Clinical Genomic, phenotype and epigenetic insights into the pathology, autoimmunity and weight management of patients with Myasthenia Gravis (Review)

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Abstract. Myasthenia Gravis (MG) is an autoimmune disease that affects neuromuscular junctions and is characterized by muscle weakness as a result of autoantibodies against certain proteins. As a heterogeneous disorder, MG presents with different types, including neonatal, ocular and generalized in both juveniles and adults. Different types of antibodies serve a role in how MG presents. The main biological characteristic of MG is the production of antibodies against the muscular acetylcholine receptor; however, other types of antibody have been associated with the disorder. The role of the thymus gland has been established and thymectomy is a possible treatment of the disease, along with traditional medication such as pyridostigmine bromide (Mestinon) and immunosuppresants. In recent years, steps have been made towards developing more sensitive diagnostic methods. Additionally, novel treatments have demonstrated promising results. Developing new assays may lead to an increased understanding of the disease and to unravelling the genetic pathway that leads to the development of neuromuscular diseases.

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Contents

1. Introduction

In 1672, English physician Thomas Willis first described signs and symptoms of a disease that affected the muscles (1), which is now known as the autoimmune disease Myasthenia Gravis ('my' means muscle and 'asthenia' means illness/weakness in Greek, 'gravis' means severe in Latin).

This heterogeneous disorder biologically is mainly caused by antibodies against the muscular acetylcholine receptor (AChR) at the neuromuscular juction and recent developments have shown that other autoantibodies are involved in the manifestation of MG (2). These antibodies lead to loss of the muscle receptors, which causes a defect in neuromuscular transmission presenting with involuntary progressive muscle weakness. Patients usually share a variety of symptoms, which most commonly include dyplopia (double vision), dysphagia, drooping (ptosis) of one or both eyelids, weakness in the neck, arms and legs (3).

The diagnosis of MG is primarily based on the serological detection of AChR antibodies (4) and the presence of the thymus gland in adults is taken under consideration as the thymus plays a profound role in the pathogenesis of MG and thymectomy is one of the current therapeutic options.

As a complex genetic disorder, MG has some shared associations with other autoimmune disorders with the most reproducible finding being the association of the 8.1 ancestral haplotype of HLA-DR3 for class II and HLA-B8 and A1 for class I (5) in Caucasians. Phenotypically, Myasthenia Gravis presents most commonly in its generalized form with anti-AChR antibodies as an early-onset disorder, presented before the age of 40 years, usually in females. Other forms of the disease can be described in both adults and juveniles, as analyzed below.

Several polymorphisms have been identified in GWS studies in relation with myasthenia gravis and the important role of epigenetics is yet to be discovered. Along with genetic predisposition, environmental factors can lead to the development of autoimmunity as well as affect the severity of autoimmune diseases.

The symptoms of MG can be treated very successfully with different drugs, even leading to remission in some cases. Harriet Edgeworth, a myasthenic herself, in 1930 discovered that ephedrine sulfate improved her condition and muscle functionality and a few years later, in 1934 Mary Walker was the first to successfully administer physostigmine salicylate, and later neostigmine bromide, to a patient with MG (1). Since then, other drugs have been added in the fight against MG, from the anticholinesterase pyridostigmin bromide to the latest monoclonal antibodies (mAb) drugs.

2. Biology of autoimmune generalized myasthenia gravis, MG related antibodies and the thymus gland

Autoimmune myasthenia gravis (MG) is a chronic autoimmune disease of the neuromuscular junction, characterized by a post-synaptic blockade of the nervous transmission and presenting clinically with varying degrees of weakness of the skeletal muscles and generalized fatigue. MG has been identified and reported in literature centuries ago without too much progress in regard to finding a cure or efficient therapy (Fig. 1). It is considered to be a rare disease with a prevalence of 8-30 patients per 100,000 population in Caucasians that has doubled over the last decades and continues to increase. A 10% - 15% of all patients are estimated through studies to be pediatric patients with the percentage going up to 50% among Asian populations.

Both pediatric and adult patients usually share a variety of symptoms, which most commonly include dyplopia (double vision), dysphagia, drooping of one or both eyelids (ptosis), nasal or impaired speech, change of facial expressions, weakness in the neck (head droop), arms and legs. It has the potential of a life-threating disease when there is involvement of the respiratory muscles. The symptoms may be acute or subacute and periods of remission or relapse may occur. Despite the symptoms' pool that most patients share, MG is considered to be a heterogeneous disease.

As a heterogeneous disorder, myasthenia may present as ocular or generalized in adults and juveniles along with transient neonatal myasthenia and congenital myasthenic syndromes. Among patients with generalized myasthenia gravis, there are different human leukocyte antigen (HLA) associations between early-onset and late-onset generalized MG (2).

The main characteristic of MG biologically is the production of antibodies against the muscular acetylcholine receptor (AChR). In ~5% of patients anti-MUSK (muscle-specific kinase) antibodies can be present and there is a small percentage (~10%) of seronegative patients. The need to discover and detect new antigenic targets is significant in these patients in order to identify them and establish new therapeutic treatments. Towards this direction the past few years new assays have been used and it has been possible to identify new autoantibodies in some MG patients, like the anti-Titin antibodies or the low-density lipoprotein receptor-related protein 4 (LRP4) as an autoantigen in MG. Achieving identification of the autoantibodies present in each MG patient allows not only for a correct diagnosis but also for a more targeted therapeutic strategy with advanced antigen-specific treatment (3).

MG with anti- AChR antibodies. An approximate 80% of patients present with anti-AChR antibodies in their serum, which target the Ach-gated cation channel, nicotinic α1 AChR. Acetylcholine receptors (AChRs) are found on the surface of the muscle cells and are concentrated between the muscle cells and the nerve cells (6). The five protein chains in adults and neonates which compose AChRs, form a 'bridge' shaped as a long tube that crosses the cell membrane and have binding sites for acetylcholine on the external side. They contain the immunogenic region that is recognized by anti-AChR antibodies and when acetylcholine binds on these chains, an alteration in the receptor's shape is caused, the channel formed by the 'tube' opens and positively charged ions cross the membrane, causing the muscles to contract. Patients with generalized anti- AChR antibodies MG have a lower density of AChR at the neuromuscular junction, which leads to involuntary muscle weakness, as the signal for the muscle to work does not go through (7).

Patients in this category can be divided in those with ocular and generalized form of the disease. The generalized form can be further divided in groups according to the age of onset. Early-onset myasthenia (EOMG) patients are under the age of 50 and usually present with a high number of anti- AChR antibodies in their serum and hyperplasia of the thymus. In this category the vast majority of patients are women, and the role of sex hormones should be furtherly investigated. Late-onset (LOMG) patients are over the age of 50 and usually present with a thymoma (malignant) and are more prone to severe respiratory crises. Over the age of 60, a category of mostly male patients does not present with thymoma and is considered to be a very late-onset form of MG. Both forms can be also found in juvenile myasthenia gravis (JMG).

The muscle AChR composes of five subunits and each one has an intracellular domain that is partially structured, one highly structured extracellular domain and four transmembrane domains. The antibodies target the extracellular domain and the heterogeneity of the disease is that in a single patient antibody against all subunits can be detected. Despite this, an approximate 50% of the antibodies bind to the α subunit and these appear to be more pathogenic compared to others (8). The α subunit is directly involved in neuro transmission due to its involvement in acetylholine binding. Furthermore, it presents with a known functional polymorphism at the protein

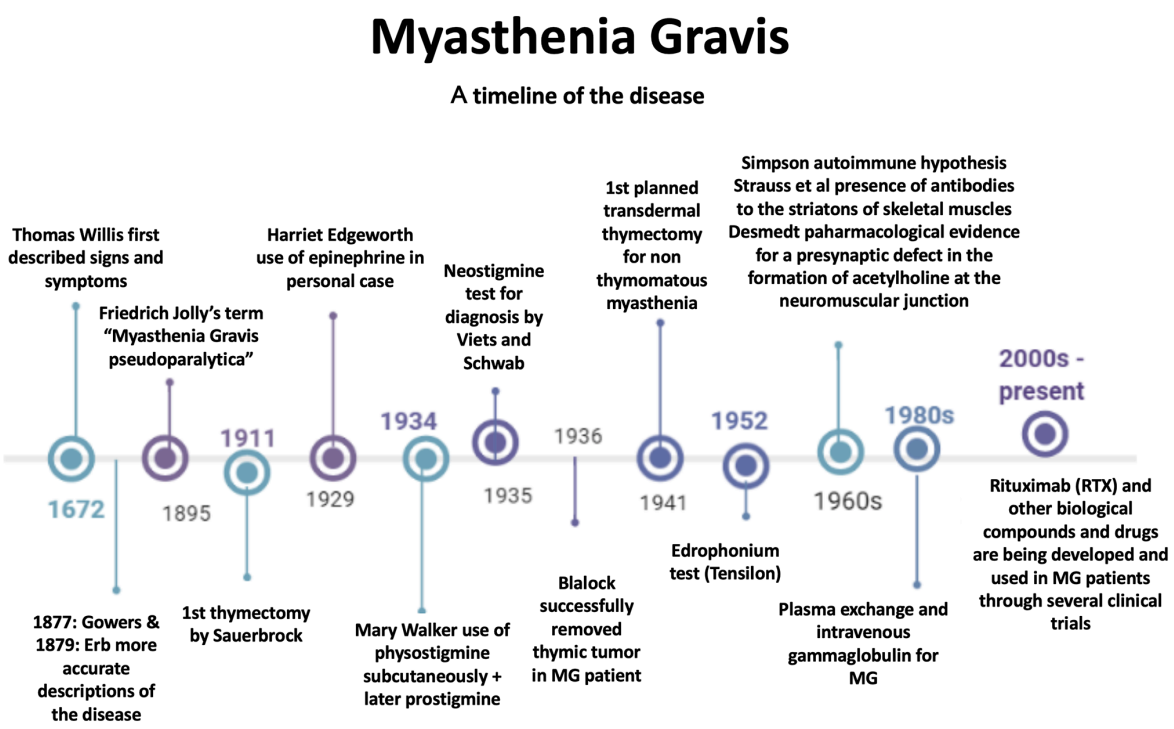

Figure 1. Important steps towards discovering the mechanisms and treatment options of MG (42). MG, Myasthenia Gravis.

level that derives from an alternative splicing of an additional P3A exon (9). The AChR antibodies can cause AChR loss by activating complement at the postsynaptic membrane, as they mostly belong to the IgG1 and IgG3 subclasses (10). The signal transduction is therefore lost and, furthermore, there is an interference with receptor activation by acetylholine when antibodies bind close to the ligand binding site.

The diagnosis of MG is primarily based on the serological detection of AChR antibodies. A blood test is usually the first step along with physical examination and an assessment of response to acetylcholinesterase (AChE) inhibitors. The serological testing provides a title of AChR antibodies, which does not always correlate with the severity of the disease in some patients but can be related to the response to therapy (11).

It is suggested by physicians to monitor the levels of AChR antibodies in order to monitor a patient's progress and guide the disease management by modifying, if necessary, drug dosage. Concerning transient neonatal myasthenia, maternal antibody titers have been found to correlate with the onset and/or severity of the disorder in infants.

MG with anti-MuSK antibodies. The Muscle Specific Kinase (MuSK) protein has an essential role in the neuromuscular junction and is important for the clustering of the AChRs. In the early 21st century, anti-MuSK antibodies were identified in about 40% of patients with negative anti-AChR antibodies (7). These patients often present with severe symptoms, including bulbar dysfunction, respiratory insufficiency and atrophy of the facial and tongue muscles.

In animal models of MuSK MG histopathological studies have revealed that anti-MuSK antibodies cause contraction of motor terminals, significant loss of acetylcholine receptor (AChR) expression and a reduction in synaptic folds at the postsynaptic membrane in the absence of complement involvement. Failure of neuromuscular transmission at pre- and postsynaptic membranes of the neuromuscular junctions has been observed in both patients and animal models of MuSK MG (12). Anti-MuSK antibodies in MG patients mainly belong to the IgG4 subclass, which has distinct properties directly associated with the MG pathology.

Seronegative MG. Patients that are seronegative have a similar clinical presentation of the condition with anti-AChR antibodies patients. Although anti-AChR antibodies are not detected by the classical assay, they appear to be present and to predominantly belong to the IgG1 isotype that activates the complement.

MG and other antibodies. Recent developments have shown that the protein Titin plays a role in sarcomere assembly and elasticity. The Ryanodin receptor (RyR) is a calcium channel participating in the muscle contraction through releasing calcium from the sarcolemma into the cytoplasm.

Antibodies against LRP4 were recently reported in MG patients without detectable AChR or MuSK antibodies, but that percentage varies among populations examined and assays used from 2% to 33% (13). LRP4 is a transmembrane protein with a central role in synaptic development and LRP4 antibodies have been shown to activate the complement and therefore playing a role in MG as well as in other disorders, like ALS (amyotrophic lateral sclerosis) (14).

In more rare cases, antibodies against cytokines, interferon-α, interferon-ω and IL-12 can be present in patients with thymoma or LOMG, but their pathogenic role is not yet clear.

Thymus hyperplasia. The thymus plays a profound role in the pathogenesis of MG. A large percentage of patients

have pathological changes in their thymic tissue with >70% of them showing hyperplasia and about 10-15% having a thymoma.

Over half the patients with AChR-MG have a hyperplastic thymus characterized by production of antibodies by B cells, which can be decreased by immunosuppressive drugs, production of AChR antibodies and the presence of anti-AChR-reactive T cells. $CD4^+$ $CD25^+$ Treg cells play an important role in the control of both the autoimmune response and the immune response and in MG patients they appear to be defective (15). The antibodies created exit the thymus and generate an autoimmune reaction in the neuromuscular junction against AChRs. However, the exact mechanism of the attack against AChRs is still unclear. A viral mechanism can also be taken under consideration as latest data show that poliovirus and EBV have been detected in the thymus.

Thymoma. Thymoma is observed in about 10% of adult MG patients and is caused by the abnormal development of epithelial cells whereas children have rarely been found to develop thymomas. Thymomas are categorised in 5 types according to the nature of the cortical or medullary epithelial cells involved in the tumor: Type A, B1, B2, B3 or AB, with the most common types being B1 and B2 in patients over 40 years of age. Autoimmune mechanisms have a strong link with thymoma development, as over half the patients that have a thymoma also present in MG or other autoimmune syndromes (16).

In contrast to thymic hyperplasia, B cells and germinal centers are not present and thymomas lack medulla. Also, the major histocombatibility complex (MHC class II) antigens and the autoimmunity regulator factor (AIRE) are deficient in thymoma and that could suggest that there is a difference in the autoimmunisation process (17). The existing scientific data suggest that T cell selection may be affected in different ways by a thymoma and that the lack of $CD4^+$ $CD25^+$ Tregs along with a change in expression of AChR subunits may also play a role.

Thymectomy. In early-onset Myasthenia Gravis, thymectomy is one of the current therapeutic options. Studies show that the removal of the thymus has a positive effect on patients with generalized MG, (18) who usually have a stabilization of their symptoms 3-5 years after the surgery and often present with reduced severity or remission, especially if the procedure is performed soon after the onset of symptoms. It is not suggested for seronegative or late-onset patients. In children it may have an effect for those unresponsive to treatment and may, also, protect patients with ocular form of JMG to undergo into the generalized form. The improvement that occurs after thymectomy may be related to the elimination of thymic B cells but the exact pathway is not clear yet.

3. Clinical Genomics and phenotypes of the disorder

Clinical Genomics. As a complex genetic disorder, MG has some shared associations with other autoimmune disorders. The most reproducible finding is the association of the 8.1 ancestral haplotype of HLA-DR3 (DRB1*03) for class II and HLA-B8 and A1 for class I with early-onset AchR-MG and thymic hyperplasia in Caucasians (5), with a 60% frequency in patients and the risk associated with haplotype increases about 7-times for two copies. The Myasthenia gravis with thymus hyperplasia (MYAS1) locus is one of the three loci of the HLA region identified and encompasses 36 genes in a region of 1.2Mb at the boundaries of class I on the telomeric side and class III, confirming that the B8 allele is predominant over the DR3 (19). The other two loci have effects on serum titers of AchR antibodies: a QTL associated with elevated titers, overlapping with MYAS1 and a locus mapped toward the class I region, which suppresses the enhancing effect of the QTL. HLA-DR3 and DR7 may have opposing effects on the MG phenotype, with the first associated with EOMG and the second associated with LOMG with Titin antibodies. Using a high-resolution haplotype map as reference, a panel of 1472 single-nucleotide polymorphisms (SNPs) was genotyped across the classic MHC region in MG recently: The strongest association arose from the class I region specifically in the vicinity of the HLA complex protein 5 (HCP5) gene, while the strongest associated HLA allele was HLA-C*0701; paucity of significant signals was detected in the class II region. MuSK-MG has a suggested association with LA-DR14-DQ5 while no reproducible MHC associations have been reported in MG with thymoma (20).

The 1858T (rs2476601) functional SNP located in the PTPN22 gene is a minor allele that may weaken T cell receptor signaling and is represented in patients without a thymoma and with anti-Titin antibodies, who also present a lower IL-2 expression (7). Also, the CHRNA1 locus encodes the α subunit of the AChR and a minor G allele of a functional SNP is associated with myasthenia Gravis. CHRNA1 is directly involved in acetylcholine binding and therefore in neuro transmission. It also presents a functional polymorphism, the only one that is known at the protein level in the AChR -subunit and that results from an alternative splicing of the additional P3A exon. This exon codes for 25 amino-acids in the N-terminal extra-cellular domain that disrupt the main immunogenic region and impair the channel function of the AChR pentamer. These features made the CHRNA1 gene that codes for the α-subunit a good candidate for influencing the genetic susceptibility to MG.

A number of SNPs have been associated with EOMG and LOMG through genome-wide association and other studies (21). Rs231770, rs4263037, rs9270986 as well as rs601006 and rs9271850 in the HLA region were analyzed in one GWAS (21) in order to identify their influence in AChR antibody-positive MG patients.

Apart from the HLA complex, other unlinked genetic loci have been investigated for their involvement in autoimmune disorders and specifically in MG. Interferon regulatory factor 5 (IRF-5), which induces IFN gene expression and upregulates cytocines like IL-6, TNF-α, IL-12, is influenced by SNP rs10954213 in the 3' UTR and by rs60344245 in an IRF-5 exon. The TNFα-induced protein 3 (TNFAIP3) has an inhibitory effect on on NF-κB signaling and the SNP rs13207033 in the 6p23 region probably affects regulatory DNA elements (22). Interleukin-10 (IL-10) is an anti-inflammatory cytocine that stimulates TH2 cells and suppresses TH1 cells at the same time (23) and has been suggested to cause an increase of anti-AChR antibody levels in MG. Three SNPs in the human IL-10 gene, rs45552637, rs1800872 and rs1800896 determine

the formation of three haplotypes (GCC, ACC, and ATA) with the ACC/GCC genotype being the most frequently observed in MG patients (19).

Several other genes were reported to be associated (encoding β2-adrenergic receptor, IL-1β, IL-10, IFN-γ, TCRα, Ig heavy chain, Ig κ-chain, TNF-α, TNF-β, type 2 receptors for Fc fragment of IgG and cysteine protease cathepsin V) with various subtypes of MG, but there is no confirmation yet.

Phenotypes of the disorder. Most commonly generalized myasthenia Gravis with anti-AChR antibodies is an early-onset disorder, presented before the age of 40 years, usually in females. It is rarer to appear before the age of 20 and is usually accompanied by thymus hyperplasia. Often patients develop within a decade of the MG onset other autoimmune diseases, such as thyreoditis and diabetes or may also have them prior to MG.

In children, Transient Neonatal Myasthenia (TNM), Juvenile MG and Congenital Myasthenic Syndromes (CMS) may appear. The CMS are a group of disorders characterized by fatigue in the skeletal muscles with an onset shortly after or at birth with variable severity. TMN affects newborns born to mothers with MG and is a temporary form of the disease. It shares some similarities with the adult and juvenile forms of MG, like affecting the respiratory system, and occurs due to the transplacental passage of antibodies, usually against the AChR antigen. Maternal antibodies recognize the fetal form of the AChR and inhibit neuromuscular transmission in the baby, leading to a transient MG at birth (24). In a small percentage of cases, AChR antibodies developing during pregnancy may target fetal AChRs and result in severe arthrogryposis of the fetus even if the mother has no clinical signs. Neonatal myasthenia is a form of the disease presenting with AChR mutations. Maternal antibodies recognize the fetal form of the AChR and inhibit neuromuscular transmission in the baby, leading to a transient MG at birth (24). In this case, the blocking effects appear to trigger neonatal MG and are correlated with the severity of the disease in the child.

Patients with anti-MuSK antibodies are not so common and tend to be mainly female with no thymic pathology (thymoma is exceptional) (25). Clinically these patients have an oculopharyngeal onset rapidly progressing into dysphonia, dysphagia and difficulty in chewing. Many patients develop respiratory failure but there is rarely limb weakness in contrast to AChR-MG. Another phenotype includes only ocular and neck weakness, that can rapidly evolve into a myasthenic crisis with respiratory failure.

Late-onset MG and MG with a thymoma are phenotypes defined by the presence of anti-Titin, anti-RyR and anti-cytocine (IL-12, IFN-α) antibodies. These phenotypes also present with severe oropharyngeal and neck muscle involvement, along with respiratory crisis and low response to thymectomy.

Ocular myasthenia can be characterized as the primary onset in about half the patients that will develop generalized MG, especially in cases with no diagnosis and no treatment. The generalized form of the disease is developed withing 6-24 months after the ocular myasthenia and a functional SNP in the regulatory region of the DAF gene (decay-accelerating factor) associates with ocular myasthenia.

In certain populations familial cases of myasthenia gravis have been observed, suggesting a role of familial factors in the pathogenesis of the disease. In 2013 in Taiwan a population-based study (N=23,422,955) revealed that 0.064% of individuals had at least one first-degree relative with MG with a familial prevalence of 0.205% in the first-degree relatives with a family history (26). In Buenos Aires, a case study of 190 MG patients in one public hospital was performed, in which familial autoimmune Myasthenia Gravis represented 3.2% of the cases (6 patients had a first-degree family member also affected by MG) (27).

4. The role of Epigenetics

Alterations in genome architecture can result in autoimmune diseases. Several polymorphisms have been identified in GWS studies in relation with myasthenia gravis, but the manifestation of the disease can not solely be described by these gene abnormalities. Epigenetics play an important role and studies in twins have shown that epigenetic deregulation contributes to the severity and even the manifestation of this autoimmune disorder.

Through the development of each individual, a set of epigenetic factors can differentiate the activity of specific genes or even whole genomic regions, including methylations in the DNA sequence and modifications of histone proteins. These modifications are not only the result of heritable epigenetic changes that happen during gene expression, but environmental factors also have a great influence. Dietary habits and stress are examples of environmental factors that may cause the manifestation of autoimmune disorders and their severity. In other autoimmune diseases, like Rheumatoid Arthritis, research indicates that dietary habits may be a risk factor and that environmental and genetic factors interact during the pathogenesis of autoimmune diseases years before clinical onset (28). Along with genetic predisposition, environmental factors can lead to the development of autoimmunity and the break of immune tolerance to self-antigens (29). Multiple environmental factors along diet and stress, such as air-pollution, infection and smoking may contribute to the development of chronic illnesses (30).

Studies of several complex human diseases have shown differences in DNA methylation in peripheral normal blood cell populations ($CD4^+$ T cells, $CD19^+$ B cells etc.) and the analysis of these differences has led to the understanding of the role of epigenetics in autoimmunity. Patients with disorders like multiple sclerosis (MS) and myasthenia Gravis (MG) that are related with the production of autoantibodies can be benefited by fully understanding the role of the environmental conditions, like stress. Epigenetic differences have been presented in twin studies and these differences become clearer with age and this can potentially explain the phenotypic differences. It is possible that genetic factors and environmental triggers along with epigenetic factors lead to autoimmune disorders. The environment can lead to overexpression of silenced genes caused by subvert epigenetic regulatory pathways. As children grow up and are exposed to radiation, drugs, chemicals, dietary changes and stress through their life, autoimmune diseases may become more and more common in the coming decades.

Given the key role that B cells have in many autoimmune disorders and the alterations described by several studies that deal with DNA methylation (31), a need for further investigation emerges to confirm whether and how epigenetics is involved in these disorders. The direct or indirect role of environmental factors in the pathogenesis of myasthenia Gravis is essential for the better understanding of the disease and the ways to design new drugs that will lead to full remission or even prevent MG from manifestating.

5. Obesity and weight management in MG

Obesity is considered to be a chronic disease itself caused by an imbalance between the energy spent by a person and the energy ingested in food. Fat cells store the excess energy and that causes their enlargement and/or increase causing a pathological etiology for obesity (32). The medical hazards of obesity include diabetes mellitus, hypertriglyceridemia, changes in the levels of high and low-density lipoprotein cholesterol, gallbladder disease, sleep apnea and degenerative joint disease and an elevated risk for coronary heart disease along with malignancies among the most common.

In Myasthenia gravis patients' excess weight is a rather common problem. Some factors that lead towards patients gaining weight are lifestyle and drug related. It is important for MG patients to maintain a relaxed lifestyle without extreme workouts or activities that include heavy lifting, repetitive nerve stimulation or any muscle force that may lead to muscular skeleton weakness and increase other symptoms like cramps, unintelligible speech, swallowing or breathing problems and lead to generalized fatigue. This may result in involuntary gain weight, especially if patients do not follow a dietary plan specially designed for their needs.

Another factor that may lead to obesity is the use of certain drugs that are connected with alterations of the body's appetite-regulating mechanisms resulting in excessive weight gaining. Corticosteroids lead to improvement or remission of the symptoms in a large percentage of patients as mentioned and prednisone, for example, is widely used as an MG treatment as it suppresses the immune system and helps control myasthenia gravis. One of the main side effects of oral corticosteroids is weight gain with fat deposits in the abdomen area and the face. This can cause a deterioration of MG as an adverse reaction as the patient's compliance with prescribed medication may be jeopardised and the excess weight may itself lead to less exercise, more stress on the body and a deterioration of the disease's progress.

A number of other drugs may change body weight as an adverse consequence of their therapeutic use such as antidepressants and mood stabilizers, both commonly taken among patients with autoimmune and other chronic diseases. Studies have shown associations that suggest that autoimmune disorders are important etiologic factors of mood disorders, such as depression and anxiety-related disorders, especially in patients that are hospitalized (33). Antidepressants such as tricyclic antidepressants and monoamine oxidase (MAO) inhibitors are most often associated with significant weight gain.

Overweight increases the risk of mortality as a growing body of research tells us and a logical anticipation would be that intentional weight loss may reduce mortality and certainly reduce the risk of other obesity related diseases. A body mass index (BMI kg/m^2), the formula used to assess body weight and is an indicator of obesity, is the main criteria for evaluating normal weight. In 1995, WHO published a technical report establishing for categories: an individual with a BMI <19.9 is considered to be underweight, to have normal weight with the BMI 20-24.9, overweight if the BMI is between 25 - 29.9 and obese with a BMI >30.

Intentional weight loss improves individual risk factors, reduces the risk of diabetes mellitus and leads to changes in blood pressure and dyslipidemia. Therefore, maintaining a normal weight (BMI <25) is important for everyone and can be furtherly proven important in MG patients of all age groups as it helps stabilize the disease, ensures proper drug intake and optimal exercise, which lead to a better and healthier lifestyle with a more controlled form of the disease. Successful weight loss maintenance can be achieved by dietary restrictions and with the addition of gradually increased physical activity up to the level of not causing MG deterioration. While weight can be a complicated issue for many of us, it is important for MG patients to make an effort to reach and maintain a long-term healthy weight. Further studies evaluating the risks of MG and obesity can be proven valuable.

6. Treatment options

Medical therapies are different between neonatal MG and Juvenile and adult MG. In infants the treatment used has a much smaller duration, varying from a few days to a few months depending on the duration of the symptoms while in JMG and in adults the treatment is very often a life-long commitment.

The first treatment is pyridostigmin bromide (brand name Mestinon), an acetylcholinesterase inhibitor. Pyridostigmin as a half-life of 20 min after an oral 60 mg dose and the dosage is usually prescribed depending on each patient's clinical assessment and his/her age. Mestinon timespan is an extended-release formula, available in 180 mg tablets that has been recently developed. A typical adult takes 30-60 mg orally every 4-6 h daily, while a typical pediatric dose vary, from 1-7 mg/kg/day divided to up to 6 doses. There is a risk of a cholinergic crisis if the dose of AChEI is too high, which may result in excessive saliva, extreme fatigue and even respiratory failure, sometimes making it difficult to differentiate from a myasthenic crisis. Neostigmine is another acetylcholinesterase inhibitor used, but Pyridostigmine has a longer duration of action and is, therefore, preferred. Side effects include increased salivation, diaphoresis, muscle cramps, broncial secretions and gastrointestinal issues.

If the patient remains symptomatic after the maximum pyridostigmindosage,thenextstepistobeginimmunosuppressive therapy, usually with prednisone, azathioprine, cyclosporine, tacrolimus and IVIG (34). Corticosteroids lead to improvement or remission of the symptoms in a large percentage of patients. intravenous immunoglobulin (IVIG) and plasma exchange (PLEX) as immunomodulating therapies are very effective in treating MG exacerbations, myasthenic crisis and, also, as a treatment prior to surgery and thymectomy. Azathioprine is recommended in cases where long-term immunosuppression

is necessary, but the effect of this substance is delayed for as long as 12 months. Cyclosporine is usually given to patients who have a low tolerance for azathioprine and tacrolimus is an immunosuppressive agent with a similar mechanism to cyclosporine and appears to be a promising agent in patients with RyR antibodies, as it increases RyR-related calcium release.

New classes of biological compounds and drugs are currently in a clinical experimentation state, opening the possibility of personalized medicine and specific-targeted treatment options (35). They are divided in three major categories: complement inhibitors, anti-B cell therapies and nFcR (Neonatal Fc Receptor) antagonists. Complement inhibitors include Eculizumab (ECU), a humanized monoclonal antibody that targets complement protein C5 preventing the effect of micro-destruction of the post-synaptic membrane and has a good safety profile, demonstrating improvement in AChR positive patients, especially in perceived fatigue (tiredness, difficulty concentrating and lack of energy that differentiates from muscle weakness) (36).

Rituximab (RTX) is a very recent development in the treatment of MG symptoms that falls into the category of anti-B cell therapies. RTX is a chimeric mouse/human monoclonal antibody that targets CD20 B lymphocytes and is, at this point, prescribed in cases with drug-resistant MG, especially with MuSK antibodies (37). This new theurapeutic approach has been increasingly suggested, but there are yet not enough studies to describe the impact in the quality of life and to establish its benefits in comparison to the other available therapy options (37).

Finally, neonatal Fc Receptor (nFcR) antagonists are used for the first time in MG, offering a new therapeutic option for the disease, if their capacity to reduce circulating Igs is proven effective. This category of specific-targeted treatment options is subdivided into three groups, Recombinant Fc multimers, Neonatal Fc receptor antagonists and antiFcgR antagonists. Under current investigation through clinical trials are compounds, such as Efgartigimod (an engineered IgG1-derived Fc fragment), Rozanolixizumab (a humanized monoclonal antibody), Nipocalimab (M281- a fully humanized deglycosylated monoclonal antibody to nFcR) and RVT-1401 (a human recombinant anti-nFcR monoclonal antibody) (35). The mechanism of these compounds allows treatment for both AChR and MuSK- positive MG patients.

7. Discussion

Pediatric myasthenia gravis can present in infants as CMS or TNM or in adolescents as JMG which turns into adult MG, with either ocular or generalized form. Understanding the mechanisms involved in the pathophysiology of the autoimmune disease generalized myasthenia gravis is crucial in order for effective therapies to be introduced. The AChR pathogenic autoantigen was identified in the 1970s but the genetic basis is not clear. The number of patients has increased during the last twenty years and it is important to explain the mechanisms underlying MG. The study of MYAS1 could lead to a better understanding of the HLA B8 DR3 haplotype in the pathogenesis of this disease and perhaps many other autoimmune diseases. The difference in the genetic basis between Early and Late Onset MG confirms that there are more variants to be explored. The need for better prevention, easiest diagnosis and more efficient treatment is important in this era.

The first therapy of MG is basically a symptomatic treatment. Other than cholinesterase inhibitors, usually sufficient in mild cases in the beginning of the disease, corticosteroids and variable degrees of immunosuppression are needed for the majority of patients. Nevertheless, reducing the use of corticosteroids in patients is a current need, as they are a risk factor for other conditions, such as osteoporosis, metabolic, endocrine and cardiovascular complications. A new era is beginning with the introduction of new biological compounds directed against specific aspects of the autoimmune process in MG.

The relationship between certain drug categories that are forbidden or are considered cautionary drugs for myasthenia patients and the biological pathways behind the disease, is an area that needs to be further explored, especially in children. Beta- blockers used for hypertension and heart disease may worsen MG, as well as fluoroquinolones and macrolide antibiotics (e.g., azithromycin and erythromycin), statins used to reduce serum colesterol, botulinum toxin and other sunbstances like quinine (used to treat malaria but also found in tonic water) and magnesium. Other than patients knowing the side-effects and using these drugs with caution (if at all), the biochemical information connecting these drugs with a neuromuscular disease like MG can prove even life-saving in some cases.

Myasthenia Gravis has lately been associated in 3 cases with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and observers propose that a viral infection of nerve cells may cause MG symptoms (38). More investigation in this direction for other viruses should also be planned in the future as new emerging pathogens could create new threats for MG. Novel drug design pipelines should be considered for further studies especially on ssRNA viruses under the prism of MG (39-41). Research in this direction could be proven valuable at all levels of investigation, starting from in silico and computational work, to *in vitro* and *in vivo* and eventually reaching clinical trials.

The study of epigenetic mechanisms, like DNA methylation and histone modifications, and discovering microRNAs that are MG-specific along with comprehending environmental factors that might play a role in myasthenia, may lead to valuable information about the genetic and biological profile of myasthenia and could, furthermore, lead to the discovery of new and more effective therapeutic treatments.

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Availability of data and materials

Not applicable.

Authors' contributions

RG and DV conceived the current study. RG, LP, AE, FB, GPC, EE and DV wrote, drafted, revised, edited and reviewed the manuscript All authors have read and approved the final manuscript. Data sharing is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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