly more common in males. Thymic hyperplasia primarily affects young females[2,7], suggesting that hormones have a role in MG pathogenesis and even may influence the response to therapy such as thymectomy. Oestrogens can influence anti-inflammatory and pro-inflammatory responses, depending on their dose, timing and the microenvironment[37]. Moreover, oestrogen and testosterone might affect the expression of thymic transcription factors such as autoimmunie regulator (AIRE) and therefore the risk of developing MG with AChR antibodies[38].

Environmental risk factors for MG are nearly completely unknown. The thymus is sensitive to infections, and involvement of an infectious agent in MG pathogenesis is possible. B cells infected with Epstein–Barr virus were reported in the thymus of patients with MG, but this finding was not confirmed in later control studies[39,40]. Other viruses, such as West Nile virus and Zika virus, have also been associated with MG[41]. Cancer immunotherapy can trigger MG, in addition to other autoimmune and rheumatic diseases[42,43]. This association is especially true for immune checkpoint inhibitors of programmed cell death and cytotoxic T lymphocyte associated protein 4 (REFS[42,43]).

Mechanisms/pathophysiology

MG is the most studied and best understood autoantibody-mediated neurological disease. The induction of experimental MG in rabbits by immunization with muscle-type AChR[44] was already observed in 1973, followed by the identification of anti-AChR antibodies in patients with MG a few years later[44,45], and the transmission of MG by immunoglobulin G (IgG) from patients[46]. MG-associated autoantibodies can be classified into two major groups: those to transmembrane or extracellular autoantigens and those to intracellular autoantigens. Some of the antibodies are clearly pathogenetic, whereas others are most probably not.

Transmembrane or extracellular proteins

Antibodies against extracellular or transmembrane proteins are pathogenetic for MG and either directly (such as anti-AChR antibodies) or indirectly (such as anti-MuSK antibodies and anti-LRP4 antibodies) affect

AChR function at the neuromuscular junction, leading to impairment of ionic transport across the muscle membrane and reduced muscle contraction (FIG. 1).

AChR. Nicotinic AChR of the muscle is the most common autoantigen in MG and is concentrated at the tips of the folds of the postsynaptic membrane[2,44,47]. The nicotinic AChR is a transmembrane pentameric glycoprotein of 250 kDa and is composed of two α1-subunits, one β1-subunit, one δ-subunit and either one γ-subunit (in the embryonic AChR) or ε-subunit (in the adult AChR) (FIG. 2). The subunits form the cation channel, which opens with ACh binding to the two binding sites on the α1-subunits, to allow cation (Na^+, Ca^{2+} and K^+) translocation across the membrane[48] (FIG. 2a).

Anti-AChR antibodies are detected in 80% of patients with MG[1]. The anti-AChR response is polyclonal, with antibodies binding to extracellular domains of the AChR; therefore, these antibodies can impair signal transduction. The epitopes for most anti-AChR antibodies are conformational (that is, they depend on the exact 3D structure of the AChR molecule in vivo), which hinders epitope studies, yet a main immunogenic region (MIR) has been identified as the target for >50% of antibodies[49]. The MIR represents a group of overlapping epitopes around the AChR central core that are formed by the amino acids α1(67–76) of the α-subunits[50,51]. Anti-MIR antibodies are highly pathogenetic in model systems[50].

One important mechanism for the pathogenetic effect of anti-AChR antibodies is through complement activation. Most antibodies are capable of activating the complement cascade upon antigen binding, leading to the formation of the associated membrane attack complex and damage of the postsynaptic membrane[52] (FIG. 3). A second important pathogenetic mechanism is through antigenic modulation, with acceleration of AChR internalization and destruction mediated by the crosslinking of AChRs by bivalent antibodies[53]. The antigenic crosslinking leads to a loss of AChR at the postsynaptic membrane. This loss is not fully compensated for by the increased AChR synthesis that occurs as a response to the increased autoantibody-induced AChR degradation. Infrequently, some anti-AChR antibodies block the ACh binding site and thereby AChR signalling[47,54,55]. Anti-AChR antibodies that target the AChR α-subunit are more pathogenetic for MG than antibodies that target other AChR subunits, and their epitope pattern influences disease severity[53].

MuSK. MuSK is a transmembrane single-subunit protein that is responsible for the clustering of AChR at the neuromuscular junction and the maintenance of the postsynaptic membrane[56] (FIG. 2b). MuSK is activated through phosphorylation induced by the LRP4–agrin complex, after which AChR clustering is induced (FIG. 1). The process of AChR clustering involves the protein rapsyn, a scaffold protein that bridges the AChR with the cytoskeleton[57].

Anti-MuSK antibodies are detected in 1–10% of patients with MG[47,54,58]. Most anti-MuSK antibodies belong to the IgG4 subclass, which is unable to activate complement and is unable to induce antigenic modulation because they are functionally monovalent[59]. Thus, their mode of action differs from that of the AChR antibodies. Anti-MuSK antibodies mask binding sites on MuSK that allow interactions with

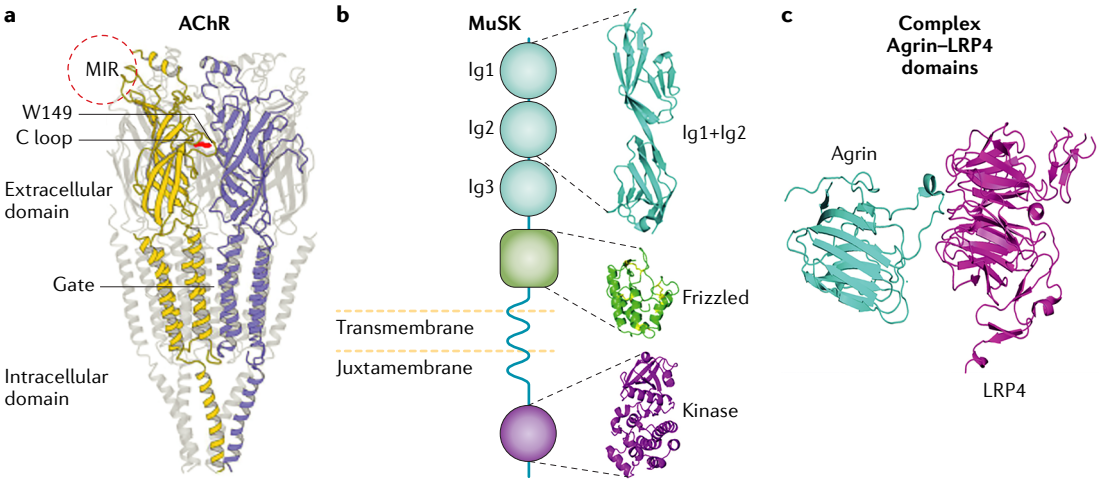

Fig. 2 | **Structures of the main autoantigens in MG. a** | The structure of the Torpedo (a fish, the Pacific electric ray) acetylcholine receptor (AChR), the only available structure of the intact muscle-type AChR, is shown[205]; the site of one of the two main immunogenic regions (MIRs) is marked on the top left. **b** | A schematic drawing of muscle-specific kinase (MuSK) is shown on the left, with domains of known structures that interact with other key proteins on the right[206]. **c** | Lipoprotein-receptor-related protein 4 (LRP4)–agrin complex domains. LRP4 binds to the extracellular matrix proteoglycan agrin[207], triggering MuSK activation and the signalling cascade leading to AChR clustering and postsynaptic differentiation. Ig, immunoglobulin; MG, myasthenia gravis. Part **a** adapted with permission from Unwin, N. Nicotinic acetylcholine receptor and the structural basis of neuromuscular transmission: insights from Torpedo postsynaptic membranes. *Q. Rev. Biophys.* **46**(4), 283–322 (2013). Part **b** adapted from REF.[54], Springer Nature Limited, and with permission from REF.[206], Elsevier. Part **c** adapted from REF.[54], Springer Nature Limited, and with permission from Zong, Y. N. et al. Structural basis of agrin LRP4 MuSK signaling. *Genes Dev.* **26**, 247–258 (2012). © Cold Spring Harbor Laboratory Press.

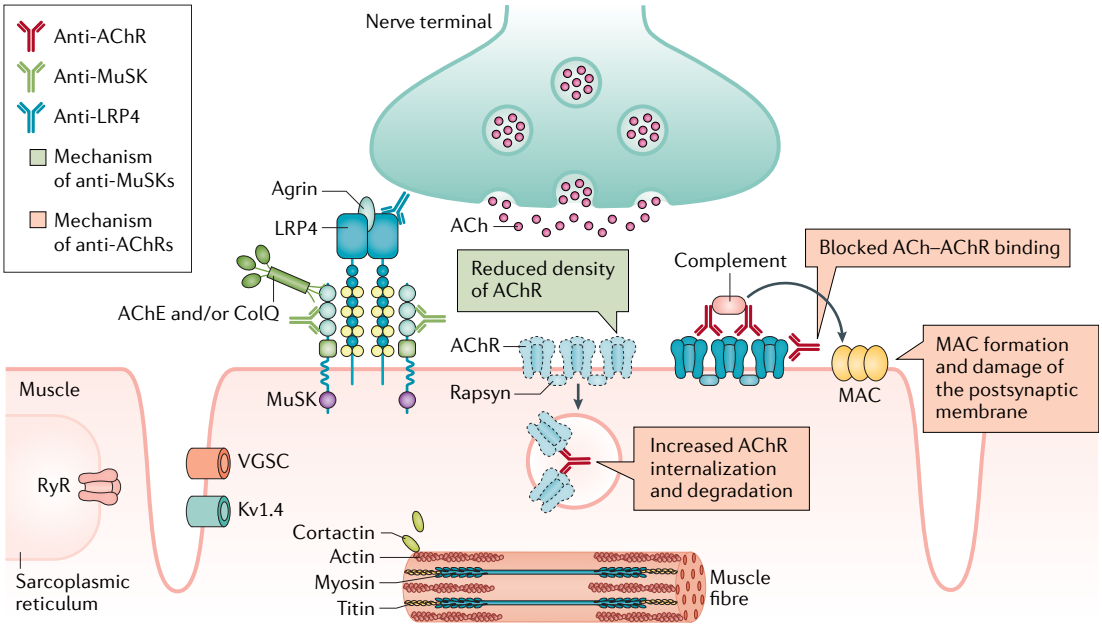

Fig. 3 | **Pathophysiology of MG at the neuromuscular junction.** Anti-acetylcholine (ACh) receptor (AChR) antibodies activate complement, leading to damage of the postsynaptic membrane at the neuromuscular junction through production of the membrane attack complex (MAC). Anti-AChR antibodies can also crosslink AChRs, leading to their accelerated internalization and degradation rate. Some antibodies can directly block the ACh binding site. Anti-muscle-specific kinase (MuSK) antibodies do not activate complement and typically prevent the interaction of MuSK and lipoprotein-receptor-related protein 4 (LRP4), among other proteins, leading to reduced AChR clustering on the postsynaptic membrane. The pathogenicity of anti-LRP4 antibodies in myasthenia gravis (MG) remains to be established. Additional antibodies, such as anti-collagen Q (ColQ), anti-titin, anti-ryanodine receptor (RyR), anti-cortactin and anti-voltage-gated potassium channel (Kv1.4) have been demonstrated in patients with MG, although any pathogenetic significance remains unknown. AChE, acetylcholinesterase; VGSC, voltage-gated sodium channel.

its binding proteins, including LRP4 and collagen Q (ColQ), thereby inactivating MuSK[60]. Inactivating MuSK leads to a reduced postsynaptic density of AChRs and impairs their alignment in the postsynaptic membrane[59]. Most anti-MuSK antibodies bind to the immunoglobulin-like domains of MuSK[58,61]. Changes in MuSK antibody titre over time within patients usually reflect disease activity[17]. Patients with MG who are double seropositive for both MuSK and AChR antibodies are exceptionally rare[62].

LRP4. LRP4 is a single-subunit transmembrane protein with a large extracellular domain that contains multiple low-density lipoprotein repeats[63]. In adult skeletal muscle, LRP4 is concentrated at the neuromuscular junction, where it binds to agrin normally secreted from the nerves. As previously mentioned, the LRP4–agrin complex triggers MuSK activation (FIG. 2c).

Anti-LRP4 antibodies in patients with MG have been detected with various frequencies, depending on the assay used and the examined population[6,21,64]. In the largest study, 19% of patients with MG who are double seronegative (that is, those with neither anti-AChR nor anti-MuSK antibodies) had anti-LRP4 antibodies (range of 7–33% in the ten participating countries)[21]. In addition, anti-LRP4 antibodies were identified in 8% of patients with anti-AChR antibodies, in 15% of those with anti-MuSK antibodies, in 4% of patients with other neuroimmune diseases and in no healthy controls ($n = 56$)[21]. Interestingly, anti-LRP4 antibodies have been detected in 10–23% of patients with amyotrophic lateral sclerosis (ALS)[65]. The IgG1 subclass is predominant both in MG and ALS and is capable of complement binding[21]. The pathogenicity of anti-LRP4 antibodies in MG remains to be fully established.

Anti-LRP4 antibodies are pathogenetic in LRP4-immunized mice and induce muscle weakness[66]. The antibodies exerted their action through the disruption of the interaction between LRP4 and agrin, leading to inhibition of AChR-mediated neuromuscular transmission in this model system through inhibited agrin-induced MuSK activation and AChR clustering[66]. In addition, complement and IgG deposits at the neuromuscular junction might contribute to pathogenicity[67]. The disease in mice immunized with LRP4 was similar to that seen in mice immunized with AChR and with MuSK[68]. To date, no induction of MG in animals by passive transfer of human anti-LRP4 antibodies has been shown.

Agrin. Agrin binds to proteins in the muscle membrane, such as LRP4, dystroglycan and laminin, thereby regulating the formation, maintenance and regeneration of the neuromuscular junction[69]. Anti-agrin autoantibodies are detected in some patients with MG, in those with or without anti-AChR antibodies[70]. Such antibodies inhibit MuSK phosphorylation and AChR clustering in vitro[70]. Mice immunized with neural agrin showed similarities to human MG, with muscle weakness[71]. However,

whether anti-agrin antibodies play any pathogenetic role in the human disease is unclear.

ColQ. ColQ concentrates and anchors acetylcholinesterase (which breaks down ACh) in the extracellular matrix of the neuromuscular junction. Anti-ColQ antibodies have been detected in the serum of 3% of patients with MG (including in five patients seronegative for anti-AChR and anti-MuSK antibodies) and in 2.3% of healthy controls[72]. Any pathogenetic role of these antibodies has not been shown. Mutations in ColQ can lead to myasthenic syndromes.

Kv1.4. Antibodies against the α-subunit of the voltage-gated potassium channel Kv1.4 in skeletal muscle have been detected in 10–20% of Japanese and European patients with MG[73,74]. Kv1.4 channels are concentrated in axonal membranes and are also found in the endocardium[75]. Anti-Kv1.4 antibodies might cross-react with voltage-gated potassium channels in heart muscle in patients with MG. In the Japanese patients, anti-Kv1.4 antibodies were associated with severe MG and cardiac complications, although these complications were not observed in the European patients[73,74]. At present, anti-agrin, anti-ColQ and anti-Kv1.4 antibodies have no role in the clinic.

Intracellular proteins

Antibodies against intracellular antigens are unlikely to be pathogenetic for MG but are clinically useful as markers for MG characteristics, such as disease severity, presence of thymoma and myopathy, particularly anti-titin antibodies.

Titin. Titin is abundant in skeletal muscle cells and is essential for muscle contractility[76] (FIG. 1). Anti-titin antibodies are detected in 20–30% of patients with MG and anti-AChR antibodies, mostly in those with thymoma or late-onset MG[77,78]. In fact, anti-titin antibodies are a marker of thymoma in early-onset MG, with both a sensitivity and specificity of ~90%[77]. The presence of these antibodies indicates a more-severe form of MG associated with mild myopathy. Most anti-titin antibodies bind to a region located near the A–I junction in muscle[79].

Ryanodine receptor. The ryanodine receptor (RyR) is the calcium channel in the sarcoplasmic reticulum. This channel opens upon sarcolemma depolarization and is involved in muscle contraction through the release of calcium from the sarcolemma into the cytoplasm[80]. Anti-RyR antibodies are present in 70% of thymoma MG and in 14% of patients with late-onset MG[77,81]. The presence of anti-RyR antibodies is a marker of thymoma and indicates severe MG[77,82].

Cortactin. Cortactin binds to actin in skeletal muscle, promotes actin assembly and is involved in AChR clustering mediated by MuSK[83]. Anti-cortactin antibodies have been detected in 20% of double-seronegative patients with MG and in 5–10% of patients with AChR MG. However, these antibodies were also detected in up to 5% of healthy controls and in 10–15% of patients with other autoimmune disorders[83,84], including 20% of patients with polymyositis[85]. This lack of specificity makes them inadequate for diagnostic purposes, and these antibodies are at present not used as biomarkers for MG.

Mechanisms of autoantibody production

The mechanisms leading to the selective production of muscle autoantibodies in MG are unclear. The thymus is affected in most patients with AChR MG, with thymoma in 10% of patients or with thymic follicular hyperplasia in >80% of patients with early-onset MG[7,86]. Thymectomy for patients with hyperplasia often results in considerable clinical improvement[87]. The hyperplasia is characterized by the presence of a high number of germinal centres (sites of B cell development and maturation)[7]. Germinal centres are normally found in B cell-producing organs, are almost absent in the normal thymus and are not present in skeletal muscle[7]. Thus, the thymus seems to be the inflamed tissue in AChR MG. Indeed, the presence of many germinal centres with anti-AChR-antibody-producing B cells in the thymus of patients with AChR MG supports that the thymus is the site responsible for the loss of immune tolerance to AChR[7,37,88]. Thymus epithelial cells, myoid cells and professional antigen-presenting cells all contribute in the immunization process, leading to the formation of germinal centres. This immunization has been elucidated in some detail, but not yet so that prevention or inhibition is possible[7,77,89].

The thymus is the organ of T cell maturation and is responsible for the development of central tolerance by the deletion of self-reactive T cells[77,90] (FIG. 4). Self-reactive T cells that escape central tolerance are normally controlled by regulatory T (T_{reg}) cells and through peripheral tolerance (FIG. 4). Such self-reactive T cells directed against muscle antigens can be detected in all MG subgroups but also in healthy controls. CD8-positive ($CD8^+$) T cells represent important players during the initiation of MG[91]. The immunoregulatory defects that are observed in patients with AChR and MuSK MG are due to the impairment of both T_{reg} cells and conventional cells[37,92]. Indeed, T_{reg} cell defects have been demonstrated in several autoimmune diseases[93], including MG with thymic hyperplasia[94]; the changes in T_{reg} cell markers are moderate, but the suppressor function of these cells is impaired[37]. However, whether T_{reg} cell dysfunction is a primary causal event or is a result of disease development, defective T_{reg} cells should be important for MG initiation or progression[95]. A T cell subset expressing high levels of Fas is strongly enriched in the thymus and participates in the AChR response[37]. In thymoma MG, we strongly believe that the tumour induces the loss of tolerance to AChR. The lack of intratumour myoid cells, T_{reg} cells and AIRE expression is likely to result in abnormal T cell selection[96]. Additional regulatory factors, such as interferons, other cytokines and major histocompatibility complex (MHC) class II antigens, are important in thymoma MG[37], as symptomatic MG in most patients develops a long time after tumour development. In late-onset MG, the role of the thymus is unclear, as the organ is without detectable inflammation[7]. The thymus probably has no distinct role in MuSK MG[97].

Diagnosis, screening and prevention

Clinical features

The diagnosis of MG should start with a clinical picture compatible with the disorder (FIG. 5). The typical feature is muscle weakness, which increases with repetitive muscle use and as the day progresses. Ocular muscles are commonly involved with diplopia and ptosis caused by weakness of the extraocular muscles and the levator palpebrae superioris, respectively (FIG. 6). Ocular muscle weakness is often asymmetrical. The facial, neck, limb and truncal muscles can be involved in those with generalized disease and nearly always symmetrically. Bulbar and respiratory weakness can be life threatening and requires intensive care support.

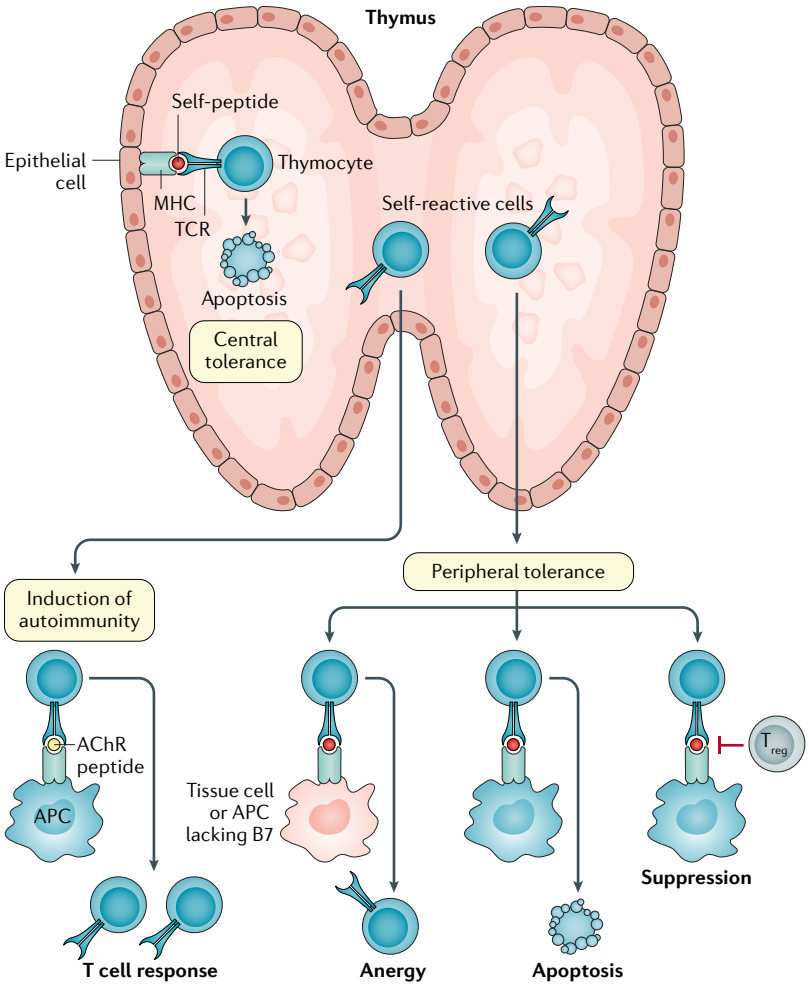

Fig. 4 | **The process of normal immune tolerance.** The recognition of self-antigens in the thymus is facilitated by multigene transcription factors, such as AIRE (autoimmune regulator), which is expressed in thymic medulla. AIRE leads to the expression of major peripheral proteins on the surface of thymic epithelial cells, after which T cells that recognize these proteins (or 'self-antigens') are targeted for negative selection and undergo apoptosis. Self-reactive T cells that escape central tolerance enter the periphery, where they can undergo apoptosis, enter into a state of anergy or undergo suppression (peripheral tolerance). The central and peripheral tolerance of T and B cells to self-antigens is crucial for health and development. In myasthenia gravis, there is a failure of central tolerance, occurring within the thymus in most patients, leading to the development of self-reactive cells. AChR, acetylcholine receptor; APC, antigen-presenting cell; MHC, major histocompatibility complex; TCR, T cell receptor; T_{reg} cell, regulatory T cell.

The clinical examination is expected to support the clinical history by demonstrating muscle weakness associated with impairment in specific tasks. If MG is mild, muscle weakness is apparent only when muscles are fatigued, such as the development of ptosis after sustained upgaze or arm weakness after prolonged exercise, in studies sometimes defined as 4 minutes of full arm abductions or 20 abductions. Muscle fatigability may become apparent during the consultation and, in particular, speech may become progressively worse in some patients. Patients with MG can have a completely normal clinical examination.

Ptosis can be isolated or associated with eye movement abnormalities. The diagnosis of ocular MG can be challenging, as this disorder can occur in patients with negative antibody assays and normal neurophysiological investigations. However, other clinical tests can support a diagnosis of ocular MG, for example, Cogan's lid twitch test sign (a brief overshoot twitch of the eyelid when downgaze is followed by the return of gaze to the primary position) has a sensitivity of 50–75% and specificity of >90% for MG, similar for all groups of MG but diagnostically most important in the ocular subgroup[98,99]. This sign is not always associated with symptomatic ptosis. The predictive value of the test depends on the population tested and may be lower in clinical practice and in ophthalmology clinics than in neurology clinics. In addition, an improvement of >2 mm in ptosis after 5 minutes of orbital cooling using an ice pack is indicative of MG, with a sensitivity and specificity of >90%[100,101]. Along the same lines as this test, an iced drink test for bulbar myasthenia has been proposed[101]. These cooling tests accord with the worsening of MG symptoms with heat. The 'curtain sign' describes the worsening of ptosis in the least-affected eye when the lid of the most-severely affected eye is lifted, typical for MG. In addition, furrowing of the frontalis and an eyebrow lift can be seen. Functional ptosis not due to any neuromuscular deficit is commonly misdiagnosed as MG but should be identified by noting that the eyebrow of the affected eye is lower than the eyebrow of the unaffected side, whereas the opposite is typical in MG (with elevation of the eyebrow in an effort to lift the lid). In functional ptosis in those without MG, the tarsal skin fold of the upper eyelid is also still visible, and the distance between the tarsal fold and the eyelashes is not increased.

MG muscle weakness can be immediately reversed by the intravenous administration of a fast-acting acetylcholinesterase inhibitor and represents a specific and sensitive diagnostic test in patients with MG who have observable pareses. All individuals will experience cholinergic adverse effects, therefore there is a marked placebo effect on subjective well-being, hence the reason why objective pareses such as, for example, ptosis are needed as a parameter. Similarly, the response to peroral acetylcholinesterase inhibitory treatment provides diagnostic information.

Antibody tests

All patients with a clinical history suggestive of MG should be tested for antibodies. Most patients have anti-AChR antibodies, and those without will have anti-MuSK antibodies, anti-LRP4 antibodies, antibodies to

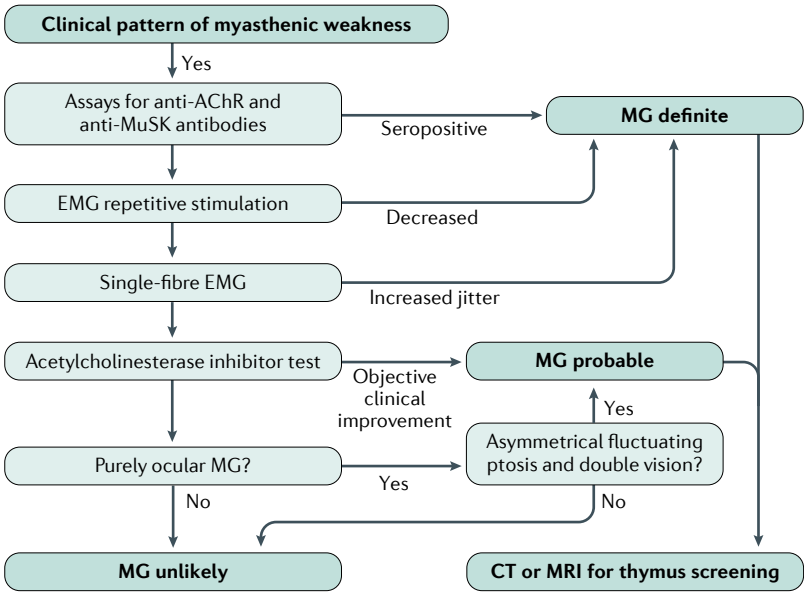

Fig. 5 | **A simplified diagnostic algorithm for MG.** Evaluation of clinical symptoms and signs, antibody testing, neurophysiology, thymus imaging and response to therapy are key elements in the diagnostic work-up of individuals with suspected myasthenia gravis (MG). AChR, acetylcholine receptor; EMG, electromyography; MuSK, muscle-specific kinase.

little-studied or unidentified antigens or, in a minority, no detectable muscle antibodies at all. Nearly all patients with MG with thymoma have detectable anti-AChR antibodies[102]. Anti-AChR and anti-MuSK antibodies are tested routinely, and all patients with symptoms and clinical signs indicating MG without anti-AChR antibodies should be tested for anti-MuSK antibodies.

The specificity and relevance of anti-agrin, anti-ColQ and anti-Kv1.4 antibodies are still unclear. Standardized assays that have been evaluated in large patient cohorts are needed for these antibodies and for anti-LRP4 antibodies. Antibodies to intracellular antigens are also detected but almost exclusively in patients with AChR MG[47,103].

Anti-AChR assays. The anti-AChR antibody assay offered by most diagnostic laboratories is a radioimmunoassay kit with almost 100% specificity[2]. When this test is positive in individuals with muscle weakness, no further diagnostic tests are required and a diagnosis of MG can be made. However, an unexpected positive result should be retested as technical mistakes always can appear in biomarker tests. Anti-AChR antibodies are also detected by commercial enzyme-linked immunosorbent assay (ELISA) kits, which are easier to use for most diagnostic laboratories. These ELISAs have acceptable sensitivity and specificity for MG and provide anti-AChR antibody titres equivalent to the radioimmunoassay. However, they are inferior to radioimmunoassay in terms of both sensitivity and specificity[6,47]. A supplementary test if standard tests for AChR and MuSK antibodies are negative is a live-cell-based assay, in which AChRs are clustered[6,47,104]. Anti-AChR antibodies that have a too low affinity for soluble AChR can be detected when AChR antigen is expressed in a more native form and at higher concentration. However, this test is not routinely available, as no commercial kits have been produced and the test procedure is complicated. Patients with MG and anti-AChR antibodies that are detected using cell-based assays often have only milder MG with a better prognosis than patients with MG with AChR antibodies detected by standard tests[105]. The aim is to develop more-sensitive tests for MG, but maintaining the specificity is highly important.

Anti-MuSK assays. IgG4 antibodies against MuSK should be tested using a specific radioimmunoassay or ELISA in patients lacking anti-AChR antibodies. MuSK MG usually has more bulbar involvement than AChR MG, although other phenotypes, including ocular MG, can occur[17]. The MuSK radioimmunoassay is sensitive and detects low antibody concentrations, although some anti-MuSK antibodies are conformation-dependent and do not bind to the soluble MuSK extracellular domain used in this assay[106]. An anti-MuSK cell-based assay has the advantage of detecting such conformation-dependent antibodies, but the challenge is to keep the same specificity as for the radioimunoassay[106,107]. The selective detection of only the IgG MuSK antibodies (exclusion of the IgM antibodies) increases the specificity of this assay[108].

Other assays. The presence of IgG antibodies against LRP4 has been confirmed by several laboratories, and diagnostic assays that detect these antibodies may soon become available. Anti-LRP4 antibodies are usually detected by cell-based assays that express the whole recombinant protein[21]. ELISAs are also used[23], although the degree of correlation between the cell-based assays and the ELISAs is not yet clear. LRP4 is a large protein with some unidentified epitopes, and testing for antibodies against only a few peptides is inappropriate. Detection of anti-LRP4 antibodies is not pathognomonic for MG, as they have been detected in some patients with other disorders as well[21,23,65].

Anti-titin antibodies are often detected by a commercial ELISA. A more-sensitive radioimmunoassay can detect anti-titin antibodies in anti-AChR-antibody-seronegative patients with MG[103], but this assay is not yet routinely available. Anti-titin antibody testing can be valuable in all patients with MG and anti-AChR antibodies. For example, anti-titin antibodies in patients with early-onset MG indicate the presence of a thymoma[54], and such antibodies can indicate more-severe MG in all MG subgroups[54].

Anti-RyR antibodies can indicate the presence of thymoma and severe MG[77]. Although tests for anti-RyR antibodies are offered by a few diagnostic laboratories, no commercial kits are available and these antibodies are rarely tested for in clinical practice.

Neurophysiological tests

Neurophysiological testing is important when antibody tests are negative and confirmation of a primary neuromuscular junction disorder is required.

Repetitive nerve stimulation at a frequency of 3 Hz shows a gradual decline in the compound muscle action potential (CMAP) in patients with MG. A smooth decrease in CMAP on a stable baseline is specific for MG. A decrement of >10% from the first to the fourth

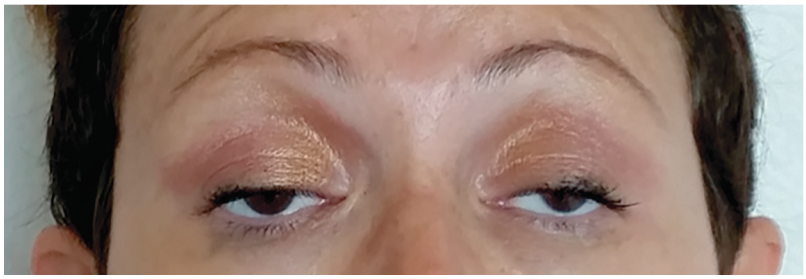

Fig. 6 | **Typical asymmetrical bilateral ptosis in a patient with MG.** Ptosis (that is, drooping of the upper eyelid) is common in patients with myasthenia gravis (MG).

CMAP is regarded as abnormal[109]. All tests should be performed in weak muscles, as results can be normal in strong and unaffected muscles. However, results from repetitive nerve stimulation can be normal even in patients with severe MG[109]. The overall sensitivity of repetitive nerve stimulation in generalized MG is reported to be up to 80%, but lower immediately after an acute onset[109], whereas the sensitivity is 50% in MuSK MG[110]. The LRP4 MG subgroup has rarely pathological neurophysiological tests[111]. Repetitive nerve stimulation represents a fast and non-invasive procedure.

Single-fibre electromyography (SFEMG) is more sensitive but less specific for MG than repetitive nerve stimulation[112]. SFEMG abnormalities can be observed in other neuromuscular conditions — notably mitochondrial disorders and motor neuron disease. Two types of SFEMG are used to diagnose MG: voluntary SFEMG measures the variability in activation time ('jitter') between muscle fibres that are innervated by the same motor axon when the patient voluntary contracts the muscle, whereas stimulation SFEMG measures variability between the time of nerve stimulation and muscle response ('jitter')[113]. The use of concentric needles has been validated for both methods. Experience is needed for SFEMG, and there are global differences regarding its use[112]. SFEMG also shows a high sensitivity in MuSK MG and represents an important test in such patients[110]. A mildly increased jitter only should be judged with caution.

Differential diagnoses and comorbidities

MG needs to be differentiated from the much rarer congenital myasthenic syndromes. These syndromes usually present in infancy and can show a family history[114]. However, some syndromes present later in childhood or in early adulthood, and as the majority are autosomal recessive disorders, familial involvement is often lacking. Useful features that can differentiate MG from these syndromes include the presence in MG of asymmetry of ptosis and eye movements, no ankle dorsiflexion weakness and a positive response to immunotherapies. Symptom worsening with pyridostigmine treatment can indicate a congenital myasthenic syndrome — notably the slow channel, ColQ or DOK7 syndromes[115].

Lambert–Eaton myasthenic syndrome (LEMS) causes weakness owing to dysfunction of the neuromuscular junction and is associated with antibodies against presynaptic voltage-gated calcium channels in >90% of cases[116]. Approximately 60% of patients have a malignancy, usually small-cell lung carcinoma[117]. Symptoms usually differ from MG, with muscle weakness, autonomic dysfunction and weak tendon reflexes predominating. Muscle weakness occurs proximally in the limbs with worse weakness in the legs, has relative sparing of the ocular muscles and has less fatigue and fluctuations than in MG. Neurophysiological testing characteristically shows reduced CMAPs, reductions with repetitive nerve stimulation at low frequency but an increase (by usually >100%) at high-frequency stimulation (20 Hz) or with exercise[118]. This increase can be observed when retesting strength in a weak muscle before and immediately after sustained muscle contraction in some patients with LEMS, but only very rarely in MG. Antibodies against voltage-gated calcium channels are diagnostic for LEMS but are not completely specific[119].

Other differential disorders include fatigue syndromes with major psychiatric or social aspects and postinfectious conditions. The history and impairment in such syndromes are usually out of keeping with the examination, and investigations will be normal. In MG, symptoms are more distinct, and this will usually be clear with an examination aimed at elucidating the typical MG manifestations. Other neuromuscular disorders can be differentiated by specialized investigations, which can be challenging in some children but are usually straightforward in adults. Thyroid disorders including thyroiditis with hyperthyreosis or hypothyreosis can represent both a differential diagnosis and a comorbidity of MG. Aside from ptosis, extraocular muscle involvement is typical for Graves disease[120]. The differential diagnosis of MG or other muscle disease is usually obvious after a careful clinical examination and ancillary tests. Motor neuron disease with predominant bulbar impairment represents another differential diagnosis, rarely also cerebrovascular brainstem disease.

Comorbidities of MG can represent diagnostic challenges, as it is sometimes difficult to judge whether a reduction in function, lack of quality of life and a general feeling of weakness are due to MG exacerbation, comorbid conditions or a combination of the two[27]. MG should be actively examined for in all patients with a thymoma, as one-third of all patients with thymoma have MG[7]. This examination should encompass a detailed patient history, clinical examination and testing for AChR antibodies. Patients with MG with a thymoma are as a rule AChR-antibody-positive, and thymoma patients without any clinical symptoms can have such antibodies[2].

Management

The management of MG is directed at restoring patients' muscle strength and well-being through the control of disease activity, the monitoring of treatment-related adverse events and individualized supportive measures. Disease-specific treatment generally consists of the combined use of symptomatic treatment and immunosuppressive therapies, with short-term treatments, thymectomy and monoclonal antibodies in selected patients (FIG. 7). Current knowledge of MG pathophysiology favours a personalized treatment strategy that takes into consideration disease subtyping according to thymus pathology and associated antibodies[2] (TABLE 1), weakness distribution and severity, patient

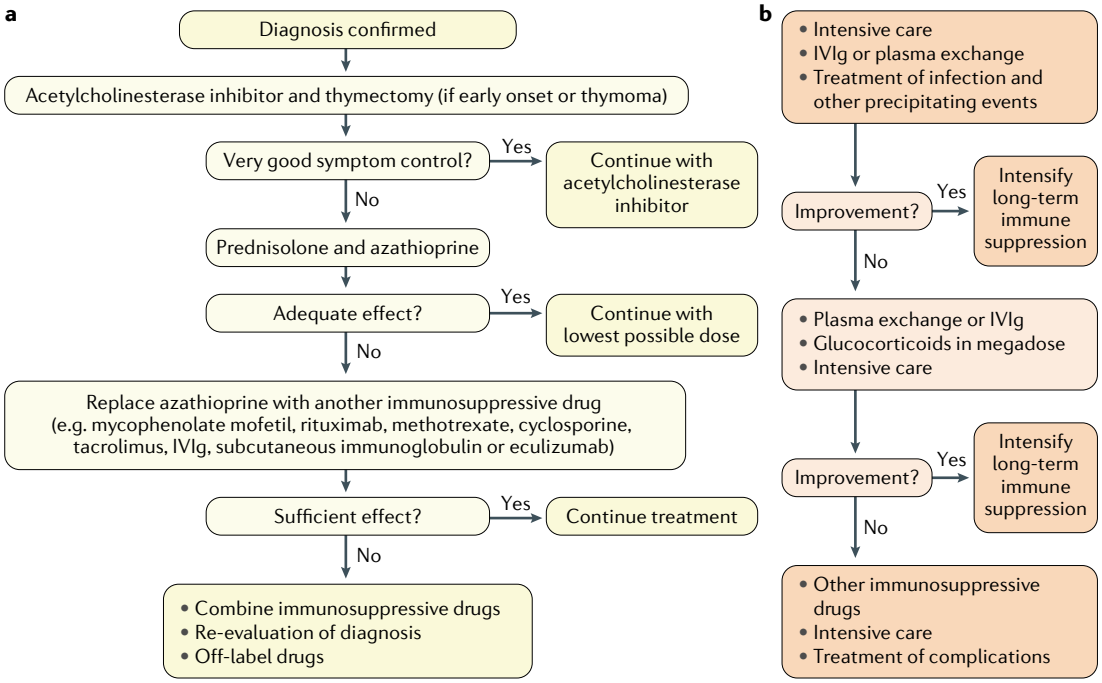

Fig. 7 | **Treatment algorithms for chronic MG and acute MG exacerbations. a** | Treatment of chronic myasthenia gravis (MG). **b** | Treatment of acute exacerbations of MG. The individualized combination of symptomatic drugs, immunosuppressive drugs, thymectomy and supportive therapy should lead to a very good outcome in patients with MG. The combination of prednisolone and azathioprine is recommended, but prednisolone alone or prednisolone combined with mycophenolate mofetil are alternative first-line immunosuppressive treatments. IVIg, intravenous immunoglobulin.

characteristics and comorbidities. Several international and national treatment guidelines are available for MG[3,121–126] and are based on expert consensus and a limited number of controlled studies.

Symptomatic treatment

Anticholinesterases (also known as cholinesterase inhibitors) enhance the bioavailability of ACh at the synaptic cleft. Pyridostigmine bromide is the preferred anticholinesterase for oral treatment and is usually the first-line medication in patients with MG. Ambenonium chloride is an alternative treatment but is rarely used because in most patients it is less effective and has variation in drug bioavailability, and this drug is not available everywhere.

All subgroups of MG respond to anticholinesterases, except for patients with anti-MuSK antibodies, with inter-individual variability in the degree of weakness relief, optimal dosage and tolerability[6]. However, muscle strength can be restored to normal levels over long periods in only a few patients with mild disease. More frequently, the persistence of weakness and clinical fluctuations after pyridostigmine dose optimization is an indication for adding disease-modifying therapy such as corticosteroids, azathioprine, mycophenolate mofetil or thymectomy. In patients using immunosuppressive therapies, a reduced need for pyridostigmine usually parallels disease stabilization and symptom remission.

Patients with MuSK MG are usually unresponsive to and often intolerant of pyridostigmine, which can induce muscle cramps and fasciculations up to a cholinergic crisis with increased muscle weakness[127]. In patients with such adverse effects, anticholinesterases should be avoided. No established symptomatic drug therapy is available for patients with MuSK MG if they do not have a positive effect with anticholinesterases, but 3,4-diaminopyridine might have a positive effect[128].

Common adverse effects of anticholinesterases mainly result from the stimulation of muscarinic AChR in the autonomic nervous system and can be controlled by dose adjusting or, when necessary for obtaining an optimal treatment result, by atropine-like agents[129]. Typical adverse effects are stomach pain, diarrhoea, nausea and increased salivation. MG worsening due to depolarization block may occur with very high pyridostigmine doses[130,131].

Conventional immunosuppression

All patients with symptoms that exert functional impairment or reduce quality of life when on optimal acetylcholinesterase inhibitor treatment should receive immunotherapy based on steroids and other immunosuppressants. Immunosuppression can include antimetabolites (such as azathioprine, mycophenolate mofetil, methotrexate or cyclophosphamide) and calcineurin inhibitors (such as cyclosporine and tacrolimus). All MG subgroups respond to conventional immunosuppression, although a higher proportion of patients with MuSK MG remain steroid-dependent than the other MG subgroups. However, patients in this subgroup usually do well on rituximab.

Corticosteroids. Oral prednisone and prednisolone are first-line immunotherapy drugs for MG owing to the rapid effect that is especially important in patients with severe MG. High-dose treatment is associated with a more

rapid response (usually within 2–4 weeks) and is used in patients with severe disease[129,132]. Alternatively, glucocorticoids can be started at low doses with gradual escalation and are usually selected in those with mild to moderate disease. As steroid initiation can induce a transient deterioration of muscle weakness, patients with bulbar weakness require close monitoring during the first week of treatment. Subsequently, alternate-day administration is often preferred, with slow tapering to the lowest effective dose. Low maintenance doses are usually well tolerated in all MG patient groups and have a favourable impact on health-related quality of life (HR-QOL)[133]. Prednisone or prednisolone at lower peak doses is an accepted first-line therapy for ocular MG[3]. High-dose daily steroid treatment in association with plasma exchange or intravenous immunoglobulin (IVIg) is recommended in patients with respiratory crises[121,122,132]. IVIg and plasma exchange can also be given to improve MG weakness before starting pharmacological immunotherapies that take longer before an effect appears.

There is limited evidence of steroid efficacy in MG in general from controlled studies. In large, retrospective studies, the mean response rate to glucocorticoid monotherapy was 74%[134]. Because of adverse effects, the degree of response and non-responders, corticosteroids are most often combined with additional immunosuppression. The EPITOME trial, although underpowered, demonstrated a clear superiority of prednisone over placebo in patients with ocular MG[135].

Long-term steroid treatment is associated with several adverse effects, including osteoporosis, weight gain, skin atrophy, impaired glucose tolerance, glaucoma, mood disorders and increased risk of infection[136]. Most patients are given steroids in combination with non-steroidal immunosuppressive therapy to prevent disease flares during dose tapering, to minimize the adverse effects of each drug and to optimize the clinical improvement. In patients who have a high risk of steroid-related adverse effects, such as osteoporosis, gastrointestinal alterations, skin changes and weight gain, glucocorticoids should be given short term for bridging until other immunosuppressive medications have gained their peak efficacy.

Non-steroidal immunosuppressants. Non-steroidal immunosuppressants have a delayed response, which is longer for azathioprine (several months) and shorter for cyclosporine (1–2 months)[134]. Such drugs can be used as monotherapy or, as previously mentioned, can be administered in combination with corticosteroids. Therapy is sometimes initiated at low doses and, in the absence of early toxicity, can be titrated upwards to the induction regimen. Long-term therapy is recommended (often lifelong), but the dose of these drugs can also sometimes be slowly reduced when the patient is in a stable remission[137]. The mechanism of action and adverse effects of the most frequently used agents are provided in TABLE 2.

Widespread clinical experience has demonstrated the efficacy of non-steroidal immunosuppressants in MG, with a response rate of 70–80% for azathioprine, mycophenolate mofetil, cyclosporine[138,139] and tacrolimus[140]. Results from controlled studies are more equivocal. The effectiveness of cyclosporine as a monotherapy and azathioprine as a steroid-sparing agent when combined with prednisolone has been demonstrated in well-controlled, prospective studies[141,142]. Two short-term studies did not demonstrate additional benefits of mycophenolate combined with prednisone over prednisone alone as initial immunosuppression in these trials[143,144]. Trials assessing the steroid-sparing effect of tacrolimus[145,146] and methotrexate[147,148] yielded conflicting results, but the direction of change was in favour of a beneficial effect of adding these two drugs as they then could use lower steroid doses. Controlled and well-powered long-term studies are generally lacking, and even more so for MG subgroups.

Medication choice is based on the risk:benefit ratio in the individual patient and the accessibility of each medication in different countries. Azathioprine is the first-choice non-steroidal immunosuppressant in MG in most European centres, whereas mycophenolate is often the first-choice non-steroidal immunosuppressant in the United States. Mycophenolate is usually preferred to cyclosporine, as it has a more favourable safety profile. Cyclophosphamide use is reserved for patients who are unresponsive to other agents owing to potentially serious adverse effects. Azathioprine is particularly favourable in women of child-bearing age, whereas mycophenolate should be avoided in this group.

Refractory disease

In 10–30% of patients, MG is deemed more or less refractory to conventional immunosuppression owing to persistent and disabling weakness despite this immunotherapy, disease relapses on treatment tapering or severe treatment-related adverse effects. Patients with severe and disease-resistant MG more frequently have thymoma-associated AChR MG or MuSK MG[2].

B cell depletion with rituximab is a preferred treatment for refractory MG. In uncontrolled reports, rituximab was effective in all subgroups of MG but with varying response rates[149–151]. Dose regimens and enrolment criteria varied between studies. In particular, rituximab provides a meaningful and prolonged benefit in patients with MuSK MG[149] and has been proposed as an early treatment option after failure of first-line therapies[1,122]. In some countries, such as those in Scandinavia, rituximab is used as a second-line therapy for patients with moderate to severe MG[1]. Although rituximab is generally well tolerated, progressive multifocal leukoencephalopathy (PML) has been reported in two patients with MG who were previously treated with conventional immunosuppressants[152,153]. The frequency of PML in those treated with rituximab for rheumatoid disease and in general has been estimated as <1 in 30,000 (REFS[41,153]). The results of a phase II trial in patients with AChR MG (NCT02110706) have recently been reported at a conference but not yet published. Rituximab was safe and well tolerated in patients with MG but did not lead to a 75% reduction in glucocorticoid dose (main end point).

Eculizumab (which inhibits terminal complement activation) did not demonstrate significant superiority over placebo in the primary end point, which was improvement of MG activities of daily living (MG-ADL) scores, in patients with refractory AChR

Table 2 | **Overview of the most commonly used immunosuppressive drugs in MG**

Drug	Mechanism of action	Adverse effects (listed in order of frequency)	Contraindications
Azathioprine	• Purine analogue • Interferes with DNA synthesis • Reduces T cell and B cell proliferation	Hepatic enzyme increase, nausea, macrocytosis, leukopenia, thrombocytopenia, pancreatitis, hair loss and idiosyncratic reaction	Impaired liver function, leukopenia, haematological malignancies or low TPMT activity
Mycophenolate mofetil	• Prodrug • Inhibits de novo purine synthesis • Reduces T cell and B cell proliferation	Nausea, diarrhoea, leukopenia, liver enzyme increase and hypertension	Cancer, leukopenia, before conception or during pregnancy
Cyclosporine	• Calcineurin inhibitor • Reduces IL-2 transcription • Blocks T cell activation	Nephrotoxicity, hypertension, tremor, hypertrichosis, gingival hyperplasia, dizziness, headache and encephalopathy	Impaired kidney function, severe hypertension or cancer
Tacrolimus	• Calcineurin inhibitor • Reduces IL-2 transcription • Blocks T cell activation	Hypertension, tremor, diabetes mellitus, nephrotoxicity, myocardial hypertrophy and encephalopathy	Impaired kidney function, severe hypertension, congenital long QT syndrome or cancer
Methotrexate	• Folate analogue • Interferes with DNA synthesis • Reduces T cell and B cell proliferation	Stomatitis, nausea, hair loss, leukopenia, macrocytic anaemia, thrombocytopenia, oligospermia and hepatic enzyme increase	Impaired liver function, bone marrow dysfunction, before conception or during pregnancy
Cyclophosphamide	• Alkylating agent • Interferes with DNA replication • Suppresses B cells more than T cells	Nausea, vomiting, fever, hair loss, liver dysfunction, liver enzyme increase, cystitis, oligospermia and myelosuppression	Impaired liver function, bone marrow dysfunction, before conception or during pregnancy
Rituximab	• Anti-CD20 monoclonal antibody • Depletes B cells through cytotoxicity and induction of apoptosis	Infusion reactions, hypotension, leukopenia, minor infections, arrhythmias, dyspnoea, reactivation of HBV, HCV and JCV and heart failure	Neutropenia, ischaemic heart disease, congestive heart failure, before conception or during pregnancy

HBV, hepatitis B virus; HCV, hepatitis C virus; JCV, John Cunningham virus; MG, myasthenia gravis; TPMT, thiopurin-S-methyltransferase.

MG in a phase III randomized controlled trial. However, post hoc sensitivity analysis of MG-ADL scores together with secondary end points, such as the quantitative MG score for muscle weakness, demonstrated a clear but moderate significant efficacy of this drug[154]. In some patients, the drug had a pronounced effect. Eculizumab has been approved by the European Medicines Agency (EMA), the US FDA and in Japan for refractory AChR MG, but the very high costs seem prohibitive for its use in very many such patients[155]. As eculizumab does not modify MG immunopathology, concomitant immunosuppression is required.

Other treatments for refractory MG have demonstrated mixed results. Belimumab, a human IgG1 monoclonal antibody against B cell-activating factor, demonstrated no significant additional effect in a phase II controlled study in patients with generalized MG receiving standard-of-care treatment[156], and this drug is not recommended for MG. Pulsed intravenous cyclophosphamide was effective in a small controlled study compared with placebo[157]. High-dose cyclophosphamide[158] and autologous haemopoietic stem cell transplantation[159] are effective rescue treatments in patients with severe, refractory disease. In addition, refractory MG can be managed with periodic plasma exchange or IVIg treatment (see below).

Short-term treatments

Plasma exchange and IVIg are fast-acting treatments and are typically used in patients with acute severe MG when a rapid response is crucial (FIG. 7). As their effect is short-lived (4–12 weeks), additional immunotherapy is usually needed.

Plasma exchange removes antibodies, complement, cytokines and adhesion molecules from the circulation. IVIg has multiple effects, including inhibition of complement deposition, blockade of activating Fc receptors and neutralization of cytokines and antibodies[160]. In controlled studies, plasma exchange and IVIg had comparable efficacy for the treatment of myasthenic crisis with the need for respiratory support[161] and for disease exacerbations[162]. In addition, both treatments are successfully used to prevent or minimize MG deterioration at the start of steroid therapy or in preparation for thymectomy and other surgeries[121,122]. Plasma exchange and IVIg can be used as periodic treatments in patients who are unresponsive or intolerant to immunosuppression[2,122]. The choice between the two therapies is mostly based on the individual patient comorbidity that can increase the risk of adverse effects, in addition to availability, resources and experience at the treatment unit. IVIg is generally well tolerated, with the exception in patients with IgA deficiency and antibodies against this subclass. IVIg is made from human plasma; therefore, the source is limited, although in most countries the availability is still satisfactory. Plasma exchange complications are predominantly related to the central venous access and include catheter problems, infection, replacement fluid reactions and hypotension. Plasma exchange should not be given to haemodynamically unstable patients or to patients who are allergic to any of its constituents.

Immunoadsorption removes circulating IgG antibodies from the circulation but leaves other plasma components unaltered and is a valid alternative to IVIg or plasma exchange, particularly for repeated treatment cycles[129]. Clinical experience with subcutaneous immunoglobulin is still limited, although retrospective studies and a single open-label trial have reported good responses both in chronic disabling MG[163] and in disease exacerbations[164].

Thymectomy

Thymectomy is indicated for nearly all patients with thymoma and in many other patients with MG with AChR antibodies. Previously, a trans-sternal approach was used, but in many centres this has now been replaced by less-invasive surgery, such as thoracoscopic and robotic thymectomy[165,166]. Thymomas with growth invading into surrounding tissues require additional treatment, such as radiotherapy and chemotherapy. As a rule, oncological treatments do not have a negative impact on MG. In all patients eligible for thymectomy, stable control of MG symptoms should be achieved preoperatively through appropriate treatment.

Thymectomy is undertaken in patients with MG with anti-AChR antibodies, with weakness that is not limited to the ocular muscles and who are <50 years of age and with short MG duration. For patients 50–65 years of age, those with longer disease duration, ocular symptoms only or full pharmacological remission on anticholinesterase alone, thymectomy should be considered in individual patients. Small and slowly growing thymomas in elderly and frail patients can be observed with regular CT or MRI examinations and do not require surgery.

In patients with a non-neoplastic thymus, thymectomy for the improvement of MG has been performed for several decades, supported by extensive evidence on the pathogenetic role of the thymus in AChR MG and by a large number of studies showing a higher remission rate after thymectomy than without thymectomy in comparable patient cohorts[121,167]. Many of these studies included control groups, but none were randomized and prospective. A recent, well-controlled trial demonstrated significantly better disease status and lower alternate-day prednisone requirements, and improvements in several secondary outcomes, with thymectomy plus prednisone than with prednisone alone. The study included patients with non-thymoma generalized AChR MG, some even up to 65 years of age[87]. The results from this study favour early thymectomy in young adults with generalized AChR MG. Benefit from thymectomy in patients >50 years of age and in those with long disease duration is still controversial. Some reports indicate a long-term benefit of thymectomy in patients with ocular MG with anti-AChR antibodies, with a reduced risk of generalization of symptoms, but thymectomy should not be regarded as a routine therapy for ocular MG[3]. Thymectomy is generally not recommended in MG subgroups without anti-AChR antibodies. Thymectomy does not have an effect in MuSK MG, whereas thymectomy has not been systematically examined in patients with MG who have no detectable antibodies. Thymectomy does not cure MG, and the long-term effect on antibody concentrations, T cell subsets and immune responses is very modest.

Supportive measures

Tailored physical exercise should be encouraged in all patients with MG. Avoiding a sedentary lifestyle is important, and this can also prevent comorbid pathologies. Although specific studies are sparse, recent observations have reported good tolerance, improved quality of life and better muscle strength in patients with mild disease undergoing supervised training compared with standard treatment as before[168,169]. A multicentre, randomized and controlled trial (MGEX) is ongoing (NCT02066519).

Treatment-related adverse events

Before starting immunosuppressive therapy, patients should undergo screening for active and latent severe infections, such as viral hepatitis, tuberculosis and opportunistic infections, and infections in patients should be actively treated. Long-term immunosuppressive therapy conveys a slightly increased risk of infections in all autoimmune disorders, including MG[41]. Risk factors are high drug doses, combination therapy, severe disease, older age and comorbidity[41]. Vaccinations are generally recommended for available infections, including for influenza[41]. Immunosuppressive medication may influence vaccination policy. Sleep apnoea has a high frequency in patients with MG and should be actively investigated and treated[170].

Patients using steroids should be prescribed a generally healthy diet. Bone mineral density is usually measured before starting glucocorticoids and at periodic intervals thereafter. Calcium and vitamin D supplementation should be considered in all patients, and bisphosphonate therapy should be given when appropriate. Monitoring for the development of cataracts and increased ocular pressure is recommended in patients using glucocorticoids.

Individuals using immunosuppressive treatments should be strictly monitored during the first months, and at regular intervals subsequently, for the development of leukopenia, thrombocytopenia, hepatic dysfunction (particularly with azathioprine treatment) or renal dysfunction (more common with cyclosporine treatment), which require dose reduction or a treatment switch. Patients receiving immunosuppressants should reduce sun exposure and undergo periodic screening for skin cancers[171]. Aside from skin cancers, these patients do not seem to have an increased cancer risk[172,173].

Pyridostigmine, prednisolone and azathioprine are safe during pregnancy and breastfeeding[122,174,175], whereas methotrexate, mycophenolate mofetil and cyclophosphamide are teratogenic and should be avoided in women of child-bearing age. Current practice is to stop rituximab 6 months before a planned pregnancy in women with MG to be on the safe side. IVIg and plasma exchange are safe during pregnancy.

Several drugs have been associated with worsening of MG and should be used with caution in MG, and only if they are clearly necessary. Association of drug use and MG exacerbation can be causal or by chance. Patients with MG should always be warned about the possibility of adverse effects, including MG exacerbation, when starting new therapies. The antibiotic telithromycin should be avoided, in addition to, if possible, fluoroquinolones, macrolides and aminoglycosides[41]. Botulinum toxin, quinine, procainamide, D-penicillamine, magnesium and β-blockers should be used with caution. Statins can induce myositis but do not act differently in patients with MG and those without

MG and probably do not have a higher risk of inducing myositis in patients with MG[27].

Quality of life

Patient-reported outcomes, including HR-QOL measures, are well suited for estimating the patient's experience with MG and disease development because the manifestations are often more evident to the patient than the physician (such as dysphagia and chewing fatigue), fluctuate over time and worsen later in the day. A handful of patient-reported tools for MG are available, such as the MG-ADL[176], Myasthenia Gravis Impairment Index (MGII)[177] and Myasthenia Gravis Quality of Life-15 (MG-QOL15) scales[178,179]. The scales measure the patient's experience with the symptoms, limitations in function and effects on social and psychological well-being of MG in a parsed, validated and standardized way. The 15-item MG-QOL15 is a disease-specific HR-QOL instrument that is quick to deliver and easy to administer and interpret. This instrument has been validated in many languages and in multiple settings and cohorts of patients with MG. The revised version, the MG-QOL15r, was more recently developed and validated using modern psychometric statistical techniques[180].

Studies have illustrated the usefulness of these scales in the everyday clinic, clinical trials and in scientific studies of patients with MG[133,181–183]. Indeed, studies have revealed that most patients with MG report limitations in social activity and in their ability to enjoy fun activities and perform work, including work at home. Patients are at least 'somewhat' frustrated, as well as sometimes overwhelmed or depressed, by their MG[178–180]. Many of these observations might otherwise go unidentified during a routine clinic or study visit but are important aspects of the patient experience and should be assessed to best estimate the patient's true clinical status. Disease-specific patient-reported scales allow assessment of the patient experience over time, in contrast to the examination, which provides only a snapshot at one moment in time.

Outcome measures

Quantifying the response to therapy relies on validated, generally accepted measures. Quantitative MG and MG composite scoring systems are based on testing sentinel muscle groups[184,185]. Changes in these scores, between baseline and any time after a therapeutic intervention, provide measures of treatment efficacy. In addition, the Myasthenia Gravis Foundation of America post-intervention status categorizes patient clinical status, considering changes in both clinical manifestations and MG medications[184]. Patient-reported data are crucial.

Although therapeutic advances have substantially reduced MG-related mortality[25,26], complete stable remission remains infrequent. In many patients, sustained control of symptoms requires chronic and even lifelong immunosuppressive treatment. In one long-term study, only 7% of the patients obtained a complete stable remission with no need for treatment after 10 years[5]. However, as many as 75% had an optimal outcome with remission, ocular symptoms only or mild symptoms, whereas only 3% had a poor outcome with severe symptoms. A post-intervention status of minimal manifestations or better, with no or only mild medication adverse effects, as the treatment target has recently been proposed by an international consensus[122] (BOX 1).

Outlook

The major challenges for MG research are to identify the primary cause of the disease and to develop an antigen-specific therapy that restores tolerance for the key muscle antigen. New sensitive and specific diagnostic tools and biomarkers to predict the course of disease and the response to therapy would also be useful. MG is one of the few autoimmune disorders for which detailed knowledge is available regarding both the target antigens and contributing factors, including thymus pathology, genetic predisposition and external factors such as pregnancy and the role of some specific drugs.

Identifying the primary cause of MG

The differences in age of onset, sex ratio, HLA associations, thymic abnormalities and serum autoantibodies indicate that early-onset MG, late-onset MG and thymoma-associated MG have different pathogeneses that share a common final pathway resulting in the production of anti-AChR antibodies[186,187]. Most likely, the onset of clinical signs and symptoms is determined by multiple factors, such as a viral, bacterial or parasitic infection in individuals with a specific genetic background and in some with a predisposing hormonal constellation[188]. Recently, it has been suggested that the high frequency of childhood-onset MG in China is due to a specific Japanese encephalitis vaccination.

Box 1 | **Classification of MG severity and response to therapy**

Complete stable remission
No symptoms or signs of myasthenia gravis (MG) for at least 1 year and no MG therapy during that time. Isolated weakness of eyelid closure accepted.

Pharmacological remission
Criteria as for complete stable remission except that the patient continues to use drug therapy for MG. Patients taking cholinesterase inhibitors are excluded.

Minimal manifestations
No functional limitations from MG but some weakness on examination.

Improved
A substantial decrease in pretreatment clinical manifestations or a substantial reduction in MG medication.

Unchanged
No substantial change in pretreatment clinical manifestations and no reduction in MG medication.

Worse
A substantial increase in pretreatment clinical manifestations or a substantial increase in MG medication.

Exacerbation
Fulfilled criteria of complete stable or pharmacological remission, or minimal manifestations, but subsequently more extensive clinical manifestations.

Death
Died of MG manifestations, complications of MG therapy or within 30 days after thymectomy.

Table 3 | **Potential therapeutic drugs for patients with MG (adapted from REF.[186])**

Target	Function	Therapeutics	Ongoing MG trial
Neuromuscular synapse	Acetylcholinesterase inhibition	Pyridostigmine	Phase IV (NCT03510546)
Multiple	Potassium channel blocker	3,4-Diaminopyridine	Phase III NCT03304054
	Immunoregulation of the immune system	Haematopoietic stem cells	Phase I, nine patients (NCT00424489)
	Folic acid antagonist	Methotrexate	Phase II (NCT00814138); negative results
	General immunosuppression	Prednisone, azathioprine	Phase IV (NCT00987116)
CD80 and CD86	T cell inhibition	Abatacept	Phase I (NCT03059888)
CD20	Deplete B cells	Rituximab	Phase II (BeatMG study) (NCT02110706); negative results
		Rituximab	Phase III (Rinomax study) (NCT02950155)
CD40L pathway	Inhibit B cell activation	CFZ533	Phase II (NCT02565576)
BAFF pathway	Inhibit B cell survival	Belimumab	Phase II (NCT01480596); negative results[156]
Proteasome	Deplete antibody-producing B cells	Bortezomib	Phase II (NCT02102594)
IgG degradation	Block FcRn	Efgartigimod	Phase III (ADAPT study) (NCT03669588)
Complement	Block FcRn	UCB7665	Phase II (NCT03052751)
	Block FcRn	M281	Phase II (NCT03772587)
	IgG-degrading enzyme	Imlifidase	None
	Diminish membrane destruction	Eculizumab	Drug approved (NCT01997229)
	Peptide inhibitor of C5	RA101495	Phase II (NCT03315130)

FcRN, neonatal Fc receptor; IgG, immunoglobulin G; MG, myasthenia gravis.

This hypothesis has not been confirmed but is supported by vaccination studies in mice[189].

New techniques that enable deep sequencing of single cells could provide information on abnormal clonal expansions, skewed gene usage or distinctive antigen-binding-region properties[190], among other characteristics of these immune cells. The study of microRNAs in the thymus and sera might also provide new clues for the aetiological or pathophysiological mechanisms that are involved in MG[191,192]. Together, these studies should elucidate the specific factors that are involved in the onset of MG.

Improving diagnostics

A large number of diagnostic tests are available, including clinical, electrophysiological and laboratory antibody tests. Nevertheless, results from these tests can be negative in some patients, and diagnosing pure ocular MG can be a challenge. The ongoing development of cell-based antibody assays with a higher sensitivity should partially overcome this diagnostic problem[193]. Further improvements would be the development of more non-radioactive diagnostic tests to detect anti-AChR antibodies, using, for example, regular ELISA or immunostick ELISA[194].

Thus far, no test exists that directly examines the function of the extraocular eye muscles. The recently described electrophysiological test assessing ocular vestibular myogenic potentials might be a promising addition to the diagnostic armamentarium to overcome this problem[195]. Except for some older CT studies and an MRI case study, extraocular muscles have not been studied in MG using modern imaging tools[196]. New and more powerful quantitative MRI techniques to measure structural changes in muscles and neuromuscular junctions might be another option.

The diagnosis of thymoma and thymic hyperplasia in AChR MG is lacking, both in sensitivity and specificity. New magnetic resonance protocols are being developed, and, combined with antibody markers, they should improve precision[197].

New therapies

Therapeutic interventions in MG should aim to weaken the autoimmune response, strengthen the neuromuscular synapse or a combination of both strategies.

One set of new therapies is based on developments of older, existing treatment modalities. These therapies include subcutaneous immunoglobulins[198], replacing total IgG apheresis with selective depletion of MG-specific autoantibodies[199], comparing new tapering strategies of prednisone and studying the use of 3,4-diaminopyridine or amifampridine phosphate.

In addition, a large number of new drugs are available or are being evaluated for MG[186] (TABLE 3). Some drugs have shown a beneficial effect in oncology or other autoimmune diseases, such as rheumatoid arthritis, psoriasis or systemic lupus erythematosus. Complement is crucial for an important pathophysiological mechanism for AChR MG, and eculizumab (an inhibitor of complement activation through inhibiting C5 protein) was recently approved for use in MG. Clinical trials of another complement inhibitor are ongoing. Neonatal Fc receptor (FcRn) antagonists are promising for the treatment of MG. Efgartigimod blocks FcRn, thereby shortening the IgG half-life[200]. Multiple dosing of

efgartigimod resulted in a 75% drop of IgG serum concentration in healthy volunteers[200]. By contrast, IVIg has multiple mechanisms of action in addition to the inhibitory effect on pathogenetic IgG, which could be an advantage in some patients with autoimmune disease. The use of the FcRn system to degrade specific antibodies might form another attractive future option. Indeed, a proof of principle has been demonstrated using a new type of engineered antibody-based reagents ('Seldegs'). The use of Seldegs has not been tested for MG, but specific clearance of antibodies recognizing other proteins has been demonstrated[201]. Imlifidase, which is based on an IgG-degrading enzyme, cleaves IgG specifically and has been tested successfully in patients with a kidney transplant. This drug might also be suitable for MG[202].

For patients with severe or life-threatening MG despite the continued use of intensive immunosuppressive therapies, autologous haematopoietic stem cell transplantation may be an option[159]. Thus far, results of transplantation have been described for seven patients with MG, all of whom achieved a durable, complete stable remission with no residual MG symptoms and freedom from any ongoing MG therapy. However, further prospective studies are needed to determine the indications for this intervention.

Only a few studies have examined the possibility of strengthening the neuromuscular synapse. Salbutamol and ephedrine have shown a clear beneficial effect in congenital myasthenia[115] and a moderate effect in a small trial of patients with anti-AChR-antibody-mediated MG[203]. Tirasemtiv is a selective activator of the fast skeletal muscle troponin complex and has demonstrated positive results in an initial study in patients with MG[204], but no follow-up study has yet been reported.

The new complement inhibitors and FcRn blocking agents could alone or in combination temporarily induce a rapid serum IgG lowering in patients with MG. This therapy could be combined with the slower-acting, traditional or new immunosuppressive agents to inhibit long-term autoantibody production. Owing to the plethora of therapies that are now in development for MG, and the costs of the new drugs, we will need a critical review to prioritize the use of future drugs in MG.

Published online: 02 May 2019

1. Gilhus, N. E. Myasthenia gravis. *N. Engl. J. Med.* **375**, 2570–2581 (2016).
2. Gilhus, N. E. & Verschuuren, J. J. Myasthenia gravis: subgroup classification and therapeutic strategies. *Lancet Neurol.* **14**, 1023–1036 (2015).
This review article covers most MG aspects.
3. Kerty, E., Elsais, A., Argov, Z., Evoli, A. & Gilhus, N. E. EFNS/ENS Guidelines for the treatment of ocular myasthenia. *Eur. J. Neurol.* **21**, 687–693 (2014).
4. Al-bassam, W. et al. Characteristics, incidence, and outcome of patients admitted to the intensive care unit with myasthenia gravis. *J. Crit. Care* **45**, 90–94 (2018).
5. Andersen, J. B., Gilhus, N. E. & Sanders, D. B. Factors affecting outcome in myasthenia gravis. *Muscle Nerve* **54**, 1041–1049 (2016).
6. Hong, Y. et al. Multiple antibody detection in 'seronegative' myasthenia gravis patients. *Eur. J. Neurol.* **24**, 844–850 (2017).
7. Marx, A. et al. The different roles of the thymus in the pathogenesis of the various myasthenia gravis subtypes. *Autoimmun. Rev.* **12**, 875–884 (2013).
8. Carr, A. S., Cardwell, C. R., McCarron, P. O. & McConville, J. A systematic review of population based epidemiological studies in Myasthenia Gravis. *BMC Neurol.* **10**, 46 (2010).
9. Park, S. Y., Lee, J. Y., Lim, N. G. & Hong, Y. H. Incidence and prevalence of myasthenia gravis in Korea: a population-based study using the National Health Insurance Claims Database. *J. Clin. Neurol.* **12**, 340–344 (2016).
10. Heldal, A. T., Owe, J. F., Gilhus, N. E. & Romi, F. Seropositive myasthenia gravis: a nationwide epidemiologic study. *Neurology* **73**, 150–151 (2009).
11. Zieda, A. et al. A nationwide epidemiological study of myasthenia gravis in Latvia. *Eur. J. Neurol.* **25**, 519–526 (2018).
12. Andersen, J. B., Heldal, A. T., Engeland, A. & Gilhus, N. E. Myasthenia gravis epidemiology in a national cohort; combining multiple disease registries. *Acta Neurol. Scand.* **129**, 26–31 (2014).
13. Hong, Y. et al. Juvenile-onset myasthenia gravis: autoantibody status, clinical characteristics and genetic polymorphisms. *J. Neurol.* **264**, 955–962 (2017).
14. Matsuki, K. et al. HLA antigens in Japanese patients with myasthenia-gravis. *J. Clin. Invest.* **86**, 392–399 (1990).
15. Matsuki, K., Maeda, H., Nomura, Y. & Segawa, M. Distortion of HLA gene transmission in childhood-onset myasthenia-gravis. *Lancet* **340**, 796–796 (1992).
16. Popperud, T. H., Boldingh, M. I., Rasmussen, M. & Kerty, E. Juvenile myasthenia gravis in Norway: clinical characteristics, treatment, and long-term outcome in a nationwide population-based cohort. *Eur. J. Paediatr. Neurol.* **21**, 707–714 (2017).
17. Evoli, A. et al. Myasthenia gravis with antibodies to MuSK: an update. *Ann. NY Acad. Sci.* **1412**, 82–89 (2018).
This article represents a comprehensive review of an important MG subgroup.
18. Hong, Y. et al. Autoantibody profile and clinical characteristics in a cohort of Chinese adult myasthenia gravis patients. *J. Neuroimmunol.* **298**, 51–57 (2016).
19. Hong, Y., Li, H. F., Romi, F., Skeie, G. O. & Gilhus, N. E. HLA and MuSK-positive myasthenia gravis: a systemic review and meta-analysis. *Acta Neurol. Scand.* **138**, 219–226 (2018).
20. Huda, S., Woodhall, M. R., Vincent, A. & Heckmann, J. M. Characteristics of acetylcholine-receptor-antibody-negative myasthenia gravis in a South African cohort. *Muscle Nerve* **54**, 1023–1029 (2016).
21. Zisimopoulou, P. et al. A comprehensive analysis of the epidemiology and clinical characteristics of anti-LRP4 in myasthenia gravis. *J. Autoimmun.* **52**, 139–145 (2014).
This large, multinational study establishes the presence, frequency and some clinical characteristics of anti-LRP4 MG.
22. Yan, M., Xing, G. L., Xiong, W. C. & Mei, L. Agrin and LRP4 antibodies as new biomarkers of myasthenia gravis. *Ann. NY Acad. Sci.* **1413**, 126–135 (2018).
23. Zhang, B. et al. Autoantibodies to lipoprotein-related protein 4 in patients with double-seronegative myasthenia gravis. *Arch. Neurol.* **69**, 445–451 (2012).
24. Boldingh, M. I. et al. Prevalence and clinical aspects of immigrants with myasthenia gravis in Northern Europe. *Muscle Nerve* **55**, 819–827 (2017).
25. Owe, J. F., Daltveit, A. K. & Gilhus, N. E. Causes of death among patients with myasthenia gravis in Norway between 1951 and 2001. *J. Neurol. Neurosurg. Psychiatry* **77**, 203–207 (2006).
26. Hansen, J. S. et al. Mortality in myasthenia gravis: a nationwide population-based follow-up study in Denmark. *Muscle Nerve* **53**, 73–77 (2016).
This population-based registry study proves the excellent prognosis of MG with modern treatment.
27. Gilhus, N. E., Nacu, A., Andersen, J. B. & Owe, J. F. Myasthenia gravis and risks for comorbidity. *Eur. J. Neurol.* **22**, 17–23 (2015).
28. Ogawa, Y. et al. Survey of epidemiology, clinical picture and current treatments for elderly-onset (>=65 years) patients with myasthenia gravis in Nagano Prefecture. *Japan. Neurol. Clin. Neurosci.* **5**, 107–112 (2017).
29. Pedersen, E. G., Hallas, J., Hansen, K., Jensen, P. E. H. & Gaist, D. Late-onset myasthenia not on the increase: a nationwide register study in Denmark, 1996–2009. *Eur. J. Neurol.* **20**, 309–314 (2013).
30. Boldingh, M. I. et al. Geographical distribution of myasthenia gravis in Northern Europe - results from a population-based study from two countries. *Neuroepidemiology* **44**, 221–231 (2015).
31. Cea, G. et al. Clinical and epidemiological features of myasthenia gravis in Chilean population. *Acta Neurol. Scand.* **138**, 338–343 (2018).
32. Ramanujam, R., Pirskanen, R., Ramanujam, S. & Hammarstrom, L. Utilizing twins concordance rates to infer the predisposition to myasthenia gravis. *Twin Res. Hum. Genet.* **14**, 129–136 (2011).
33. Li, F., Yuan, W. Z. & Wu, X. S. Association of CTLA-4 polymorphisms with increased risks of myasthenia gravis. *Ann. Hum. Genet.* **82**, 358–369 (2018).
34. Giraud, M. et al. An IRF8-binding promoter variant and AIRE control CHRNA1 promiscuous expression in thymus. *Nature* **448**, 934–937 (2007).
35. Punga, A. R. & Punga, T. Circulating microRNAs as potential biomarkers in myasthenia gravis patients. *Ann. NY Acad. Sci.* **1412**, 33–40 (2018).
36. Boldingh, M. I. et al. Increased risk for clinical onset of myasthenia gravis during the postpartum period. *Neurology* **87**, 2139–2145 (2016).
37. Berrih-Aknin, S. & Le Panse, R. Myasthenia gravis: a comprehensive review of immune dysregulation and etiological mechanisms. *J. Autoimmun.* **52**, 90–100 (2014).
This article explains the major immunological mechanisms in MG.
38. Dragin, N. et al. Estrogen-mediated downregulation of AIRE influences sexual dimorphism in autoimmune diseases. *J. Clin. Invest.* **126**, 1525–1537 (2016).
39. Cavalcante, P. et al. Epstein-barr virus persistence and reactivation in myasthenia gravis thymus. *Ann. Neurol.* **67**, 726–738 (2010).
40. Meyer, M. et al. Lack of evidence for epstein-barr virus infection in myasthenia gravis thymus. *Ann. Neurol.* **70**, 515–518 (2011).
41. Gilhus, N. E., Romi, F., Hong, Y. & Skeie, G. O. Myasthenia gravis and infectious disease. *J. Neurol.* **265**, 1251–1258 (2018).
42. Kao, J. C., Brickshawana, A. & Liewluck, T. Neuromuscular complications of programmed cell death-1 (PD-1) inhibitors. *Curr. Neurol. Neurosci. Rep.* **18**, 63 (2018).
43. Benfaremo, D., Manfredi, L., Luchetti, M. M. & Gabrielli, A. Musculoskeletal and rheumatic diseases induced by immune checkpoint inhibitors: a review of the literature. *Curr. Drug Safety* **13**, 150–164 (2018).

44. Patrick, J. & Lindstrom, J. Autoimmune response to acetylcholine receptor. *Science* **180**, 871–872 (1973).
45. Lindstrom, J. M., Seybold, M. E., Lennon, V. A., Whittingham, S. & Duane, D. D. Antibody to acetylcholine-receptor in myasthenia-gravis - prevalence, clinical correlates, and diagnostic value. *Neurology* **26**, 1054–1059 (1976).
46. Toyka, K. V., Drachman, D. B., Pestronk, A. & Kao, I. Myasthenia-gravis - passive transfer from man to mouse. *Science* **190**, 397–399 (1975).
47. Zisimopoulou, P., Brenner, T., Trakas, N. & Tzartos, S. J. Serological diagnostics in myasthenia gravis based on novel assays and recently identified antigens. *Autoimmun. Rev.* **12**, 924–930 (2013).
48. Changeux, J. P. The nicotinic acetylcholine receptor: the founding father of the pentameric ligand-gated ion channel superfamily. *J. Biol. Chem.* **287**, 40207–40215 (2012).
49. Luo, J. et al. Main immunogenic region structure promotes binding of conformation-dependent myasthenia gravis autoantibodies, nicotinic acetylcholine receptor conformation maturation, and agonist sensitivity. *J. Neurosci.* **29**, 13898–13908 (2009).
50. Tzartos, S. J. et al. Anatomy of the antigenic structure of a large membrane autoantigen, the muscle-type nicotinic acetylcholine receptor. *Immunol. Rev.* **163**, 89–120 (1998).
51. Noridomi, K., Watanabe, G., Hansen, M. N., Han, G. W. & Chen, L. Structural insights into the molecular mechanisms of myasthenia gravis and their therapeutic implications. *eLife* **6**, e23043 (2017).
This study clarifies the pathogenetic effect of anti-AChR antibodies.
52. Tuzun, E. & Christadoss, P. Complement associated pathogenic mechanisms in myasthenia gravis. *Autoimmun. Rev.* **12**, 904–911 (2013).
53. Kordas, G., Lagoumintzis, G., Sideris, S., Poulas, K. & Tzartos, S. J. Direct proof of the in vivo pathogenic role of the AChR autoantibodies from myasthenia gravis patients. *PLOS ONE* **10**, e0117673 (2015).
54. Gilhus, N. E. et al. Myasthenia gravis - autoantibody characteristics and their implications for therapy. *Nat. Rev. Neurol.* **12**, 259–268 (2016).
55. Hara, H. et al. Detection and characterization of blocking-type antiacetylcholine receptor antibodies in sera from patients with myasthenia-gravis. *Clin. Chem.* **39**, 2053–2057 (1993).
56. Messeant, J. et al. MuSK frizzled-like domain is critical for mammalian neuromuscular junction formation and maintenance. *J. Neurosci.* **35**, 4926–4941 (2015).
57. Li, L. et al. Enzymatic activity of the scaffold protein rapsyn for synapse formation. *Neuron* **92**, 1007–1019 (2016).
58. Hoch, W. et al. Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. *Nat. Med.* **7**, 365–368 (2001).
59. Koneczny, I. et al. IgG4 autoantibodies against muscle-specific kinase undergo Fab-arm exchange in myasthenia gravis patients. *J. Autoimmun.* **77**, 104–115 (2017).
60. Huijbers, M. G. et al. MuSK IgG4 autoantibodies cause myasthenia gravis by inhibiting binding between MuSK and Lrp4. *Proc. Natl Acad. Sci. USA* **110**, 20783–20788 (2013).
This study represents an achievement in understanding the role of anti-MuSK antibodies in disease development.
61. Huijbers, M. G. et al. Longitudinal epitope mapping in MuSK myasthenia gravis: implications for disease severity. *J. Neuroimmunol.* **291**, 82–88 (2016).
62. Poulas, K., Koutsouraki, E., Kordas, G., Kokla, A. & Tzartos, S. J. Anti-MuSK- and anti-AChR-positive myasthenia gravis induced by d-penicillamine. *J. Neuroimmunol.* **250**, 94–98 (2012).
63. Kim, N. et al. Lrp4 is a receptor for agrin and forms a complex with MuSK. *Cell* **135**, 334–342 (2008).
64. Higuchi, O., Hamuro, J., Motomura, M. & Yamanashi, Y. Autoantibodies to low-density lipoprotein receptor-related protein 4 in myasthenia gravis. *Ann. Neurol.* **69**, 418–422 (2011).
65. Tzartos, J. S. et al. LRP4 antibodies in serum and CSF from amyotrophic lateral sclerosis patients. *Ann. Clin. Transl Neurol.* **1**, 80–87 (2014).
66. Shen, C. Y. et al. Antibodies against low-density lipoprotein receptor-related protein 4 induce myasthenia gravis. *J. Clin. Invest.* **123**, 5190–5202 (2013).
67. Ulusoy, C., Cavus, F., Yilmaz, V. & Tuzun, E. Immunization with recombinantly expressed LRP4 induces experimental autoimmune myasthenia gravis in C57BL/6 mice. *Immunol. Invest.* **46**, 490–499 (2017).
68. Mori, S. et al. Immunization of mice with LRP4 induces myasthenia similar to MuSK-associated myasthenia gravis. *Exp. Neurol.* **297**, 158–167 (2017).
69. Witzemann, V., Chevessier, F., Pacifici, P. G. & Yampolsky, P. The neuromuscular junction: selective remodeling of synaptic regulators at the nerve/muscle interface. *Mech. Dev.* **130**, 402–411 (2013).
70. Gasperi, C. et al. Anti-agrin autoantibodies in myasthenia gravis. *Neurology* **82**, 1976–1983 (2014).
71. Yan, M. et al. Induction of anti-agrin antibodies causes myasthenia gravis in mice. *Neuroscience* **373**, 113–121 (2018).
72. Cossins, J. et al. in *Myasthenia Gravis and Related Disorders II: 12th International Conference* Vol. 1275 (eds Wolfe, G. I., Meriggioli, M. N., Ciafaloni, E. & Ruff, R. L.) 123–128 (Wiley, 2012).
73. Romi, F. et al. Anti-voltage-gated potassium channel Kv1.4 antibodies in myasthenia gravis. *J. Neurol.* **259**, 1312–1316 (2012).
74. Suzuki, S. et al. Cardiac involvements in myasthenia gravis associated with anti-Kv1.4 antibodies. *Eur. J. Neurol.* **21**, 223–230 (2014).
75. O'Connell, K. M. S., Whitesell, J. D. & Tamkun, M. M. Localization and mobility of the delayed-rectifer K^+ channel Kv2.1 in adult cardiomyocytes. *Am. J. Physiol. Heart Circ. Physiol.* **294**, H229–H237 (2008).
76. Powers, K. et al. Titin force enhancement following active stretch of skinned skeletal muscle fibres. *J. Exp. Biol.* **220**, 3110–3118 (2017).
77. Romi, F., Hong, Y. & Gilhus, N. E. Pathophysiology and immunological profile of myasthenia gravis and its subgroups. *Curr. Opin. Immunol.* **49**, 9–13 (2017).
78. Szczudlik, P. et al. Antititin antibody in early- and late-onset myasthenia gravis. *Acta Neurol. Scand.* **130**, 229–233 (2014).
79. Gautel, M. et al. Titin antibodies in myasthenia-gravis - identification of a major immunogenic region of titin. *Neurology* **43**, 1581–1585 (1993).
80. Santulli, G., Nakashima, R., Yuan, Q. & Marks, A. R. Intracellular calcium release channels: an update. *J. Physiol.* **595**, 3041–3051 (2017).
81. Mygland, A. et al. Ryanodine receptor autoantibodies in myasthenia-gravis patients with a thymoma. *Ann. Neurol.* **32**, 589–591 (1992).
82. Romi, F., Skeie, G. O., Gilhus, N. E. & Aarli, J. A. Striational antibodies in myasthenia gravis - Reactivity and possible clinical significance. *Arch. Neurol.* **62**, 442–446 (2005).
83. Gallardo, E. et al. Cortactin autoantibodies in myasthenia gravis. *Autoimmun. Rev.* **13**, 1003–1007 (2014).
84. Illa, I., Cortes-Vicente, E., Martinez, M. A. & Gallardo, E. Diagnostic utility of cortactin antibodies in myasthenia gravis. *Ann. NY Acad. Sci.* **1412**, 90–94 (2018).
85. Labrador-Horrillo, M. et al. Identification of a novel myositis-associated antibody directed against cortactin. *Autoimmun. Rev.* **13**, 1008–1012 (2014).
86. Strobel, P. et al. The ageing and myasthenic thymus: a morphometric study validating a standard procedure in the histological workup of thymic specimens. *J. Neuroimmunol.* **201**, 64–73 (2008).
87. Wolfe, G. I. et al. Randomized trial of thymectomy in myasthenia gravis. *N. Engl. J. Med.* **375**, 511–522 (2016).
This randomized trial confirms the efficacy of thymectomy in some MG subgroups with anti-AChR antibodies.
88. Cron, M. A. et al. Thymus involvement in early-onset myasthenia gravis. *Ann. NY Acad. Sci.* **1412**, 137–145 (2018).
89. Valencia, X. & Lipsky, P. E. $CD4^+$ $CD25^+$ $FoxP3^+$ regulatory T cells in autoimmune diseases. *Nat. Clin. Pract. Rheumatol.* **3**, 619–626 (2007).
90. Cheng, M. & Anderson, M. S. Thymic tolerance as a key brake on autoimmunity. *Nat. Immunol.* **19**, 659–664 (2018).
91. Weis, C. A., Schalke, B., Strobel, P. & Marx, A. Challenging the current model of early-onset myasthenia gravis pathogenesis in the light of the MGTX trial and histological heterogeneity of thymectomy specimens. *Ann. NY Acad. Sci.* **1413**, 82–91 (2018).
92. Yi, J. S. et al. Characterization of CD4 and CD8 T cell responses in MuSK myasthenia gravis. *J. Autoimmun.* **52**, 130–138 (2014).
93. Buckner, J. H. Mechanisms of impaired regulation by $CD4^+CD25^+FOXP3^+$ regulatory T cells in human autoimmune diseases. *Nat. Rev. Immunol.* **10**, 849–859 (2010).
94. Luther, C. et al. Prednisolone treatment induces tolerogenic dendritic cells and a regulatory milieu in myasthenia gravis patients. *J. Immunol.* **183**, 841–848 (2009).
95. Gradolatto, A. et al. Both Treg cells and Tconv cells are defective in the myasthenia gravis thymus: roles of IL-17 and TNF-alpha. *J. Autoimmun.* **52**, 53–63 (2014).
96. Marx, A. et al. Thymoma related myasthenia gravis in humans and potential animal models. *Exp. Neurol.* **270**, 55–65 (2015).
97. Leite, M. I. et al. Fewer thymic changes in MuSK antibody-positive than in MuSK antibody-negative MG. *Ann. Neurol.* **57**, 444–448 (2005).
98. Singman, E. L., Matta, N. S. & Silbert, D. I. Use of the Cogan lid twitch to identify myasthenia gravis. *J. Neuroophthalmol.* **31**, 239–240 (2011).
99. Van Stavern, G. P., Bhatt, A., Haviland, J. & Black, E. H. A prospective study assessing the utility of Cogan's lid twitch sign in patients with isolated unilateral or bilateral ptosis. *J. Neurol. Sci.* **256**, 84–85 (2007).
100. Chatzistefanou, K. I. et al. The ice pack test in the differential diagnosis of myasthenic diplopia. *Ophthalmology* **116**, 2236–2243 (2009).
101. Yamamoto, D. et al. Effect of local cooling on excitation-contraction coupling in myasthenic muscle: another mechanism of ice-pack test in myasthenia gravis. *Clin. Neurophysiol.* **128**, 2309–2317 (2017).
102. Richards, J. & Howard, J. F. Seronegative myasthenia gravis associated with malignant thymoma. *Neuromuscul. Disord.* **27**, 417–418 (2017).
103. Stergiou, C. et al. Titin antibodies in "seronegative" myasthenia gravis - a new role for an old antigen. *J. Neuroimmunol.* **292**, 108–115 (2016).
104. Leite, M. I. et al. IgG1 antibodies to acetylcholine receptors in 'seronegative' myasthenia gravis. *Brain* **131**, 1940–1952 (2008).
This study discovers that some MG autoantibodies are undetectable by the classical methods and introduces cell-based assays.
105. Cruz, P. M. R. et al. Clinical features and diagnostic usefulness of antibodies to clustered acetylcholine receptors in the diagnosis of seronegative myasthenia gravis. *JAMA Neurol.* **72**, 642–649 (2015).
This study reports the characteristics of all patients with anti-AChR antibodies exclusively identified by the cell-based assay.
106. Vincent, A. et al. in *Myasthenia Gravis and Related Disorders: 11th International Conference* Vol. 1132 (eds Kaminski, H. J. & Barohn, R. J.) 84–92 (The New York Academy of Sciences, 2008).
107. Tsonis, A. I. et al. MuSK autoantibodies in myasthenia gravis detected by cell based assay - a multinational study. *J. Neuroimmunol.* **284**, 10–17 (2015).
108. Huda, S. et al. IgG-specific cell-based assay detects potentially pathogenic MuSK-Abs in seronegative MG. *Neurol. Neuroimmunol. Neuroinflamm.* **4**, e357 (2017).
109. Liik, M. & Punga, A. R. Repetitive nerve stimulation often fails to detect abnormal decrement in acute severe generalized myasthenia gravis. *Clin. Neurophysiol.* **127**, 3480–3484 (2016).
110. Nikolic, A., Basta, I., Stojanovic, V. R., Stevic, Z. & Lavrnic, D. Electrophysiological profile of the patients with MuSK positive myasthenia gravis. *Neurol. Res.* **36**, 945–949 (2014).
111. Nikolic, A. V., Bojic, S. D., Stojanovic, V. M. R., Basta, I. Z. & Lavrnic, D. V. Electrophysiological findings in patients with low density lipoprotein receptor related protein 4 positive myasthenia gravis. *Eur. J. Neurol.* **23**, 1635–1641 (2016).
112. Stalberg, E., Sanders, D. B. & Kouyoumdjian, J. A. Pitfalls and errors in measuring jitter. *Clin. Neurophysiol.* **128**, 2233–2241 (2017).
This article informs about how to interpret key neurophysiological findings in MG.
113. Thornton, R. C. & Michell, A. W. Techniques and applications of EMG: measuring motor units from structure to function. *J. Neurol.* **259**, 585–594 (2012).
114. Cruz, P. M. R., Palace, J. & Beeson, D. The neuromuscular junction and wide heterogeneity of congenital myasthenic syndromes. *Int. J. Mol. Sci.* **19**, E1677 (2018).
115. Lee, M., Beeson, D. & Palace, J. Therapeutic strategies for congenital myasthenic syndromes. *Ann. NY Acad. Sci.* **1412**, 129–136 (2018).
116. Titulaer, M. J., Lang, B. & Verschuuren, J. Lambert-Eaton myasthenic syndrome: from clinical characteristics to therapeutic strategies. *Lancet Neurol.* **10**, 1098–1107 (2011).
117. Verschuuren, J., Strijbos, E. & Vincent, A. Neuromuscular junction disorders. *Handb. Clin. Neurol.* **133**, 447–466 (2016).
118. Sanders, D. B. et al. Is the decremental pattern in Lambert-Eaton syndrome different from that in myasthenia gravis? *Clin. Neurophysiol.* **125**, 1274–1277 (2014).

119. Di Lorenzo, R. et al. Low specificity of voltage-gated calcium channel antibodies in Lambert-Eaton myasthenic syndrome: a call for caution. *J. Neurol.* **265**, 2114–2119 (2018).
120. Lacey, B., Chang, W. & Rootman, J. Nonthyroid causes of extraocular muscle disease. *Surv. Ophthalmol.* **44**, 187–213 (1999).
121. Skeie, G. O. et al. Guidelines for treatment of autoimmune neuromuscular transmission disorders. *Eur. J. Neurol.* **17**, 893–902 (2010).
122. Sanders, D. B. et al. International consensus guidance for management of myasthenia gravis: executive summary. *Neurology* **87**, 419–425 (2016).
123. Sussman, J. et al. The Association of British Neurologists' myasthenia gravis guidelines. *Ann. NY Acad. Sci.* **1412**, 166–169 (2018).
124. Murai, H. et al. Rationale for the clinical guidelines for myasthenia gravis in Japan. *Ann. NY Acad. Sci.* **1413**, 35–40 (2018).
125. Pitha, J. et al. Guideline for the diagnosis and therapy of myasthenia gravis. *Cesk. Neurol. Neurochir.* **75**, 244–252 (2012).
126. Gilhus, N. E., Kerty, E., Loseth, S., Mygland, A. & Tallaksen, C. Myasthenia gravis - diagnosis and treatment [Norwegian]. *Tidsskr. Nor. Laegeforen.* **136**, 1089–1094 (2016).
127. Evoli, A. & Padua, L. Diagnosis and therapy of myasthenia gravis with antibodies to muscle-specific kinase. *Autoimmun. Rev.* **12**, 931–935 (2013).
128. Bonanno, S. et al. Amifampridine phosphate in the treatment of muscle-specific kinase myasthenia gravis: a phase IIb, randomized, double-blind, placebo-controlled, double crossover study. *Sage Open Med.* **6**, 2050312118819013 (2018).
129. Mantegazza, R., Bonanno, S., Camera, G. & Antozzi, C. Current and emerging therapies for the treatment of myasthenia gravis. *Neuropsychiatr. Dis. Treat* **7**, 151–160 (2011).
130. Meriggioli, M. N. & Sanders, D. B. Autoimmune myasthenia gravis: emerging clinical and biological heterogeneity. *Lancet Neurol.* **8**, 475–490 (2009).
131. Sieb, J. P. Myasthenia gravis: an update for the clinician. *Clin. Exp. Immunol.* **175**, 408–418 (2014).
132. Sanders, D. B. & Evoli, A. Immunosuppressive therapies in myasthenia gravis. *Autoimmunity* **43**, 428–435 (2010).
133. Utsugisawa, K. et al. Health-related quality-of-life and treatment targets in myasthenia gravis. *Muscle Nerve* **50**, 493–500 (2014).
134. Gotterer, L. & Li, Y. B. Maintenance immunosuppression in myasthenia gravis. *J. Neurol. Sci.* **369**, 294–302 (2016).
135. Benatar, M. et al. Efficacy of prednisone for the treatment of ocular myasthenia (epitome): a randomized, controlled trial. *Muscle Nerve* **53**, 363–369 (2016).
136. El-Salem, K. et al. Treatment of MuSK-associated myasthenia gravis. *Curr. Treat Options Neurol.* **16**, 283 (2014).
137. Hobson-Webb, L. D. et al. Can mycophenolate mofetil be tapered safely in myasthenia gravis? A retrospective, multicenter analysis. *Muscle Nerve* **52**, 211–215 (2015).
138. Sathasivam, S. Current and emerging treatments for the management of myasthenia gravis. *Ther. Clin. Risk Manag.* **7**, 313–323 (2011).
139. Hehir, M. K. et al. Mycophenolate mofetil in AChR-antibody-positive myasthenia gravis: outcomes in 102 patients. *Muscle Nerve* **41**, 593–598 (2010).
140. Cruz, J. L., Wolff, M. L., Vanderman, A. J. & Brown, J. N. The emerging role of tacrolimus in myasthenia gravis. *Ther. Adv. Neurol. Disord.* **8**, 92–103 (2015).
141. Tindall, R. S. A., Phillips, J. T., Rollins, J. A., Wells, L. & Hall, K. A. Clinical therapeutic trial of cyclosporine in myasthenia-gravis. *Ann. NY Acad. Sci.* **681**, 539–551 (1993).
142. Palace, J., Newsom-Davis, J. & Lecky, B. A randomized double-blind trial of prednisolone alone or with azathioprine in myasthenia gravis. *Neurology* **50**, 1778–1783 (1998).
This study still represents the scientific proof for the effect of combination immunosuppressive therapy in MG.
143. Sanders, D. B. et al. A trial of mycophenolate mofetil with prednisone as initial immunotherapy in myasthenia gravis. *Neurology* **71**, 394–399 (2008).
144. Sanders, D. B. et al. An international, phase III, randomized trial of mycophenolate mofetil in myasthenia gravis. *Neurology* **71**, 400–406 (2008).
145. Nagane, Y., Utsugisawa, K., Obara, D., Kondoh, R. & Terayama, Y. Efficacy of low-dose FK506 in the treatment of myasthenia gravis - a randomized pilot study. *Eur. Neurol.* **53**, 146–150 (2005).
146. Yoshikawa, H., Kiuchi, T., Saida, T. & Takamori, M. Randomised, double-blind, placebo-controlled study of tacrolimus in myasthenia gravis. *J. Neurol. Neurosurg. Psychiatry* **82**, 970–977 (2011).
147. Pasnoor, M. et al. A randomized controlled trial of methotrexate for patients with generalized myasthenia gravis. *Neurology* **87**, 57–64 (2016).
148. Heckmann, J. M., Rawoot, A., Bateman, K., Renison, R. & Badri, M. A single-blinded trial of methotrexate versus azathioprine as steroid-sparing agents in generalized myasthenia gravis. *BMC Neurol.* **11**, 97 (2011).
149. Tandan, R., Hehir, M. K., Waheed, W. & Howard, D. B. Rituximab treatment of myasthenia gravis: a systematic review. *Muscle Nerve* **56**, 185–196 (2017).
This article reviews the evidence thus far for rituximab as a promising therapy in MG.
150. Robeson, K. R. et al. Durability of the rituximab response in acetylcholine receptor autoantibody-positive myasthenia gravis. *JAMA Neurol.* **74**, 60–66 (2017).
151. Anderson, D., Phan, C., Johnston, W. S. & Siddiqi, Z. A. Rituximab in refractory myasthenia gravis: a prospective, open-label study with long-term follow-up. *Ann. Clin. Transl Neurol.* **3**, 552–555 (2016).
152. Kanth, K. M., Solorzano, G. E. & Goldman, M. D. PML in a patient with myasthenia gravis treated with multiple immunosuppressing agents. *Neurol. Clin. Pract.* **6**, E17–E19 (2016).
153. Afanasiev, V. et al. Resistant myasthenia gravis and rituximab: a monocentric retrospective study of 28 patients. *Neuromuscul. Disord.* **27**, 251–258 (2017).
154. Howard, J. F. 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**, 976–986 (2017).
This article reports the promising results regarding the effect of a complement inhibitory drug in MG.
155. Gilhus, N. E. Eculizumab: a treatment option for mysthenia gravis? *Lancet Neurol.* **16**, 947–948 (2017).
156. Hewett, K. et al. Randomized study of adjunctive belimumab in participants with generalized myasthenia gravis. *Neurology* **90**, E1425–E1434 (2018).
157. De Feo, L. G., Schottlender, J., Martelli, N. A. & Molfino, N. A. Use of intravenous pulsed cyclophosphamide in severe, generalized myasthenia gravis. *Muscle Nerve* **26**, 31–36 (2002).
158. Drachman, D. B., Adams, R. N., Hu, R., Jones, R. J. & Brodsky, R. A. in *Myasthenia Gravis and Related Disorders: 11th International Conference* Vol. 1132 (eds Kaminski, H. J. & Barohn, R. J.) 305–314 (The New York Academy of Sciences, 2008).
159. Bryant, A. et al. Myasthenia gravis treated with autologous hematopoietic stem cell transplantation. *JAMA Neurol.* **73**, 652–658 (2016).
160. Gelfand, E. W. Mechanisms of disease intravenous immune globulin in autoimmune and inflammatory diseases. *N. Engl. J. Med.* **367**, 2015–2025 (2012).
161. Gajdos, P., Chevret, S., Clair, B., Tranchant, C. & Chastang, C. Clinical trial of plasma exchange and high-dose intravenous immunoglobulin in myasthenia gravis. *Ann. Neurol.* **41**, 789–796 (1997).
162. Gajdos, P., Chevret, S. & Toyka, K. V. Intravenous immunoglobulin for myasthenia gravis. *Cochrane Database Syst. Rev.* **12**, CD002277 (2012).
163. Bourque, P. R., Pringle, C. E., Cameron, W., Cowan, J. & Chardon, J. W. Subcutaneous immunoglobulin therapy in the chronic management of myasthenia gravis: a retrospective cohort study. *PLOS ONE* **11**, e0159993 (2016).
164. Beecher, G., Anderson, D. & Siddiqi, Z. A. Subcutaneous immunoglobulin in myasthenia gravis exacerbation: A prospective, open-label trial. *Neurology* **89**, 1135–1141 (2017).
165. Meyer, D. M. et al. Comparative clinical outcomes of thymectomy for myasthenia gravis performed by extended transsternal and minimally invasive approaches. *Ann. Thorac Surg.* **87**, 385–391 (2009).
166. Keijzers, M. et al. Robotic thymectomy in patients with myasthenia gravis: neurological and surgical outcomes. *Eur. J. Cardiothorac Surg.* **48**, 40–45 (2015).
167. Gronseth, G. S. & Barohn, R. J. Practice parameter: thymectomy for autoimmune myasthenia gravis (an evidence-based review) - report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology* **55**, 7–15 (2000).
168. Rahbek, M. A. et al. Exercise in myasthenia gravis: a feasibility study of aerobic and resistance training. *Muscle Nerve* **56**, 700–709 (2017).
169. Westerberg, E., Molin, C. J., Lindblad, I., Emtner, M. & Punga, A. R. Physical exercise in myasthenia gravis is safe and improves neuromuscular parameters and physical performance-based measures: a pilot study. *Muscle Nerve* **56**, 207–214 (2017).
170. Nicolle, M. W. et al. Sleep apnea in patients with myasthenia gravis. *Neurology* **67**, 140–142 (2006).
171. Pedersen, E. G. et al. Risk of non-melanoma skin cancer in myasthenia patients treated with azathioprine. *Eur. J. Neurol.* **21**, 454–458 (2014).
172. Pedersen, E. G. et al. Myasthenia and risk of cancer: a population-based case-control study. *Eur. J. Neurol.* **21**, 773–778 (2014).
173. Owe, J. F., Cvancarova, M., Romi, F. & Gilhus, N. E. Extrathymic malignancies in thymoma patients with and without myasthenia gravis. *J. Neurol. Sci.* **290**, 66–69 (2010).
174. Norwood, F. et al. Myasthenia in pregnancy: best practice guidelines from a UK multispecialty working group. *J. Neurol. Neurosurg. Psychiatry* **85**, 538–543 (2014).
This article is a useful consensus document regarding treatment for MG for women of child-bearing potential and during pregnancy.
175. Gilhus, N. E. & Hong, Y. Maternal myasthenia gravis represents a risk for the child through autoantibody transfer, immunosuppressive therapy and genetic influence. *Eur. J. Neurol.* **25**, 1402–1409 (2018).
176. Wolfe, G. I. et al. Myasthenia gravis activities of daily living profile. *Neurology* **52**, 1487–1489 (1999).
177. Barnett, C., Bril, V., Kapral, M., Kulkarni, A. & Davis, A. M. Development and validation of the Myasthenia Gravis Impairment Index. *Neurology* **87**, 879–886 (2016).
178. Burns, T. M., Grouse, C. K., Conaway, M. R. & Sanders, D. B. Construct and concurrent validation of the MG-QOL15 in the practice setting. *Muscle Nerve* **41**, 219–226 (2010).
179. Burns, T. M. et al. The MG-QOL15 for following the health-related quality of life of patients with myasthenia gravis. *Muscle Nerve* **43**, 14–18 (2011).
180. Burns, T. M. et al. International clinimetric evaluation of the MG-QOL15, resulting in slight revision and subsequent validation of the MG-QOL15R. *Muscle Nerve* **54**, 1015–1022 (2016).
181. Padua, L. et al. Myasthenia gravis outcome measure: development and validation of a disease-specific self-administered questionnaire. *Neurol. Sci.* **23**, 59–68 (2002).
182. Mullins, L. L., Carpentier, M. Y., Paul, R. H. & Sanders, D. B. Disease-specific measure of quality of life for myasthenia gravis. *Muscle Nerve* **38**, 947–956 (2008).
183. Koopman, W. J., LeBlanc, N., Fowler, S., Hulley, D. & Nicolle, M. W. Hope, well-being, coping, and quality of life in adults with myasthenia gravis [abstract]. *Muscle Nerve* **52**, S141 (2015).
184. Jaretzki, A. et al. Myasthenia gravis - recommendations for clinical research standards. *Neurology* **55**, 16–23 (2000).
185. Burns, T. M., Conaway, M. & Sanders, D. B. The MG Composite: a valid and reliable outcome measure for myasthenia gravis. *Neurology* **74**, 1434–1440 (2010).
This article illustrates the importance of systematic testing and follow-up of patients with MG.
186. Behin, A. & Le Panse, R. New pathways and therapeutic targets in autoimmune myasthenia gravis. *J. Neuromuscul. Dis.* **5**, 265–277 (2018).
This article provides a nice overview of potential, new treatment targets in MG.
187. Mantegazza, R., Bernasconi, P. & Cavalcante, P. Myasthenia gravis: from autoantibodies to therapy. *Curr. Opin. Neurol.* **31**, 517–525 (2018).
188. Molina, V. et al. Immunomodulation of experimental pulmonary fibrosis by intravenous immunoglobulin (IVIG). *Autoimmunity* **39**, 711–717 (2006).
189. He, D. et al. Molecular and clinical relationship between live-attenuated Japanese encephalitis vaccination and childhood onset myasthenia gravis. *Ann. Neurol.* **84**, 386–400 (2018).
This article describes how specific vaccination may be involved in the onset of MG.
190. Makino, T. et al. Analysis of peripheral B cells and autoantibodies against the anti-nicotinic acetylcholine receptor derived from patients with myasthenia gravis using single-cell manipulation tools. *PLOS ONE* **12**, e0185976 (2017).
191. Cron, M. A. et al. Analysis of microRNA expression in the thymus of myasthenia gravis patients opens new

research avenues. *Autoimmun. Rev.* **17**, 588–600 (2018).
192. Sabre, L., Maddison, P., Sadalage, G., Ambrose, P. A. & Punga, A. R. Circulating microRNA miR-21-5p, miR-150-5p and miR-30e-5p correlate with clinical status in late onset myasthenia gravis. *J. Neuroimmunol.* **321**, 164–170 (2018).
193. Cruz, P. M. R., Huda, S., Lopez-Ruiz, P. & Vincent, A. Use of cell-based assays in myasthenia gravis and other antibody-mediated diseases. *Exp. Neurol.* **270**, 66–71 (2015).
194. Trakas, N. & Tzartos, S. J. Immunostick ELISA for rapid and easy diagnosis of myasthenia gravis. *J. Immunol. Methods* **460**, 107–112 (2018).
195. Valko, Y. et al. Ocular vestibular evoked myogenic potentials as a test for myasthenia gravis. *Neurology* **86**, 660–668 (2016).
196. Chan, J. W. & Orrison, W. W. Ocular myasthenia: a rare presentation with MuSK antibody and bilateral extraocular muscle atrophy. *Br. J. Ophthalmol.* **91**, 842–843 (2007).
197. Priola, A. M. et al. Comparison of CT and chemical-shift MRI for differentiating thymoma from non-thymomatous conditions in myasthenia gravis: value of qualitative and quantitative assessment. *Clin. Radiol.* **71**, E157–E169 (2016).
198. Sala, T. P. et al. Efficacy and patient satisfaction in the use of subcutaneous immunoglobulin immunotherapy for the treatment of auto-immune neuromuscular diseases. *Autoimmun. Rev.* **17**, 873–881 (2018).
199. Lazaridis, K., Dalianoudis, I., Baltatzidi, V. & Tzartos, S. J. Specific removal of autoantibodies by extracorporeal immunoadsorption ameliorates experimental autoimmune myasthenia gravis. *J. Neuroimmunol.* **312**, 24–30 (2017).
200. Ulrichts, P. et al. Neonatal Fc receptor antagonist efgartigimod safely and sustainably reduces IgGs in humans. *J. Clin. Invest.* **128**, 4372–4386 (2018). **This article describes a class of drugs that may offer a new therapeutic approach in IgG-driven autoimmune disease**.
201. Devanaboyina, S. C., Khare, P., Challa, D. K., Ober, R. J. & Ward, E. S. Engineered clearing agents for the selective depletion of antigen-specific antibodies. *Nat. Commun.* **8**, 15314 (2017).
202. Lonze, B. E. et al. IdeS (Imlifidase): a novel agent that cleaves human IgG and permits successful kidney transplantation across high-strength donor-specific antibody. *Ann. Surg.* **268**, 488–496 (2018).
203. Lipka, A. F. et al. Ephedrine treatment for autoimmune myasthenia gravis. *Neuromuscul. Disord.* **27**, 259–265 (2017).
204. Sanders, D. B. et al. A double-blinded, randomized, placebo-controlled trial to evaluate efficacy, safety, and tolerability of single doses of tirasemtiv in patients with acetylcholine receptor-binding antibody-positive myasthenia gravis. *Neurotherapeutics* **12**, 455–460 (2015).
205. Unwin, N. Nicotinic acetylcholine receptor and the structural basis of neuromuscular transmission: insights from Torpedo postsynapticmembranes. *Q. Rev. Biophys.* **46**, 283–322 (2013).
206. Stiegler, A. L., Burden, S. J. & Hubbard, S. R. Crystal structure of the frizzled-like cysteine-rich domain of the receptor tyrosine kinase MuSK. *J. Mol. Biol.* **393**, 1–9 (2009).
207. Zong, Y. N. et al. Structural basis of agrin-LRP4-MuSK signaling. *Genes Dev.* **26**, 247–258 (2012).

Author contributions

All authors have contributed to and are responsible for all parts of the Review. The first draft was written by N.E.G. (Introduction, Epidemiology and Overview of Primer); S.T. (Mechanisms/pathophysiology); J.P. (Diagnosis, screening and prevention); A.E. (Management); T.M.B. (Quality of life); and J.J.G.M.V. (Outlook).

Competing interests

N.E.G. has received speaker's honoraria from Octapharma and Alexion and consulting honoraria from Argenx and Ra Pharma. S.T. has shares in the research and diagnostic laboratory Tzartos NeuroDiagnostics. A.E. was a member of the advisory board for Alexion and is a scientific award jury member for Grifols and a safety data monitor for UCB. J.P. has received travel support or honoraria from MerckSerono, Biogen Idec, Novartis, Teva, Chugai Pharma, Bayer Schering, Alexion, Roche, Genzyme, Medimmune, Eurimmune, MedDay, Abide and Argenx and grants from MerckSerono, Novartis, Biogen Idec, Teva, Abide and Bayer Schering. T.M.B. was a member of the steering committee for Argenx. The institution of J.J.G.M.V. (Leiden University Medical Centre) has received fees from Alexion, Argenx and Ra Pharma owing to consultations by J.J.G.M.V. and has received royalties for antibody tests.

Publisher's note