Exploration of Neuroprotective Therapy

Open Exploration

Open Access Review

Overview of biomarkers in myasthenia gravis

Fatemeh Afrashteh*, Rayan Rajabi

Student Research Committee, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

***Correspondence:** Fatemeh Afrashteh, Student Research Committee, School of Medicine, Iran University of Medical Sciences, Tehran, Iran. ftmhafr98@gmail.com

Academic Editor: Vijay K. Sharma, National University of Singapore, National University Health System, Singapore

Received: August 2, 2022 **Accepted:** October 14, 2022 **Published:** October 31, 2022

Cite this article: Afrashteh F, Rajabi R. Overview of biomarkers in myasthenia gravis. Explor Neuroprot Ther. 2022;2:210–25. https://doi.org/10.37349/ent.2022.00029

Abstract

Myasthenia gravis (MG) is a rare auto-immune neuromuscular junction (NMJ) disorder which is caused by formation of autoantibodies and destruction of NMJ components. The MG diagnosis is based on the symptoms, autoantibodies detection and paraclinical tests. Given that MG patients have so many differential diagnosis and various medication responses, choosing an accurate diagnosis and the therapy plan in MG is challenging. According to the studies, there are the immunologic, genetic, microRNAs, gut microbiome, and other established or newly proposed biomarkers for diagnosis and prognosis of MG. More studies are needed to provide better collection of biomarkers in MG patients and evaluate their role in MG pathology.

Keywords

Myasthenia gravis, biomarker, autoimmune disease, neuromuscular junction

Introduction

Myasthenia gravis (MG) is a rare auto-immune neuromuscular junction (NMJ) disorder that is caused by the formation of autoantibodies and the destruction of NMJ components [1]. MG is the most common NMJ disorder with a prevalence of 150–300 per million population [2].

There are several autoantibodies against NMJ components that are sensitive and specific diagnostic markers in MG: acetylcholine receptor (AChR) antibodies [which are produced in 80% of patients and are from immunoglobulin G1 (IgG1)/IgG3/IgG2/IgG4 subclass], muscle-specific kinase (MuSK) antibodies (which are produced in 7–10% of patients and are mainly from IgG4 subclass), and lipoprotein-receptor related protein 4 (LRP4) antibodies (mostly are from IgG1/IgG2 subclass) [3]. Agrin is a presynaptic protein secretion from NMJ, which activates the agrin/LRP4/MuSK/downstream of kinase 7 (Dok-7) signaling pathway. These pathways lead to the clustering of AChR on the postsynaptic membrane [4]. Destruction of each part of this pathway by autoantibodies could impair neuromuscular transmission. These pathologic mechanisms are seen as a decremental response during repetitive nerve stimulation (RNS) in MG patients. Although serum autoantibody detection is the most specific diagnostic tool for MG diagnosis, electromyography, RNS, and clinical response to cholinesterase inhibitors are useful in patients who are suspected to have other NMJ

disorders. Currently, the treatment of MG is based on cholinesterase inhibitors, corticosteroids, biological medications (belimumab, rituximab, etc.), thymectomy in some patients, plasmapheresis, and Igs [5]. The role of genetic predisposing factors, consuming some medications [statins, penicillamine, interferons (IFNs), etc.], cytokines production, T-helper 1 (Th1)/Th2 ratio alternation, B cell activation, and thymic hyperplasia are the underline possible mechanisms and triggers in MG [6].

The main MG clinical manifestations are fluctuations of muscle weakness in ocular muscles (which is presented by double vision and ptosis), bulbar muscles involvement (in patients who have dysarthria, dysphagia, and facial or jaw muscle weakness), axial muscles weakness (which causes neck flexion impairment and head) and respiratory muscles involvement. Within two years after the ocular onset of MG, up to 80% of patients will develop generalized symptoms. Alongside paraneoplastic disorders associated with thymomas such as myositis, Morvan syndrome, and pure red aplasia, MG is the most common paraneoplastic disorder in thymoma [6, 7].

MG occurs in all ages [< 50 years is early-onset MG (EOMG) and > 50 years is late-onset MG (LOMG)], even children. The symptoms of MG are sometimes unspecific for the diagnosis and some patients are seronegative which means they do not have serum auto-antibodies specific to MG. Early diagnosis of MG, prevention of progression to generalized MG, predicting the response to the treatments, and distinguishing MG from other diseases associated with muscle weakness (as well as other NMJ diseases) are challenging issues. This is why biomarkers are promising tools to help us diagnose and find the best treatment plan. Here, the known biomarkers in MG disease are described.

Immunologic biomarkers of MG

Detection of the autoantibodies against every five subunits of muscle AChR by laboratory tests is the first step of MG diagnosis [1, 8]. The AChR antibodies mostly are from IgG1 or IgG3 subclasses and radioimmunoprecipitation assay (RIPA) is a widely used method to detect them [with a specificity of 99%, a sensitivity of 85% in generalized MG, and 50% in ocular MG (OMG)] [9, 10]. Although the titer of AChR antibodies does not relate to MG severity (unlike MuSK antibodies [11]), some studies evaluate that there is a correlation between the titer of the IgG1 subclass and MG severity. Since the AChR antibody was detected up to 2 years before the onset of MG symptoms, it could be used to diagnose MG earlier [12].

The majority of MuSK antibodies are produced against the extracellular domains which are cysteine-rich Ig-like regions [13]. MuSK antibodies are detected by RIPA in 6% of all MG patients, 40% in the AChR antibody-negative patients, and 0.5–12.5% in the AChR antibody-positive (AChR+) MG patients [13]. MuSK antibodies are from the IgG4 subclass which binds to the first Ig-like domain of MuSK and restrains the agrin-induced and agrin-independent AChR clustering pathways through inhibition of interactions between MuSK and collagen Q or LRP4; interestingly, MuSK antibodies do not activate complement cascade [14, 15]. One of the best examples of indicating the role of biomarkers to predict medication response is in the MuSK antibody-positive (MuSK+) MG patients who usually progress high severity of MG, generalized MG, and are prone to adverse effects with pyridostigmine, less usefulness of thymectomy, excellent treatment response to rituximab, and getting more plasma exchange benefits [16–18].

LRP4 is a transmembrane protein, containing several low-density lipoprotein domains. LRP4 acts as a muscle receptor for agrin-induced AChR clustering at the NMJ, by passing the signal to MuSK [19]. The autoantibodies against LRP4 are mostly from the IgG1 subclass and have complement activation features to play a role in MG pathology. As well as MG, LRP4 has been detected in the cerebrospinal fluid of amyotrophic lateral sclerosis patients [20]. Based on the used method and the source of antigen, and the ethnicity of the patients the prevalence of LRP4 antibody-positive (LRP4+) MG samples in MG patients varies from 2% to 45% [1]. LRP4 antibody positivity has been found in 15–20% and 7.5% of MuSK+ MG and AChR+ MG patients, respectively [21, 22].

The other precious prognostic biomarkers are autoantibodies against titin and ryanodine receptor (RyR). Titin is a huge abundant filamentous protein located in the striated muscles which is one of the sarcomere proteins and RyR is a calcium (Ca^{2+}) channel located in the sarcoplasmic reticulum membrane which plays a

role in Ca^{2+} release from the sarcolemma to the cytoplasm to launch excitation-contraction coupling [23, 24]. Among the AChR+ MG patients, 20–40% of them also have a positive test for titin antibodies. This prevalence shifts from 6% in EOMG to 50–80% in non-thymomatous LOMG patients [25]. RyR antibodies are not detected in the EOMG but 40% of LOMG have a positive result for it. Titin and RyR antibodies are worthy biomarkers of having thymoma in EOMG patients since 50–95% of them have a positive test for titin antibodies and 75% for RyR antibodies [26–28]. Therefore, the positivity of titin and RyR antibodies is correlated with having more severe disease and thymoma.

By using RIPA, 14.6% of the MuSK+ MG and 16.4% of the LRP4+ MG patients have also positive results for titin antibodies. Although enzyme-linked immunosorbent assay (ELISA) did not detect any positive result for titin antibody in the seronegative MG (SNMG) patients, RIPA has been found in 13.4% of SNMG patients [29]. So it could be an invaluable biomarker for detecting MG in SNMG patients.

As mentioned before, agrin is a key proteoglycan involved in the signaling cascade activation resulting in AChR clustering on the postsynaptic membrane. Most MG patients who produce antibodies against AChR, MuSK, or LRP4, also have a positive result for agrin antibody and it has been detected in 2–15% of all MG patients who develop mild to severe MG and who have a mild response to medications [22, 30]. Although the prevalence of agrin antibodies in MG patients is low, the lack of agrin antibodies in other neurologic diseases or healthy people is one of the strong reasons to use agrin antibodies as a specific biomarker for MG.

There are some obstacles to interpreting the level of biomarkers in different populations. One of the pieces of evidence for this statement is the evaluation of the antibody production against Kv1.4, a subunit of voltage-gated potassium (K^+) channels, which has a function to regulate presynaptic acetylcholine release. In Japanese MG patients, 11% to 18% of patients showed a positive result of antibodies against Kv1.4, and most of them had severe symptoms, myasthenic crises, myocarditis, abnormal electrocardiogram (ECG), and thymoma [31, 32]. In contrast, 17% of Caucasian MG people showed Kv1.4 antibody positive result which was mostly found in females, LOMG, patients with mild symptoms, and OMG [33].

To find out whether a biomarker is suitable to be used to identify patients or not, one of the important factors is its specificity and sensitivity. Rapsyn is an intracellular scaffold protein located in the muscle cells, which connects the AChR to the cytoskeleton inside the cell. Although 15% of MG patients had Rapsyn antibodies in their serum, they could be present in the serum of patients with other diseases [34–36]. Similar to this, the antibody against cortactin is found in 23.7% of SNMG patients, 9.5% of seropositive MG, 12.5% of patients with other autoimmune diseases, 7.6–26% of skin disorders (polymyositis, dermatomyositis, and immune-mediated necrotizing myopathy), and 5.2% of healthy controls [37–40]. Cortactin is one of the NMJ proteins located in the cytoplasm which has a role in actin assembly and AChR clustering. The detection of cortactin autoantibodies in other patients and healthy controls diminish its role as a specific MG biomarker.

Some studies evaluated the presence of antibodies against collagen Q, an extracellular matrix protein at the NMJ which has interactions with MuSK, collagen XIII, and acetylcholinesterase in the serum of MG patients. These antibodies had no significant value for being MG biomarkers and also they have been detected in other disorders [41–44].

In addition to autoantibodies, subgroups of lymphocytes in the blood and thymus of MG patients could be interesting biomarkers. Follicular regulatory T (Tfr) cells are a subset of regulatory T cells (Tregs) that inhibit the high activation of follicular helper T (Tfh) cells and B cells in germinal centers. The Tfr/Tfh ratio is negatively related to the severity of MG. The depletion of $CD4^+$ C-X-C chemokine receptor type 5 $(CXCR5)^+$ forkhead box P3 $(FOXP3)^+$ Tfr-like cells and an increased number of $CD4^+CXCR5^+FOXP3^-$ Tfh-like cells are seen in MG patients. This means that B cells and T cell activation control are disrupted by Treg cell imbalance in MG [45].

Th1-like Th17 [Th1/17; IFN-γ^+ interleukin-17 $(IL\text{-}17)^+$ $CD4^+CD3^+$] cells are pro-inflammatory trigger cells of Th17 group cells which are increased in the AChR+ MG patients and are decreased faster than Th17 cells in the good responder's immunosuppression treatment as in the non-responders [46].

MG patients had significantly fewer granulocyte-monocyte colony-stimulating factor (GM-CSF)-expressing cells or GM-CSF-expressing Th cells and ThCD103 than controls in the blood and

these cells are also collected in the thymus of the AChR+ MG patients. These subgroups of T cells could be potential biomarkers for the disease severity of MG patients since they are correlated inversely with the MG severity [47]. This study also suggests the tumor necrosis factor (TNF)-producing ThCD103 cell subset could be a valuable promising candidate as a biomarker of MG severity.

The surface lymphocyte molecules are another biomarker in MG. Inducible costimulator (ICOS)/ICOS ligand (ICOSL) are expressed on the activated or memory T cells and in the primary and secondary follicles of lymph nodes, respectively. These markers are responsible for the conversion of types of antibodies and B cell activation [48]. Programmed death protein-1 (PD-1)/PD-1 ligand (PD-L1) are inhibitors of B cell proliferation which are expressed on the surface of $CD4^+$/$CD8^+$ T cells, B cells, dendritic cells (DCs), activated T cells, and non-hematopoietic cells [49]. The PD-1/PD-L1 suppression and ICOS/ICOSL activation lead to inflammatory pathology in autoimmune diseases like MG [50]. This means that these molecules are involved in MG pathology and could be used as therapeutic/diagnostic markers. In addition to addressing T cell surface molecules, we take a glance at the B cell surface molecules. T cell Ig and mucin domain-1 (Tim-1) is expressed on the B cells and has a role in keeping immune tolerance. Tim-1 is down-regulated in B cells in MG patients and inversely correlates with MG severity [51]. Another study measured the CD72 expression on the B cells in MG, multiple sclerosis (MS), and healthy controls [52]. CD72 showed low expression in MG and MS patients. Intercellular adhesion molecule 1 (ICAM-1) and CD25 were introduced as new biomarkers in MG patients but in the same study, soluble forms of CD28, CD80, CD86, and CD152 were not significantly different in MG *vs.* controls [53].

ILs are renowned biomarkers that let us diagnose and foresee the prognosis of disease precisely. The increased levels of IL-17A, IFN-γ, and IL-21 are mainly the feature of MuSK+ MG, and IL-17A, IL-21, IL-4, and IL-10 are increased in AChR+ MG. After immunosuppressive therapy, IL-10 increases but IFN-γ decreases in AChR+ MG patients [54, 55]. In addition, another more recent study showed that in the generalized AChR+ MG patients, IL-4, IL-5, IL-2, and IL-12-P70 increased [56]. IL-6 is associated with MG severity in AChR+ MG patients and the use of anti-IL-6 therapy, like tocilizumab, causes a decreased level of IL-6 [57]. IL-33, proliferation-inducing ligand, IL-19, IL-20, IL-28A, IL-35, IL-27, and IL-36γ are other novel biomarkers that are increased in MG patients [58–60]. In addition, other inflammatory proteins increase in MG and could be used as biomarkers alongside other specific MG biomarkers: metalloproteinase 10, transforming growth factor-α (TGFα), S100 Ca^{2+}-binding protein A12 (S100A12), IL-6, IL-8, C-C motif ligand 19, and C-X-C motif ligand 1 [61].

Free light chains (FLC) of autoantibodies, were introduced as novel biomarkers in MG. Recently, an interesting study showed that κ FLC, but not λ FLC, increases in the SNMG and OMG. The authors concluded the κ/λ ratio could be used as a novel biomarker for MG patients who do not have another condition associated with FLC alternation [62].

The summary of unveiled novel immunologic biomarkers of MG is illustrated in Figure 1 and listed in Table 1.

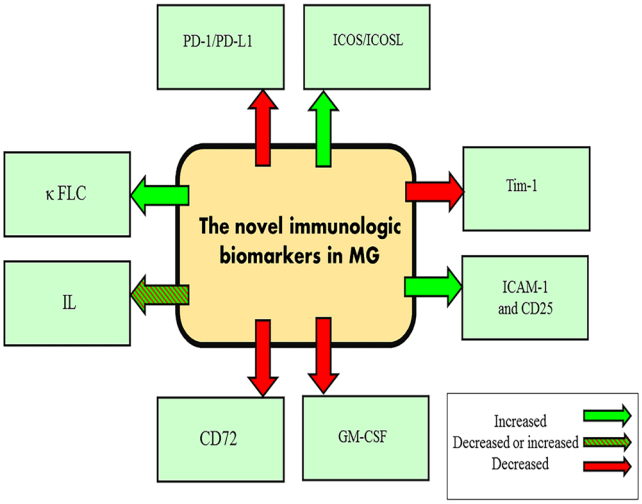

Figure 1. The novel immunologic biomarkers in MG

Table 1. The immunologic biomarkers of MG

Auto-antibodies	Immune cells and cytokines	Molecules
AChR antibodies	Th1/17	PD-1/PD-L1 suppression
LRP4 antibodies	Tfr/Tfh ratio	ICOS/ICOSL activation
MuSK antibodies	$CD4^+CXCR5^+FOXP3^+$ Tfr-like	Metalloproteinase 10
FLC of autoantibodies	$CD4^+CXCR5^+FOXP3^-$ Tfh-like	TGFα
Collagen Q antibodies	GM-CSF-expressing Th	S100A12
Collagen XIII antibodies	TNF-producing ThCD103	C-C motif ligand 19
Acetylcholinesterase antibodies	IL-17A, IL-21, IL-10, IL-14, IL-2, IL-5, IL-12, IL-P70, IL-33, IL-6, IL-8, IL-19, IL-20, IL-28A, IL-35, IL-27, IL-36γ	C-X-C motif ligand 1
Rapsyn antibodies	IFN-γ	ICAM-1
Cortactin antibodies	-	CD25 and CD72
Titin antibodies	-	Tim-1
Kv1.4 antibodies	-	-
Agrin antibodies	-	-
RyR antibodies	-	-

-: blank cells

Genetic factors in MG

The role of genetic factors associated with MG is influenced by population study. Cytotoxic T-lymphocyte antigen-4 (*CTLA-4*) is expressed on the immune cells including T cells and B cells and represses inflammation. In the Chinese cohort, MG patients showed higher *rs733618*C* allele frequency of this gene than healthy controls. In addition, in OMG *rs231775*A* allele frequency was lower than in controls and generalized MG. The *rs3087243*A* allele also was less frequent in OMGs than in generalized MG. The combination of *rs733618*C*, *rs231775*G*, and *rs3087243*G* alleles increased the risk of MG and OMG type in this cohort [63]. In another study in China, *CTLA-4* methylation and *CTLA-4* expression in the peripheral blood of MG patients were higher and lower than in controls, respectively and these results were the possible reasons for related cytokines secretion in MG [64].

Genetic factors could be used as prognosis factors in thymoma-associated MG (TAMG). *CXCR4*, is expressed highly in cancerous tissues, unlike healthy ones [65]. The overexpression of *CXCR4* is an independent biomarker of TAMG's poor prognosis and low survival [66].

LOMG is a major subgroup of MG and its prevalence is growing. A meta-analysis concluded human leukocyte antigen DRB1 (*HLA-DRB1*) is the most related genotype with LOMG. Increased risk of LOMG was related to *DRB1 07* and *0403* alleles. *DRB1*0301* and *1301* alleles were introduced as protective factors [67]. Another study measured *DQA1*0103* with lower frequency in the OMG group compared to the control group. The MG patients with thyroid-associated ophthalmopathy had higher *DQA1*0301* and lower *DQB1*0601* frequency. Also, *DQB1*0501* was more found in the OMG and OMG+ thyroid-associated ophthalmopathy compared to the control group [66].

The analysis of 598,375 single nucleotide polymorphisms (SNPs) in MG and normal controls, suggested three genes have value to be as MG biomarkers: Ca^{2+} voltage-gated channel subunit alpha1 S (*CACNA1S*), signaling lymphocytic activation molecule family 1 (*SLAMF1*), and *RyR* [68].

Recently, genome-wide and transcriptome-wide association studies on 1,873 AChR+ MG patients and 36,370 healthy controls were done [69]. This study proved there is a genetic overlap between MG and other autoimmune diseases such as thyroid-related diseases, rheumatoid arthritis, and MS. Cholinergic receptor nicotinic alpha 1 (*CHRNA1*) and cholinergic receptor nicotinic beta 1 (*CHRNB1*) signals unlike epsilon and delta subunits, were related with MG since they are targets of AChR antibodies. In this evaluation, MG-associated signals were found on chromosomes *2q31.1*, *10p14*, and *11q2*, and the major histocompatibility complex locus was also forcefully associated with an increased risk of LOMG. In addition, the genes of protein tyrosine phosphatase non-receptor type 22 protein (*PTPN22*), TNF receptor superfamily member 11a protein (*TNFRSF11A*), and *HLA-DQA1*/*HLA-B* showed MG-associated signals. A genome-wide

association study including 1,401 MG patients and 3,508 healthy controls, also showed an association between *HLA-DRB1*/*HLA-B* and *TNFRSF11A* gene [70]. Like the previously discussed study, autoimmune diseases like type 1 diabetes, rheumatoid arthritis, late-onset vitiligo, and autoimmune thyroid disease were related to MG in this study. Interestingly, they also suggested that the agrin gene (*AGRN*), is a novel MG susceptibility gene. A list of genetic factors associated with MG is shown in Table 2.

Table 2. Genetic factors associated with MG

The association	Study
CTLA-4 gene: *rs733618*C* allele frequency was higher in MG than controls, *rs231775*A* allele frequency was lower in OMG than controls and generalized MG, *rs3087243*A* allele frequency was lower in OMG than generalized MG. The combination of *rs733618*C*, *rs231775*G*, and *rs3087243*G* alleles was more frequent in MG and OMG than in other patients.	Cai et al. [63]
CTLA-4 methylation and *CTLA-4* expression were higher in MG than in controls.	Fang et al. [64]
Overexpression of *CXCR4* in poor prognosis TAMG.	Yang et al. [66]
DRB1 07 and *0403* alleles as risk factors in LOMG, *DRB1*0301* and *1301* alleles as protective factors in LOMG.	Ling et al. [67]
Lower *DQA1*0103* frequency in OMG than in the control group. Higher *DQA1*0301* and lower *DQB1*0601* frequency in MG patients with thyroid-associated ophthalmopathy. Higher *DQB1*0501* frequency in the OMG and OMG+ thyroid-associated ophthalmopathy than in the control group.	Yang et al. [66]
RyR, *CACNA1S*, and *SLAMF1* as MG biomarkers.	Na et al. [68]
CHRNA1, *CHRNB1*, chromosomes *2q31.1*, *10p14*, and *11q2*, *PTPN22*, *TNFRSF11A*, and *HLA-DQA1*/*HLA-B* were associated with MG.	Chia et al. [69]
HLA-DRB1/*HLA-B*, *TNFRSF11A*, and *AGRN* were associated with MG.	Topaloudi et al. [70]

MicroRNAs as MG biomarker

MicroRNAs (miRNAs) are small and non-coding endogenous RNA molecules that regulate gene expression via various mechanisms [71]. Recently, researchers evaluated the role of miRNAs as valuable biomarkers for detecting diseases or their complications and forecasting medication response. In addition, miRNAs were found in the extra-cellular space, blood plasma, serum, amniotic fluid, cerebrospinal fluid, peritoneal/pleural fluids, breast milk, urine, and tear called circulating miRNAs [72].

Although the comparison of gene expression profiles between MG subgroups has revealed many differences in the expression of miRNAs (Table 3), we should evaluate which ones are more specific for detecting subgroups of MG or medication response. Quantitative reverse transcription polymerase chain reaction (PCR) is a worthy and widely used method to determine miRNA profiles in the samples.

Table 3. miRNA markers for MG diagnosis

EOMG	TAMG	MuSK+ MG	AChR+ MG	LOMG
Down-regulation:	Up-regulation:	Down-regulation:	Down-regulation:	Up-regulation:
miR-7-5p, miR-29, miR-548k, miR-145, miR-24, and miR-143, miR-146	let-7a-5p, let-7f-5p, miR-125a-5p	miR-210-3p, miR-324-3p	miR-15b, miR-122, miR-140-3p, miR-185, miR-192, miR-20b, and miR-885-5p, miR-27a-3p	miR-30e-5p, miR-150-5p, miR-21-5p, miR-106b-3p, miR-223-5p, miR-140-5p, miR-19b-3p
Up-regulation:	Up-regulation:	Up-regulation:	Up-regulation:	-
miR-150-5p, miR-21-5p	let-7a-5p, let-7f-5p, miR-125a-5p	miR-151a-3p, let-7f-5p, let-7a-5p, miR-423-5p	miR-150-5p and miR-21-5p	
Up-regulation in PBMCs:	-	-	-	-
miR-612, miR-3654, miR-3651, and precursor miR-3651				

PMBC: peripheral blood mononuclear blood cell; -: blank cells

It has been shown that miR-150-5p is the most up-regulated one in the serum of AChR+ MG patients and it will be reduced after thymectomy or immunosuppressive treatment [73]. The functions of miR-150-5p are regulation of proliferation, apoptosis, and differentiation of natural killer (NK) cells, T,

and B cells [74]. miR-21-5p up-regulates in AChR+ MG patients and decreases after immunosuppressive therapy. Other immunologic disorders such as systemic lupus erythematosus, MS, and type1 diabetes mellitus also deregulate miR-21 [75, 76]. These two miRNAs are modulators for Treg maturation [77]. AChR+ MG has different clinical phenotypes: EOMG, LOMG, and TAMG. Some reports investigated the miRNAs profile in these subgroups.

MuSK+ MG patients have different miRNA profiles. Among them, miR-let-7 through stimulation of the Toll-like receptor 7 and thereby activation of T cells lead to MG in MuSK+ patients [78]. Other circulating RNAs were detected in MG patients. For example, peripheral blood hsa-circRNA5333-4 could be a promising biomarker for the early detection of MG [79]. Long non-coding RNAs (lncRNAs) are other circulating RNAs with more than 200 nucleotides. XLOC_003810, SNHG16, IFNG-AS1, and MALAT-1 are lncRNAs that were found in the serum of MG patients and were associated with T cell activation and PD-1/PD-L1 signaling suppression [80]. Five lncRNAs (NR_104677.1, ENST00000583253.1, NR_046098.1, NR_022008.1, and ENST00000581362.1) were significantly elevated in the serum exosome of MG patients [81].

The advantages of using circulating RNAs are their resistance to changes in temperature and PH changes because of encapsulation by a membrane [82]. The easy identification of miRNAs in the body fluids is a good tool for the early detection and subtyping of MG. In addition, the medication response in MG patients could be monitored by using circulating RNAs.

Gut microbiomes and biomarkers of MG

The human gut microbiome is a huge ecological community of microorganisms in the intestine which makes an important role in our health. Dysbiosis defines by changes in the composition of microbiota that could affect the host/microbe interaction which could increase disease susceptibility [83].

Like many autoimmune diseases, MG is associated with dysbiosis by decreasing the intestinal $FOXP3^+CD4^+$ Treg cells. $FOXP3^+CD4^+$ Treg cells inhibit B cell production and AChR antibody synthesis. Analysis of the fecal metabolites showed that some bacteria increase in MG: *Bacteroidaceae*, *Lachnospiraceae*, *Prevotellaceae*, and *Veillonellaceae*, while these bacteria population decrease in MG: *Lachnospiraceae*, *Ruminococcaceae*, *Erysipelotrichaceae*, *Clostridiaceae*, and *Peptostreptococcaceae* [84, 85]. MG subjects had significant depletion of *Clostridium* and *Lactobacillus* populations. Several studies confirmed that *Bacteroidetes* and *Actinobacteria* are increased in MG patients. The *Firmicutes*/*Bacteroidetes* (F/B) ratio is the indicator of a pro-inflammatory environment and is lower in MG patients [86].

The significant reduction in the *Clostridium* spp. maybe has a role in the pathology of MG. The *Clostridium* spp. affects the intestinal epithelium through up-regulation of 2, 3-dioxygenase and TGFβ-1 which are necessary to $FOXP3^+CD4^+$ Treg cells differentiation and restrain AChR antibody proliferation [87, 88].

The analysis of the fecal metabolites of MG patients shows short-chain fatty acids (SCFAs) are decreased compared to healthy controls. SCFAs play a role in the $FOXP3^+CD4^+$ Treg cell differentiation. Because the main source of SCFAs production is *Clostridium* spp. and these bacteria are depleted in MG patients, thereby it could be the second mechanism of MG pathology [89].

Streptococcus salivarius is another bacterium that increases significantly in MG patients. *Streptococcus salivarius* inhibits the transcriptional activity of peroxisome proliferator-activated receptor γ (PPAR γ). PPAR γ inhibition leads to $FOXP3^+CD4^+$ Treg cells decrease and antibody production [90].

The serum levels of lipopolysaccharide secretion soluble CD14 (LPS-sCD14) and endotoxin core antibody IgM (Endo-CAb-IgM) are biomarkers of the translocated microbe and alongside IL-6, secretory IgA (SIgA) and TNF-α were used to determine chronic inflammation from the inflated gut in MG. LPS-sCD14 and Endo-CAb-IgM are decreased but IL-6, SIgA, and TNF-α are increased in the MG patient's serum. Unlike other autoimmune diseases such as inflammatory bowel disease (IBD)/irritable bowel syndrome (IBS), in which the level of LPS-sCD14 and Endo-CAb-IgM is the reason for chronic inflammation, it seems that this is not the mechanism of inflammation in MG [85, 86].

The gene expression changes by the gut microbiome and pathways which lead to MG pathology are illustrated in Figure 2. There is still a need for studies on the microbiome changes in MG subgroups and treatment response.

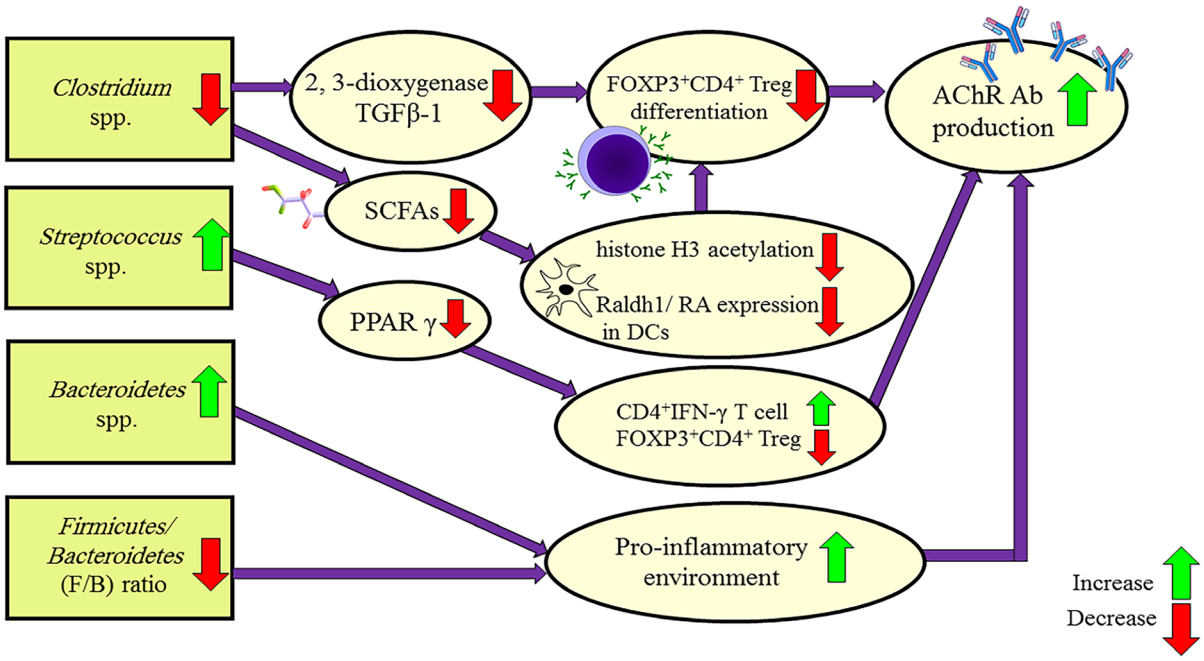

Figure 2. Mechanisms leading to MG pathology by the gut microbiome of MG patients. Raldh1: retinal dehydrogenase isoform-1; RA: retinoic acid; Ab: antibody

Other promising biomarkers

As mentioned before, the dysbiosis of gut microbiota in MG patients plays a crucial role in MG pathology. Calprotectin (CLP) is a Ca^{2+}-binding protein of the S100 family secreted by various immune cells and interacts with Toll-like receptor 4 resulting in inflammatory processes activation [91]. Since CLP previously has been shown to have a relation with dysbiosis in inflammatory diseases, a high level in MG patients exhibits this relation [92, 93].

Some studies focused on the metabolite profile in MG patients. One study demonstrated 12 specific metabolites for MG: two short-chain keto acids, two hydroxy acids, one benzenoid, five structural lipids, one organooxygen compound, and one bile acid. In this study, there were common biomarkers in MS and rheumatoid arthritis with MG [94]. Also, aspartic acid and glutamic acid show elevated levels in MG patients [95].

The receptor for advanced glycation end products (RAGE) is a membranous receptor on the various immune cells which connects to several ligands [like high-mobility group box 1 (HMGB1) and S100 family members] and proceeds inflammatory pathways resulting in impaired self-tolerance. RAGE and S100B both up-regulated in the experimental model of MG resulting in increased Th1/Th17 and decreased $FOXP3^+$ Tregs. AChR antibody production also is the other effect of high RAGE/S100B expression [96]. On the other hand, in the clinical studies, S100B had no significant different level in the MG patients but soluble form of RAGE (sRAGE), and endogenous secretory RAGE were reduced in MG [96, 97]. These forms of RAGE act as an inhibitor for RAGE and ligand interactions. Although there is a contrast between experimental MG and human MG studies, some studies demonstrated S100B increases in the peak of clinical MG presentation in rats and decreases in humans after the remission phase [97, 98]. However, HMGB1 is correlated with AChR antibody production, generalized MG, and thymoma in a study [99], but it had no significant different level in MG *vs.* controls in the other studies [97, 100]. Studies suggest maybe S100A12 is the responsible ligand for RAGE in MG in the human body [96].

Adipokines are a group of secreted peptides from adipose tissue which have a role in appetite and satiety regulation, lipid metabolism, and insulin sensitivity [101]. Leptin, resistin, and adiponectin are evaluated

biomarkers in MG patients. Leptin increased in MG patients due to glucocorticoid use, resistin increased in one study but decreased in another study, and adiponectin showed no significant different level in MG patients *vs.* controls [102, 103]. However adipokines are utile biomarkers to demonstrate the insulin sensitivity and metabolic status of MG patients, but they could be very changeful by various conditions and medications.

The urokinase plasminogen activator receptor is a fibrinolysis factor that acts by binding to a urokinase-type plasminogen activator. Recently, it has been shown that soluble urokinase plasminogen activator receptor (suPAR) increases systematic chronic inflammation [104]. In the MG patients, suPAR was correlated with disease severity and could be a potential biomarker to precipitate MG clinical course in the patients, but not detecting them from healthy ones, since there was no difference in suPAR level between MG and controls [105].

Mitochondrial proteins as energy expenditure markers could be helpful in MG diagnosis. Mitofusin 1, and 2, optic atrophy type 1, dynamin-related protein 1 and fission 1, adenosine monophosphate (AMP)-activated protein kinase, PPAR γ co-activator-1α, nuclear respiratory factor-1, and mitochondrial transcription factor A are reduced in MG patients [106]. Low plasma vitronectin level is another diagnostic biomarker in MG. Vitronectin is a glycoprotein in blood and the extracellular matrix which induces complement-dependent immune response and clot regulation. The unidentified role of this reduction in MG patients is still unknown [107].

Beyond the diagnosis, subtyping, precipitating medication response, and MG severity classification, we could apply biomarkers as prognostic factors in patients with thymoma. For example, heat shock protein 90α (HSP90α) increases in the serum in non-thymomatous MG, thymic carcinomas, thymomas, and thymic neuroendocrine tumors MG compared to those experienced thymectomy. HSP90α high level was associated with a low recurrence-free rate in patients with thymoma [108].

Conclusions

The use of various biomarkers to diagnose, treat and determine the MG prognosis could differentiate MG from its differential diagnoses and make the treatment challenges of these patients easier. Although many studies have been designed to determine new biomarkers, there are many challenges to achieving the profile of MG biomarkers. The existing limitations are the method of measuring biomarkers, the ethnicity under investigation, the time of measurement (before or after treatment), and unknown confounding factors which alter the levels of these molecules.

In this study, we gave a brief overview of immunological biomarkers, genetic biomarkers, RNAs, and other new biomarkers in MG. More studies are needed to determine the exact role of these biomarkers in the pathology of MG disease and the profile of biomarkers in different subgroups of MG, and investigate the biomarker changes after applying therapeutic plans.

Abbreviations

AChR: acetylcholine receptor

AChR+: acetylcholine receptor antibody-positive

Ca^{2+}: calcium

CTLA-4: cytotoxic T-lymphocyte antigen-4

CXCR5: C-X-C chemokine receptor type 5

Endo-CAb-IgM: endotoxin core antibody immunoglobulin M

EOMG: early-onset myasthenia gravis

FLC: free light chains

FOXP3: forkhead box P3

GM-CSF: granulocyte-monocyte colony-stimulating factor

HLA: human leukocyte antigen

HLA-DRB1: human leukocyte antigen DRB1

ICOS: inducible costimulator

ICOSL: inducible costimulator ligand

IFNs: interferons

IgG1: immunoglobulin G1

IL-17: interleukin-17

lncRNAs: long non-coding RNAs

LOMG: late-onset myasthenia gravis

LPS-sCD14: lipopolysaccharide secretion soluble CD14

LRP4: lipoprotein-receptor related protein 4

MG: myasthenia gravis

miRNA: microRNA

MS: multiple sclerosis

MuSK: muscle-specific kinase

MuSK+: muscle-specific kinase antibody-positive

NMJ: neuromuscular junction

OMG: ocular myasthenia gravis

PD-1: programmed death protein-1

PD-L1: programmed death protein-1 ligand

PPAR γ: peroxisome proliferator-activated receptor γ

RAGE: receptor for advanced glycation end products

RIPA: radioimmunoprecipitation assay

RyR: ryanodine receptor

S100A12: S100 calcium-binding protein A12

SCFAs: short-chain fatty acids

SNMG: seronegative myasthenia gravis

suPAR: soluble urokinase plasminogen activator receptor

TAMG: thymoma-associated myasthenia gravis

Tfh: follicular helper T

Th1: T-helper 1

Tim-1: T cell immunoglobulin and mucin domain-1

TNF: tumor necrosis factor

TNFRSF11A: tumor necrosis factor receptor superfamily member 11a protein

Treg: regulatory T cells

Declarations

Author contributions

FA: Conceptualization, Writing—immunologic, gut microbiome, and other biomarkers section. RR: Conceptualization, Writing—genetic factors and miRNA biomarkers section. Both of the authors contributed to manuscript revision, read and approved the submitted version.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent to publication

Not applicable.

Availability of data and materials

Not applicable.

Funding

Not applicable.

References

1. Lazaridis K, Tzartos SJ. Autoantibody specificities in myasthenia gravis; implications for improved diagnostics and therapeutics. Front Immunol. 2020;11:212.
2. Carr AS, Cardwell CR, McCarron PO, McConville J. A systematic review of population based epidemiological studies in myasthenia gravis. BMC Neurol. 2010;10:46.
3. Gilhus NE, Verschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. Lancet Neurol. 2015;14:1023–36.
4. Lacazette E, Le Calvez S, Gajendran N, Brenner HR. A novel pathway for MuSK to induce key genes in neuromuscular synapse formation. J Cell Biol. 2003;161:727–36.
5. Mantegazza R, Cavalcante P. Diagnosis and treatment of myasthenia gravis. Curr Opin Rheumatol. 2019;31:623–33.
6. Dresser L, Wlodarski R, Rezania K, Soliven B. Myasthenia gravis: epidemiology, pathophysiology and clinical manifestations. J Clin Med. 2021;10:2235.
7. Bernard C, Frih H, Pasquet F, Kerever S, Jamilloux Y, Tronc F, et al. Thymoma associated with autoimmune diseases: 85 cases and literature review. Autoimmun Rev. 2016;15:82–92.
8. Vrolix K, Fraussen J, Losen M, Stevens J, Lazaridis K, Molenaar PC, et al. Clonal heterogeneity of thymic B cells from early-onset myasthenia gravis patients with antibodies against the acetylcholine receptor. J Autoimmun. 2014;52:101–12.
9. Benatar M. A systematic review of diagnostic studies in myasthenia gravis. Neuromuscul Disord. 2006;16:459–67.
10. Rødgaard A, Nielsen FC, Djurup R, Somnier F, Gammeltoft S. Acetylcholine receptor antibody in myasthenia gravis: predominance of IgG subclasses 1 and 3. Clin Exp Immunol. 1987;67:82–8.
11. Bartoccioni E, Scuderi F, Minicuci GM, Marino M, Ciaraffa F, Evoli A. Anti-MuSK antibodies: correlation with myasthenia gravis severity. Neurology. 2006;67:505–7.
12. Strijbos E, Verschuuren JJGM, Kuks JBM. Serum acetylcholine receptor antibodies before the clinical onset of myasthenia gravis. J Neuromuscul Dis. 2018;5:261–4.

13. Hoch W, McConville J, Helms S, Newsom-Davis J, Melms A, Vincent A. Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. Nat Med. 2001;7:365–8.
14. Kawakami Y, Ito M, Hirayama M, Sahashi K, Ohkawara B, Masuda A, et al. Anti-MuSK autoantibodies block binding of collagen Q to MuSK. Neurology. 2011;77:1819–26.
15. Huijbers MG, Zhang W, Klooster R, Niks EH, Friese MB, Straasheijm KR, et al. MuSK IgG4 autoantibodies cause myasthenia gravis by inhibiting binding between MuSK and Lrp4. Proc Natl Acad Sci U S A. 2013;110:20783–8.
16. Evoli A, Bianchi MR, Riso R, Minicuci GM, Batocchi AP, Servidei S, et al. Response to therapy in myasthenia gravis with anti-MuSK antibodies. Ann N Y Acad Sci. 2008;1132:76–83.
17. Iorio R, Damato V, Alboini PE, Evoli A. Efficacy and safety of rituximab for myasthenia gravis: a systematic review and meta-analysis. J Neurol. 2015;262:1115–9.
18. Guptill JT, Sanders DB, Evoli A. Anti-MuSK antibody myasthenia gravis: clinical findings and response to treatment in two large cohorts. Muscle Nerve. 2011;44:36–40.
19. Kim N, Stiegler AL, Cameron TO, Hallock PT, Gomez AM, Huang JH, et al. Lrp4 is a receptor for agrin and forms a complex with MuSK. Cell. 2008;135:334–42.
20. Tzartos JS, Zisimopoulou P, Rentzos M, Karandreas N, Zouvelou V, Evangelakou P, et al. LRP4 antibodies in serum and CSF from amyotrophic lateral sclerosis patients. Ann Clin Transl Neurol. 2014;1:80–7.
21. Tsonis AI, Zisimopoulou P, Lazaridis K, Tzartos J, Matsigkou E, Zouvelou V, et al. MuSK autoantibodies in myasthenia gravis detected by cell based assay--a multinational study. J Neuroimmunol. 2015;284:10–7.
22. Cordts I, Bodart N, Hartmann K, Karagiorgou K, Tzartos JS, Mei L, et al. Screening for lipoprotein receptor-related protein 4-, agrin-, and titin-antibodies and exploring the autoimmune spectrum in myasthenia gravis. J Neurol. 2017;264:1193–203.
23. Skeie GO, Mygland A, Treves S, Gilhus NE, Aarli JA, Zorzato F. Ryanodine receptor antibodies in myasthenia gravis: epitope mapping and effect on calcium release *in vitro*. Muscle Nerve. 2003;27:81–9.
24. Kellermayer D, Smith JE 3rd, Granzier H. Titin mutations and muscle disease. Pflugers Arch. 2019;471:673–82.
25. Szczudlik P, Szyluk B, Lipowska M, Ryniewicz B, Kubiszewska J, Dutkiewicz M, et al. Antititin antibody in early- and late-onset myasthenia gravis. Acta Neurol Scand. 2014;130:229–33.
26. Buckley C, Newsom-Davis J, Willcox N, Vincent A. Do titin and cytokine antibodies in MG patients predict thymoma or thymoma recurrence? Neurology. 2001;57:1579–82.
27. Mygland A, Tysnes OB, Matre R, Volpe P, Aarli JA, Gilhus NE. Ryanodine receptor autoantibodies in myasthenia gravis patients with a thymoma. Ann Neurol. 1992;32:589–91.
28. Takamori M, Motomura M, Kawaguchi N, Nemoto Y, Hattori T, Yoshikawa H, et al. Anti-ryanodine receptor antibodies and FK506 in myasthenia gravis. Neurology. 2004;62:1894–6.
29. Stergiou C, Lazaridis K, Zouvelou V, Tzartos J, Mantegazza R, Antozzi C, et al. Titin antibodies in "seronegative" myasthenia gravis--a new role for an old antigen. J Neuroimmunol. 2016;292:108–15.
30. Cossins J, Belaya K, Zoltowska K, Koneczny I, Maxwell S, Jacobson L, et al. The search for new antigenic targets in myasthenia gravis. Ann N Y Acad Sci. 2012;1275:123–8.
31. Suzuki S, Baba A, Kaida K, Utsugisawa K, Kita Y, Tsugawa J, et al. Cardiac involvements in myasthenia gravis associated with anti-Kv1.4 antibodies. Eur J Neurol. 2014;21:223–30.
32. Suzuki S, Satoh T, Yasuoka H, Hamaguchi Y, Tanaka K, Kawakami Y, et al. Novel autoantibodies to a voltage-gated potassium channel Kv1.4 in a severe form of myasthenia gravis. J Neuroimmunol. 2005;170:141–9.

33. Romi F, Suzuki S, Suzuki N, Petzold A, Plant GT, Gilhus NE. Anti-voltage-gated potassium channel Kv1.4 antibodies in myasthenia gravis. J Neurol. 2012;259:1312–6.
34. Agius MA, Zhu S, Aarli JA. Antirapsyn antibodies occur commonly in patients with lupus. Ann N Y Acad Sci. 1998;841:525–6.
35. Agius MA, Zhu S, Fairclough RH. Antirapsyn antibodies in chronic procainamide-associated myopathy (CPAM). Ann N Y Acad Sci. 1998;841:527–9.
36. Agius MA, Zhu S, Kirvan CA, Schafer AL, Lin MY, Fairclough RH, et al. Rapsyn antibodies in myasthenia gravis. Ann N Y Acad Sci. 1998;841:516–21.
37. Gallardo E, Martínez-Hernández E, Titulaer MJ, Huijbers MG, Martínez MA, Ramos A, et al. Cortactin autoantibodies in myasthenia gravis. Autoimmun Rev. 2014;13:1003–7.
38. Labrador-Horrillo M, Martínez MA, Selva-O'Callaghan A, Trallero-Araguás E, Grau-Junyent JM, Vilardell-Tarrés M, et al. Identification of a novel myositis-associated antibody directed against cortactin. Autoimmun Rev. 2014;13:1008–12.
39. Illa I, Cortés-Vicente E, Martínez MÁ, Gallardo E. Diagnostic utility of cortactin antibodies in myasthenia gravis. Ann N Y Acad Sci. 2018;1412:90–4.
40. Cortés-Vicente E, Gallardo E, Martínez MÁ, Díaz-Manera J, Querol L, Rojas-García R, et al. Clinical characteristics of patients with double-seronegative myasthenia gravis and antibodies to cortactin. JAMA Neurol. 2016;73:1099–104.
41. Mappouras DG, Philippou G, Haralambous S, Tzartos SJ, Balafas A, Souvatzoglou A, et al. Antibodies to acetylcholinesterase cross-reacting with thyroglobulin in myasthenia gravis and Graves's disease. Clin Exp Immunol. 1995;100:336–43.
42. De Bellis A, Sansone D, Coronella C, Conte M, Iorio S, Perrino S, et al. Serum antibodies to collagen XIII: a further good marker of active Graves' ophthalmopathy. Clin Endocrinol (Oxf). 2005;62:24–9.
43. Tu H, Pirskanen-Matell R, Heikkinen A, Oikarainen T, Risteli J, Pihlajaniemi T. Autoimmune antibodies to collagen XIII in myasthenia gravis patients. Muscle Nerve. 2018;57:506–10.
44. Zoltowska Katarzyna M, Belaya K, Leite M, Patrick W, Vincent A, Beeson D. Collagen Q--a potential target for autoantibodies in myasthenia gravis. J Neurol Sci. 2015;348:241–4.
45. Wen Y, Yang B, Lu J, Zhang J, Yang H, Li J. Imbalance of circulating $CD4^+CXCR5^+FOXP3^+$ Tfr-like cells and $CD4^+CXCR^+FOXP3^-$ Tfh-like cells in myasthenia gravis. Neurosci Lett. 2016;630:176–82.
46. Ma Q, Ran H, Li Y, Lu Y, Liu X, Huang H, et al. Circulating Th1/17 cells serve as a biomarker of disease severity and a target for early intervention in AChR-MG patients. Clin Immunol. 2020;218:108492.
47. Ingelfinger F, Krishnarajah S, Kramer M, Utz SG, Galli E, Lutz M, et al. Single-cell profiling of myasthenia gravis identifies a pathogenic T cell signature. Acta Neuropathol. 2021;141:901–15. Erratum in: Acta Neuropathol. 2021;142:789.
48. Simpson TR, Quezada SA, Allison JP. Regulation of CD4 T cell activation and effector function by inducible costimulator (ICOS). Curr Opin Immunol. 2010;22:326–32.
49. Zamani MR, Aslani S, Salmaninejad A, Javan MR, Rezaei N. PD-1/PD-L and autoimmunity: a growing relationship. Cell Immunol. 2016;310:27–41.
50. Yan X, Gu Y, Wang C, Sun S, Wang X, Tian J, et al. Unbalanced expression of membrane-bound and soluble inducible costimulator and programmed cell death 1 in patients with myasthenia gravis. Clin Immunol. 2019;207:68–78.
51. Zhang Y, Zhang X, Xia Y, Jia X, Li H, Zhang Y, et al. CD19+ Tim-1+ B cells are decreased and negatively correlated with disease severity in myasthenia gravis patients. Immunol Res. 2016;64:1216–24.
52. Lu J, Li J, Zhu TQ, Zhang L, Wang Y, Tian FF, et al. Modulation of B cell regulatory molecules CD22 and CD72 in myasthenia gravis and multiple sclerosis. Inflammation. 2013;36:521–8.

53. Kakoulidou M, Wang X, Zhao X, Pirskanen R, Lefvert AK. Soluble costimulatory factors sCD28, sCD80, sCD86 and sCD152 in relation to other markers of immune activation in patients with myasthenia gravis. J Neuroimmunol. 2007;185:150–61.

54. Çebi M, Durmus H, Aysal F, Özkan B, Gül GE, Çakar A, et al. $CD4^+$ T cells of myasthenia gravis patients are characterized by increased IL-21, IL-4, and IL-17A productions and higher presence of PD-1 and ICOS. Front Immunol. 2020;11:809.

55. Yilmaz V, Oflazer P, Aysal F, Durmus H, Poulas K, Yentur SP, et al. Differential cytokine changes in patients with myasthenia gravis with antibodies against AChR and MuSK. PLoS One. 2015;10:e0123546.

56. Huan X, Zhao R, Song J, Zhong H, Su M, Yan C, et al. Increased serum IL-2, IL-4, IL-5 and IL-12p70 levels in AChR subtype generalized myasthenia gravis. BMC Immunol. 2022;23:26.

57. Jonsson DI, Pirskanen R, Piehl F. Beneficial effect of tocilizumab in myasthenia gravis refractory to rituximab. Neuromuscul Disord. 2017;27:565–8.

58. Wang Z, Wang W, Chen Y, Xu S, Wei D, Huang X. Elevated expression of interleukin-33 in myasthenia gravis patients. J Clin Neurosci. 2019;63:32–6.

59. Zhang QX, Li Y, Jiang SM, Zhang LJ, Yi M, Wang J, et al. Increased serum IL-36γ levels are associated with disease severity in myasthenia gravis patients. BMC Neurol. 2020;20:307.

60. Yi M, Zhang LJ, Liu XJ, Wang N, Huang CN, Liu MQ, et al. Increased serum IL-27 concentrations and IL-27-producing cells in MG patients with positive AChR-Ab. J Clin Neurosci. 2021;86:289–93.

61. Molin CJ, Westerberg E, Punga AR. Profile of upregulated inflammatory proteins in sera of myasthenia gravis patients. Sci Rep. 2017;7:39716.

62. Wilf-Yarkoni A, Alkalay Y, Brenner T, Karni A. High κ free light chain is a potential biomarker for double seronegative and ocular myasthenia gravis. Neurol Neuroimmunol Neuroinflamm. 2020;7:e831.

63. Cai GM, Gao Z, Yue YX, Xie YC, Gao X, Hao HJ, et al. Association between CTLA-4 gene polymorphism and myasthenia gravis in a Chinese cohort. J Clin Neurosci. 2019;69:31–7.

64. Fang TK, Yan CJ, Du J. CTLA-4 methylation regulates the pathogenesis of myasthenia gravis and the expression of related cytokines. Medicine (Baltimore). 2018;97:e0620.

65. Lippitz BE. Cytokine patterns in patients with cancer: a systematic review. Lancet Oncol. 2013;14:e218–28.

66. Yang HW, Wang YX, Bao J, Wang SH, Lei P, Sun ZL. Correlation of *HLA-DQ* and *TNF-α* gene polymorphisms with ocular myasthenia gravis combined with thyroid-associated ophthalmopathy. Biosci Rep. 2017;37:BSR20160440.

67. Ling CS, Shen ML, Wang Y, Cai WK, Lin XQ, Huang Q, et al. The associations of *HLA*-DRB1 gene polymorphisms with late-onset myasthenia gravis: a meta-analysis. Neurol Sci. 2020;41:1041–9.

68. Na SJ, Lee JH, Kim SW, Kim DS, Shon EH, Park HJ, et al. Whole-genome analysis in Korean patients with autoimmune myasthenia gravis. Yonsei Med J. 2014;55:660–8.

69. Chia R, Saez-Atienzar S, Murphy N, Chiò A, Blauwendraat C; International Myasthenia Gravis Genomics Consortium; Roda RH, Tienari PJ, Kaminski HJ, Ricciardi R, Guida M, De Rosa A, et al. Identification of genetic risk loci and prioritization of genes and pathways for myasthenia gravis: a genome-wide association study. Proc Natl Acad Sci U S A. 2022;119:e2108672119. Erratum in: Proc Natl Acad Sci U S A. 2022;119:e2206754119.

70. Topaloudi A, Zagoriti Z, Flint AC, Martinez MB, Yang Z, Tsetsos F, et al. Myasthenia gravis genome-wide association study implicates AGRN as a risk locus. J Med Genet. 2022;59:801–9.

71. Lu TX, Rothenberg ME. MicroRNA. J Allergy Clin Immunol. 2018;141:1202–7.

72. Sabre L, Punga T, Punga AR. Circulating miRNAs as potential biomarkers in myasthenia gravis: tools for personalized medicine. Front Immunol. 2020;11:213.

73. Punga T, Le Panse R, Andersson M, Truffault F, Berrih-Aknin S, Punga AR. Circulating miRNAs in myasthenia gravis: miR-150-5p as a new potential biomarker. Ann Clin Transl Neurol. 2014;1:49–58.
74. Smith NL, Wissink EM, Grimson A, Rudd BD. miR-150 regulates differentiation and cytolytic effector function in CD8+ T cells. Sci Rep. 2015;5:16399.
75. Fenoglio C, Cantoni C, De Riz M, Ridolfi E, Cortini F, Serpente M, et al. Expression and genetic analysis of miRNAs involved in CD4+ cell activation in patients with multiple sclerosis. Neurosci Lett. 2011;504:9–12.
76. Garchow BG, Bartulos Encinas O, Leung YT, Tsao PY, Eisenberg RA, Caricchio R, et al. Silencing of microRNA-21 *in vivo* ameliorates autoimmune splenomegaly in lupus mice. EMBO Mol Med. 2011;3:605–15.
77. Balandina A, Lécart S, Dartevelle P, Saoudi A, Berrih-Aknin S. Functional defect of regulatory $CD4^+CD25^+$ T cells in the thymus of patients with autoimmune myasthenia gravis. Blood. 2005;105:735–41.
78. Wang S, Tang Y, Cui H, Zhao X, Luo X, Pan W, et al. Let-7/miR-98 regulate Fas and Fas-mediated apoptosis. Genes Immun. 2011;12:149–54.
79. Lv J, Ren L, Han S, Zhang J, Zhao X, Zhang Y, et al. Peripheral blood hsa-circRNA5333-4: a novel biomarker for myasthenia gravis. Clin Immunol. 2021;224:108676.
80. Ghafouri-Fard S, Azimi T, Hussen BM, Taheri M, Jalili Khoshnoud R. A review on the role of non-coding RNAs in the pathogenesis of myasthenia gravis. Int J Mol Sci. 2021;22:12964.
81. Lu W, Lu Y, Wang CF, Chen TT. Expression profiling and bioinformatics analysis of exosomal long noncoding RNAs in patients with myasthenia gravis by RNA sequencing. J Clin Lab Anal. 2021;35:e23722.
82. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007;9:654–9.
83. Frank DN, Zhu W, Sartor RB, Li E. Investigating the biological and clinical significance of human dysbioses. Trends Microbiol. 2011;19:427–34.
84. Zheng P, Li Y, Wu J, Zhang H, Huang Y, Tan X, et al. Perturbed microbial ecology in myasthenia gravis: evidence from the gut microbiome and fecal metabolome. Adv Sci (Weinh). 2019;6:1901441. Erratum in: Adv Sci (Weinh). 2020;7:2001296.
85. Thye AY, Law JW, Tan LT, Thurairajasingam S, Chan KG, Letchumanan V, et al. Exploring the gut microbiome in myasthenia gravis. Nutrients. 2022;14:1647.
86. Qiu D, Xia Z, Jiao X, Deng J, Zhang L, Li J. Altered gut microbiota in myasthenia gravis. Front Microbiol. 2018;9:2627.
87. Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, et al. Induction of colonic regulatory T cells by indigenous *Clostridium* species. Science. 2011;331:337–41.
88. Nagano Y, Itoh K, Honda K. The induction of Treg cells by gut-indigenous *Clostridium*. Curr Opin Immunol. 2012;24:392–7.
89. Moris G, Arboleya S, Mancabelli L, Milani C, Ventura M, de Los Reyes-Gavilán CG, et al. Fecal microbiota profile in a group of myasthenia gravis patients. Sci Rep. 2018;8:14384.
90. Choi JM, Bothwell AL. The nuclear receptor PPARs as important regulators of T-cell functions and autoimmune diseases. Mol Cells. 2012;33:217–22.
91. Ehrchen JM, Sunderkötter C, Foell D, Vogl T, Roth J. The endogenous toll-like receptor 4 agonist S100A8/S100A9 (calprotectin) as innate amplifier of infection, autoimmunity, and cancer. J Leukoc Biol. 2009;86:557–66.
92. Halfvarson J, Brislawn CJ, Lamendella R, Vázquez-Baeza Y, Walters WA, Bramer LM, et al. Dynamics of the human gut microbiome in inflammatory bowel disease. Nat Microbiol. 2017;2:17004.

93. Stascheit F, Hotter B, Hoffmann S, Kohler S, Lehnerer S, Sputtek A, et al. Calprotectin as potential novel biomarker in myasthenia gravis. J Transl Autoimmun. 2021;4:100111.
94. Blackmore D, Siddiqi Z, Li L, Wang N, Maksymowych W. Beyond the antibodies: serum metabolomic profiling of myasthenia gravis. Metabolomics. 2019;15:109.
95. Kośliński P, Rzepiński Ł, Koba M, Gackowski M, Maciejek Z. Amino acids levels as a potential biomarker in myasthenia gravis. Folia Neuropathol. 2022;60:122–7.
96. Angelopoulou E, Paudel YN, Piperi C. Unraveling the role of receptor for advanced glycation end products (RAGE) and its ligands in myasthenia gravis. ACS Chem Neurosci. 2020;11:663–73.
97. Mu L, Zhang Y, Sun B, Wang J, Xie X, Li N, et al. Activation of the receptor for advanced glycation end products (RAGE) exacerbates experimental autoimmune myasthenia gravis symptoms. Clin Immunol. 2011;141:36–48.
98. Park KH, Jung J, Lee JH, Hong YH. Blood transcriptome profiling in myasthenia gravis patients to assess disease activity: a pilot RNA-seq study. Exp Neurobiol. 2016;25:40–7.
99. Uzawa A, Kawaguchi N, Kanai T, Himuro K, Kuwabara S. Serum high mobility group box 1 is upregulated in myasthenia gravis. J Neurol Neurosurg Psychiatry. 2015;86:695–7.
100. Moser B, Bekos C, Zimprich F, Nickl S, Klepetko W, Ankersmit J. The receptor for advanced glycation endproducts and its ligands in patients with myasthenia gravis. Biochem Biophys Res Commun. 2012;420:96–101.
101. Fasshauer M, Blüher M. Adipokines in health and disease. Trends Pharmacol Sci. 2015;36:461–70.
102. Braz NFT, Rocha NP, Vieira ÉLM, Gomez RS, Kakehasi AM, Teixeira AL. Body composition and adipokines plasma levels in patients with myasthenia gravis treated with high cumulative glucocorticoid dose. J Neurol Sci. 2017;381:169–75.
103. Zhang DQ, Wang R, Li T, Li X, Qi Y, Wang J, et al. Remarkably increased resistin levels in anti-AChR antibody-positive myasthenia gravis. J Neuroimmunol. 2015;283:7–10.
104. Rasmussen LJH, Petersen JEV, Eugen-Olsen J. Soluble urokinase plasminogen activator receptor (suPAR) as a biomarker of systemic chronic inflammation. Front Immunol. 2021;12:780641.
105. Uzawa A, Kojima Y, Ozawa Y, Yasuda M, Onishi Y, Akamine H, et al. Serum level of soluble urokinase plasminogen activator receptor (suPAR) as a disease severity marker of myasthenia gravis: a pilot study. Clin Exp Immunol. 2020;202:321–4.
106. Li L, Cai D, Zhong H, Liu F, Jiang Q, Liang J, et al. Mitochondrial dynamics and biogenesis indicators may serve as potential biomarkers for diagnosis of myasthenia gravis. Exp Ther Med. 2022;23:307.
107. Lepedda AJ, Deiana GA, Lobina O, Nieddu G, Baldinu P, De Muro P, et al. Plasma vitronectin is reduced in patients with myasthenia gravis: diagnostic and pathophysiological potential. J Circ Biomark. 2019;8:1–9.
108. Thanner J, Bekos C, Veraar C, Janik S, Laggner M, Boehm PM, et al. Heat shock protein 90α in thymic epithelial tumors and non-thymomatous myasthenia gravis. Oncoimmunology. 2020;9:1756130.