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New evidence for a genetic contribution to myasthenia gravis

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New evidence for a genetic contribution to myasthenia gravis

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ABSTRACT

Objectives To approximate the rate of familial myasthenia gravis and the coexistence of other autoimmune disorders in the patients and their families.

Design Retrospective cohort study

Setting Clinics across North America

Participants The study included 1,032 patients diagnosed with acetylcholine receptor antibody (AChR)-positive myasthenia gravis

Methods Phenotype information of 1,032 patients diagnosed with acetylcholine receptor antibody (AChR)-positive myasthenia gravis was obtained from clinics at fourteen centers across North America between January 2010 and January 2011. A critical review of the epidemiological literature on the familial rate of myasthenia gravis was also performed.

Results Among 1,032 patients, 58 (5.6%) reported a family history of myasthenia gravis. In comparing the familial and sporadic cohorts, the only feature that was different was the lower age of onset in familial cases. A history of autoimmune diseases was present in 26.6% of patients and in 28.4% of their family members.

Discussion The familial rate of myasthenia gravis was higher than would be expected for a sporadic disease. Furthermore, a high proportion of patients had a personal or family history of autoimmune disease. Taken together, these findings suggest a genetic contribution to the pathogenesis of myasthenia gravis.

ARTICLE SUMMARY

Strengths and limitations of this study

- A strength of this study is that it analyzes a large cohort of myasthenia gravis patients with complete data on each patient, allowing multiple clinical correlations to be made.
- A strength of this study is that standardized criteria were used to diagnose patients with myasthenia gravis, including establishing the specific subtype of the disease for each patient.
- A strength of this study is that the cohort of myasthenia gravis patients was sufficiently large to allow the generation of evidence confirming a genetic contribution to the disease.
- A limitation of this study is the reliance on self-reported family history status for both myasthenia gravis and other autoimmune diseases by the patients.
- A limitation of this study is its retrospective design, which precludes ascertaining additional information from individual patients.

INTRODUCTION

Myasthenia gravis is a rare autoimmune disease that is characterized by antibody-mediated interference with neuromuscular transmission at the neuromuscular junction. Originally, the role for an autoimmune attack against the acetylcholine receptor (AChR) on the postsynaptic side of the neuromuscular junction was recognized.[1] However, over the past decade, additional autoimmune targets have been identified: muscle-specific tyrosine kinase (MuSK), agrin, and lipoprotein receptor-related protein 4 (LRP4).[2-4] All of these proteins play essential roles in maintaining the structure and function of the neuromuscular junction.

Myasthenia gravis manifests clinically with muscle weakness and fatigability. Symptoms of ocular muscle weakness are observed early in 85% of myasthenic patients.[5] These symptoms include diplopia and/or ptosis. Weakness of the bulbar musculature is observed in 60% of myasthenic patients, with symptoms including dysarthria and/or dysphagia.[5]

A recent literature review of thirty-one epidemiological studies suggests that the annual incidence of myasthenia gravis may range between 3 and 30 cases per million people.[6] A common theme among myasthenia gravis epidemiological studies is that there is a significant degree of variability between cohorts. For example, a study of a Hong Kong Chinese cohort reported an incidence of 4 per million people per year, whereas a study of an English cohort reported an incidence of 30 per million people per year.[7, 8] This substantial degree of variability may be the result of inconsistent diagnostic criteria, varying case ascertainment, lack of physician awareness concerning myasthenia gravis, or it may reflect different occurrence across populations.

Historically, adult-onset myasthenia gravis has been regarded as a sporadic disease with only a minimal genetic component.[9] However, genome-wide association studies, fine-mapping studies, and epidemiological studies of myasthenia gravis suggest a genetic contribution to the disease.[10, 11] In fact, studies have described myasthenic patients with a family history of myasthenia gravis and/or a family history of autoimmune diseases. In this study, we performed a literature search of the familial rate reported by myasthenia gravis epidemiological studies and, using our cohort of 1,032 North American myasthenia gravis patients, approximated the prevalence of familial myasthenia gravis, compared the characteristics of familial disease with sporadic disease, and assessed the co-morbidity of other autoimmune diseases among patients and among their families.

METHODS

Patient ascertainment

Phenotype information of 1,032 patients diagnosed with myasthenia gravis was obtained from myasthenia gravis clinics at fourteen centers across North America between January 2010 and January 2011.[10] Patients were diagnosed by neurologists specializing in myasthenia gravis. Each myasthenia gravis diagnosis was based on standard clinical criteria that included, but was not limited to, weakness, fatigability, and electrophysiological, pharmacological (edrophonium test) and/or serological abnormalities. Inclusion criteria for this study were as follows: confirmed diagnosis of myasthenia gravis, non-Hispanic white ethnicity, and presence of anti-AChR antibodies. Patients with anti-MuSK antibodies were excluded from the study. The LRP4 antibody

was discovered after the collection of the cohort was complete. Thus, the LRP4 antibody status of the patients was not known. Family histories of myasthenia gravis and other autoimmune diseases were systematically obtained for each subject using a simple structured questionnaire. A positive family history was defined as having a first-degree (50% of DNA in common), second-degree (25% of DNA in common), or third-degree (12.5% of DNA in common) relative with the disease. DNA samples were collected from each subject and used for genetic analyses as previously reported.[10] Patients with genetic forms of myasthenia gravis were not explicitly excluded, though none of the cohort was known to have such a mutation.

Literature review methodology

To find studies about the epidemiology of familial myasthenia gravis, the PubMed and Medline databases were searched using permutations of search terms: 'epidemiology of familial myasthenia gravis', 'epidemiology of myasthenia gravis', and 'heritability of myasthenia gravis'. After reviewing thirty-one epidemiology studies between the years of 1950 through 2018, only ten studies explicitly referenced the family history of myasthenic patients. Five of those papers provided metrics about family members with myasthenia gravis.[12-16]

Patient and public involvement statement

No patients or members of the public were actively involved with co-producing the research presented in this article.

Statement of ethics approval

Written informed consent was obtained from all patients who participated in this study. Institution review board (IRB) approval was obtained at all participating institutions. Lead institutes for IRB = National Institutes of Health, protocol 03-AG-N329, https://clinicaltrials.gov/ct2/show/NCT02014246

Data availability

The data, consisting of patient family history of myasthenia gravis and other autoimmune diseases in addition to patient personal history of other autoimmune diseases, are not publicly available because of patient privacy concerns but de-identified participant data are available upon request by contacting the corresponding author. In the interests of scientific rigor, the code used for analysis of the dataset is available on GitHub: https://github.com/neurogenetics/Familial-Myasthenia-Gravis.

RESULTS

North American cohort of myasthenia gravis patients

Clinical data were collected from a total of 1,032 patients across fourteen centers in North America and were analyzed in this study. All of the patients had positive anti-AChR antibodies. The mean age of symptom onset in this clinic-based cohort was 53.5 years of age (standard deviation (SD) = 19.4). The female-to-male ratio was 1:1.27. Early-onset disease (< 40 years of age) was observed among 248 (24.0%) of the cohort. Consistent with other reports, nearly one third of the patients in our North American study cohort (305, 29.6%) had undergone thymectomy.

Fifty-eight (5.6%) patients had a family history of myasthenia gravis. Sibling-sibling (31.0%) and parent-child (32.8%) were the most common type of familial relationship, followed by uncle/aunt-nephew/niece (13.8%), cousin-cousin (12.1%) and grandparent-grandchild (5.2%) relationships. Age at symptom onset was slightly younger (50.5 years of age, SD = 19.4) among patients reporting a family history compared to patients without a positive family history (53.7 years, SD = 19.4), though this difference was not statistically significant (p-value = 0.23, Welch's independent two group t-test, figure 1). Approximately one fourth of the familial patients (n = 15) experienced disease prior to the age of 40 (table 1).

Table 1. Comparison of familial and sporadic cases among a cohort of patients diagnosed with myasthenia gravis (n = 1,032)

	Familial	**Sporadic**	***P*-value**
Number of myasthenia gravis cases (percent)	58 (5.6)	974 (94.4)	-
Mean age of disease onset (years) (standard deviation)	50.5 (19.4)	53.7 (19.4)	0.227
Number of patients with early-onset disease (< 40 years) (percent)	15 (25.9)	233 (23.9)	0.86
Number of females	25 (43.1)	429 (44.0)	0.997

(percent)

A personal history of an autoimmune disease other than myasthenia gravis was reported in 275 (26.6%) of study participants. A total of 293 (28.4%) subjects had a family history of an autoimmune disease. The prevalence of autoimmunity in the general population is ~3-9%, indicating that the rates observed among our myasthenia gravis subjects are significantly increased.[17] A breakdown of the specific autoimmune diseases for both personal history and family history of disease is shown in figure 2A and figure 2B.

The most common autoimmune diseases present concomitantly in myasthenia gravis patients were thyroid disease (n = 118, 11.4%), hematological disease (n = 33, 3.2% consisting of autoimmune hemolytic anemia and autoimmune thrombocytopenic purpura), and rheumatoid arthritis (n=28, 2.7%). This was similar to the rates previously reported by Mao et al (figure 2C).[18]

The three most common autoimmune diseases present in the families of myasthenia gravis patients were thyroid disease (n=98, 9.5%), rheumatoid arthritis (n=68, 6.6%), and type 1 diabetes (n=36, 3.5%).

Literature review

Literature review concerning the epidemiology of familial myasthenia gravis identified five studies that discussed the patient's family history of myasthenia gravis with sample sizes that ranged from 264 to 6,638 (table 2).[12-16] The frequency of familial myasthenia gravis ranged from 0.2% to 7.2% across studies, the upper limit having been reported

based on a Finnish cohort.[15] Among these, three studies reported that familial myasthenia gravis cases had an earlier symptom onset age than the sporadic cases.[12, 14, 15] Only three studies reported on the patient history of other autoimmune diseases and/or on the family history of other autoimmune diseases.[12, 14, 15] Among these, thyroid disease, rheumatoid arthritis, systemic lupus erythematosus, and Sjogren's syndrome were the most common other autoimmune diseases reported in patients' personal and/or family histories. [12, 14, 15]

Table 2. Studies reporting the rate of familial disease in myasthenia gravis.

Source	Origin	Sample size	Familial rate	Study type
12	Taiwan	6,638	0.2%	Population
13	Japan	3,141	0.7%	Population
14	Spain	462	3.5%	Clinic
15	Finland	264	7.2%	Population
16	United States	702	3.8%	Clinic

DISCUSSION

We found the prevalence of familial myasthenia gravis in our North American cohort to be 5.6%. This is comparable to the prevalence reported in other studies, where the frequency ranged from 0.2% to 7.2% of myasthenia gravis patients having a family history of myasthenia gravis depending on the cohort studied. Although the vast

majority of myasthenia gravis cases are still non-familial, a prevalence of 5.6% represents a several hundred-fold increase for a disease with an overall prevalence of 1 in 5-10,000. The two studies of myasthenia gravis based on Asian cohorts reported substantially lower rates of familial disease compared to our North American cohort and other European cohorts. For example, two studies of Taiwanese (n = 6,638) and Japanese (n = 3,141) cohorts reported rates of familial myasthenia gravis at 0.2% and 0.7%, respectively. Three studies of Spanish, American, and Finnish cohorts reported rates of 3.5%, 3.8%, and 7.2% (table 2). The familial rates reported in our cohort of over 1,000 patients were, as expected, closer in value to the rates reported among European and American cohorts. Overall, these data suggest that there is population variation in the inheritance of myasthenia gravis that warrants further study to identify the genetic contribution to disease risk.

For a number of genetic diseases, a family history is often associated with an earlier age of onset.[19, 20] Three of the studies in the literature review reported that this was also true of myasthenia gravis.[12, 14, 15] Interestingly, we also observed an earlier onset age in the familial cases (50.5 years of age) compared to the non-familial (53.7 years of age) cases in our cohort (table 1). However, this difference was not significant, a finding that is consistent with previous reports.

Another notable feature of our cohort is that males had a slightly higher prevalence of familial myasthenia gravis compared to the females (1:1.32), suggesting that there might be sex-specific differences in the occurrence of familial versus sporadic disease. This observation may reflect the different age distribution of myasthenia gravis cases in males versus females observed across all myasthenia gravis cases (mean

onset age for females = 45.6; mean onset age for males = 59.8). Indeed, our previous genome-wide association study of myasthenia gravis indicated that the genetic architecture was different among younger and older age groups.[10]

Myasthenia gravis is an immunological disorder and, generally speaking, autoimmune diseases are known to have heritable components.[21] Likewise, approximately one-third of our cohort had a personal history and/or a family history of another autoimmune disease, which is much higher than the prevalence of 3-9%, which has been historically reported in the general population.[17] We observed that familial myasthenia gravis were more likely to have a personal history of autoimmune disease than the sporadic cohort. Similar to previous reports, we found thyroid disease, rheumatoid arthritis, systemic lupus erythematous, and type 1 diabetes to be the most commonly identified comorbidities (figure 2C).[18, 21] Interestingly, the frequency of thyroid disease in this cohort (11.4%) is similar to the frequency range of thyroid disease reported in a study by Kiessling et al.[22] The increased prevalence of familial myasthenia gravis and increased prevalence of other autoimmune disorders both suggest that these autoimmune diseases may share a common predisposition that may be genetic in origin.

Our analyses provide evidence of a genetic contribution to myasthenia gravis based on the higher than expected rate of familial disease observed among our North American patient cohort, as well as the co-occurrence of autoimmune diseases known to have a genetic basis among this population. More work needs to be done to further elucidate the genetic etiology of this archetypal autoimmune disease.

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Competing interests

Mr. Green none declared.

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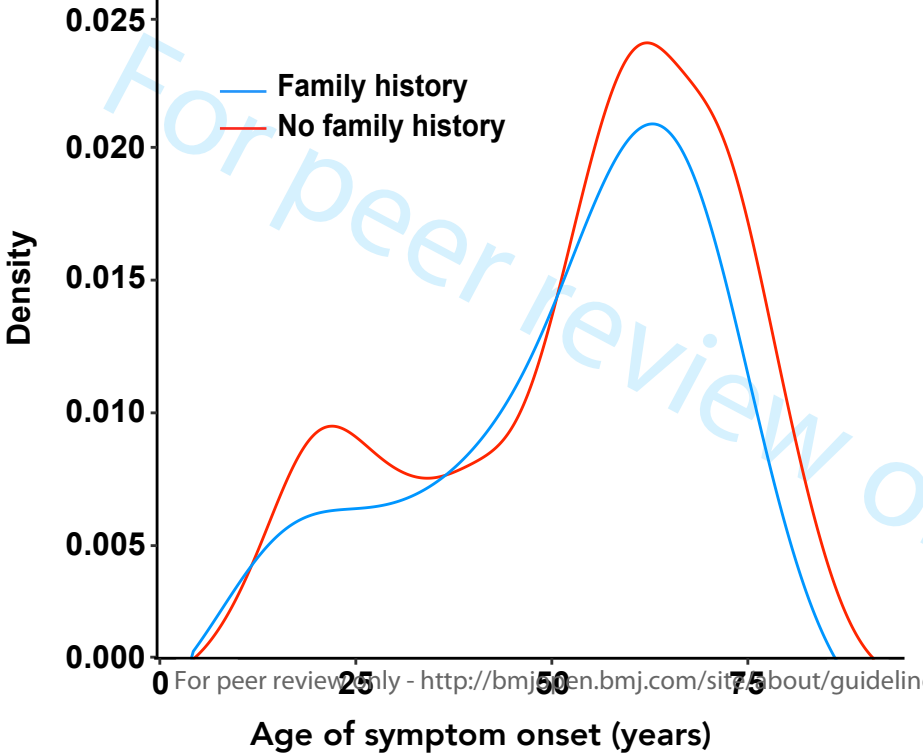
Figures

Figure 1.

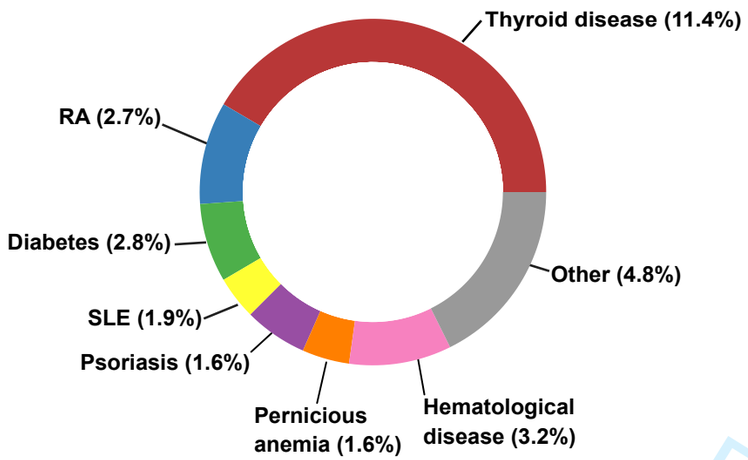
Symptom-onset age distribution of sporadic and familial cases. The density represents the relative probability of myasthenia gravis at each age point.

Figure 2.

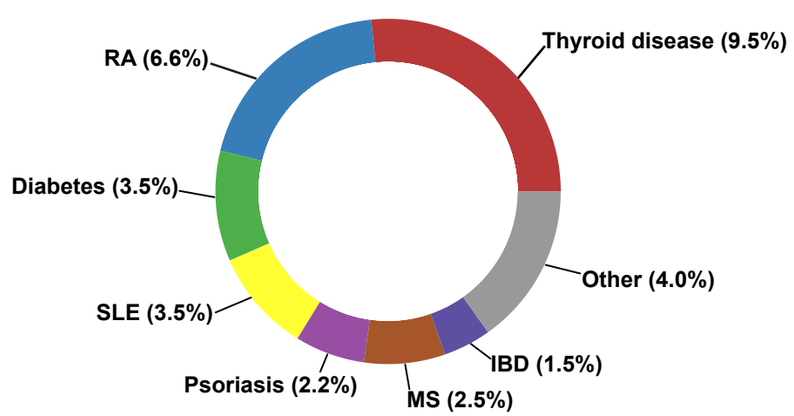
Autoimmune diseases in a cohort of 1,032 patients with myasthenia gravis. (A) Occurrence of autoimmune diseases among patients with myasthenia (n = 275). (B) Occurrence of autoimmune diseases among familial relatives of patients with myasthenia gravis (n = 293). (C) Comparisons of autoimmune diseases among patients with myasthenia gravis and among relatives. Size of circles represent the percentage previously reported by Mao et al.[18]



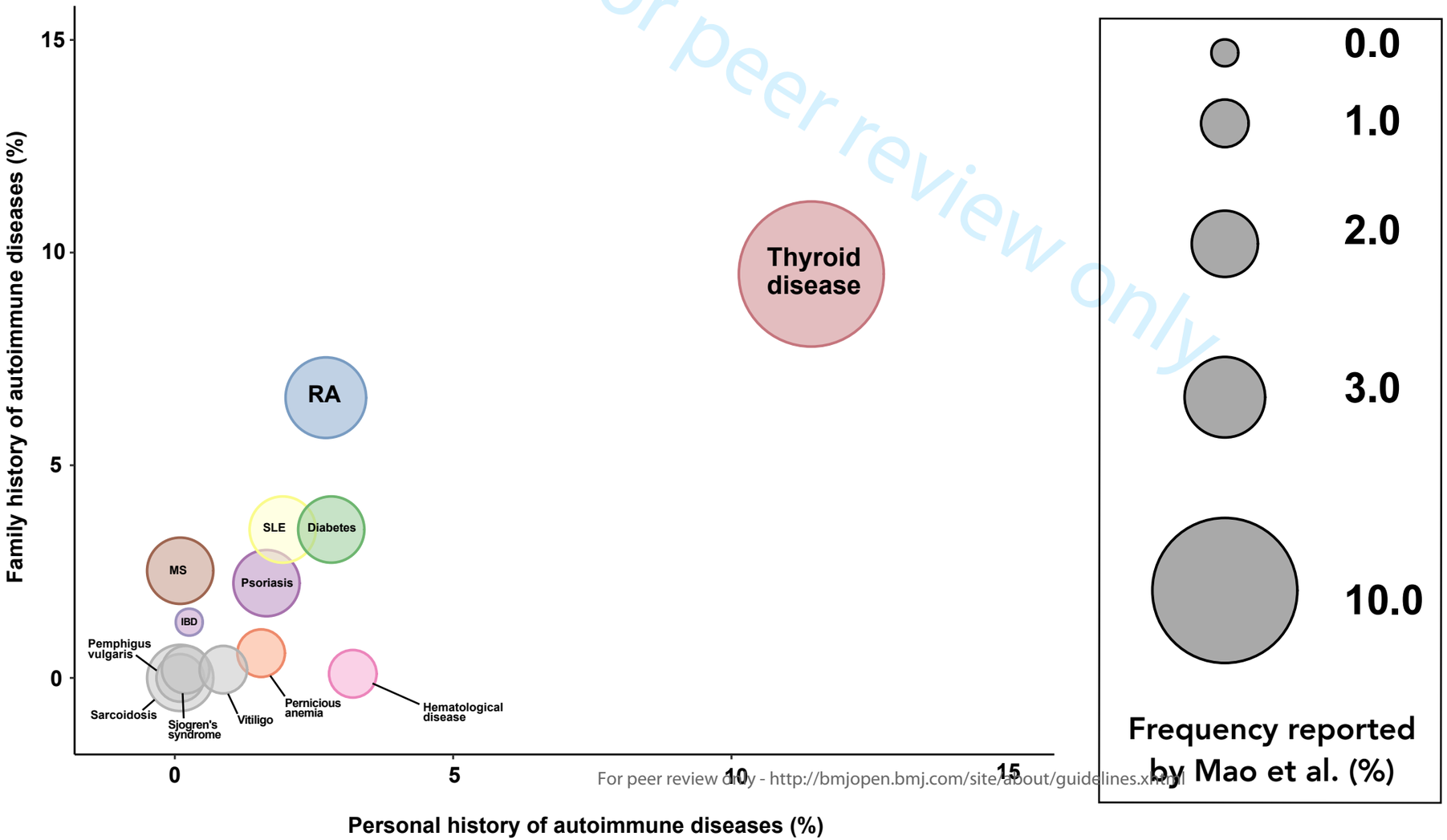
A. Personal history of autoimmune diseases



B. Family history of autoimmune diseases



C. Relationships between personal and family histories of autoimmune diseases



STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	7-8
Introduction			**10-11**
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	10-11
Objectives	3	State specific objectives, including any prespecified hypotheses	11
Methods			**11-13**
Study design	4	Present key elements of study design early in the paper	11
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	11-13
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	11
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	11-13
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	11-13
Bias	9	Describe any efforts to address potential sources of bias	11-13
Study size	10	Explain how the study size was arrived at	11-13
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	11-13
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	14-15
		(*b*) Describe any methods used to examine subgroups and interactions	14-15
		(*c*) Explain how missing data were addressed	14-15
		(*d*) If applicable, explain how loss to follow-up was addressed	14-15
		(*e*) Describe any sensitivity analyses	NA
Results			13-15

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7,11,14
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	13
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	Report numbers of outcome events or summary measures over time	13-15
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	13-15
		(*b*) Report category boundaries when continuous variables were categorized	13-15
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	13-15
Discussion			16-18
Key results	18	Summarise key results with reference to study objectives	16-18
Limitations			17-18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	17-18
Generalisability	21	Discuss the generalisability (external validity) of the study results	17-18
Other information			NA
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	23

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Epidemiological evidence for a hereditary contribution to myasthenia gravis: a retrospective cohort study of patients from North America

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ABSTRACT

Objectives To approximate the rate of familial myasthenia gravis and the coexistence of other autoimmune disorders in the patients and their families.

Design Retrospective cohort study

Setting Clinics across North America

Participants The study included 1,032 patients diagnosed with acetylcholine receptor antibody (AChR)-positive myasthenia gravis

Methods Phenotype information of 1,032 patients diagnosed with acetylcholine receptor antibody (AChR)-positive myasthenia gravis was obtained from clinics at fourteen centers across North America between January 2010 and January 2011. A critical review of the epidemiological literature on the familial rate of myasthenia gravis was also performed.

Results Among 1,032 patients, 58 (5.6%) reported a family history of myasthenia gravis. A history of autoimmune diseases was present in 26.6% of patients and in 28.4% of their family members.

Discussion The familial rate of myasthenia gravis was higher than would be expected for a sporadic disease. Furthermore, a high proportion of patients had a personal or

family history of autoimmune disease. Taken together, these findings suggest a genetic contribution to the pathogenesis of myasthenia gravis.

ARTICLE SUMMARY

Strengths and limitations of this study

- A strength of this study is that it analyzes a large cohort of myasthenia gravis patients with complete data on each patient, allowing multiple clinical correlations to be made.
- A strength of this study is that standardized criteria were used to diagnose patients with myasthenia gravis, including establishing the specific subtype of the disease for each patient.
- A strength of this study is that the cohort of myasthenia gravis patients was sufficiently large to allow the generation of evidence confirming a genetic contribution to the disease.
- A limitation of this study is the reliance on self-reported family history status for both myasthenia gravis and other autoimmune diseases by the patients.
- A limitation of this study is its retrospective design, which precludes ascertaining additional information from individual patients.

INTRODUCTION

Myasthenia gravis is a rare autoimmune disease that is characterized by antibody-mediated interference with neuromuscular transmission at the neuromuscular junction. Originally, the role for an autoimmune attack against the acetylcholine receptor (AChR) on the postsynaptic side of the neuromuscular junction was recognized.[1] However, over the past decade, additional autoimmune targets have been identified: muscle-specific tyrosine kinase (MuSK), agrin, and lipoprotein receptor-related protein 4 (LRP4).[2-4] All of these proteins play essential roles in maintaining the structure and function of the neuromuscular junction.

Myasthenia gravis manifests clinically with muscle weakness and fatigability. Symptoms of ocular muscle weakness are observed early in 85% of myasthenic patients.[5] These symptoms include diplopia and/or ptosis. Weakness of the bulbar musculature is observed in 60% of myasthenic patients, with symptoms including dysarthria and/or dysphagia.[5]

A recent literature review of thirty-one epidemiological studies suggests that the annual incidence of myasthenia gravis may range between 3 and 30 cases per million people.[6] A common theme among myasthenia gravis epidemiological studies is that there is a significant degree of variability between cohorts. For example, a study of a Hong Kong Chinese cohort reported an incidence of 4 per million people per year, whereas a study of an English cohort reported an incidence of 30 per million people per year.[7, 8] This substantial degree of variability may be the result of inconsistent diagnostic criteria, varying case ascertainment, lack of physician awareness concerning myasthenia gravis, or it may reflect different occurrence across populations.

Historically, adult-onset myasthenia gravis has been regarded as a sporadic disease with only a minimal genetic component.[9] However, genome-wide association studies, fine-mapping studies, and epidemiological studies of myasthenia gravis suggest a genetic contribution to the disease.[10, 11] In fact, studies have described myasthenic patients with a family history of myasthenia gravis and/or a family history of autoimmune diseases.[12-16] In this study, we performed a literature search of the familial rate reported by myasthenia gravis epidemiological studies and, using our cohort of 1,032 North American myasthenia gravis patients, approximated the prevalence of familial myasthenia gravis, compared the characteristics of familial disease with sporadic disease, and assessed the co-morbidity of other autoimmune diseases among patients and among their families.

METHODS

Patient ascertainment

Phenotype information of 1,032 patients diagnosed with myasthenia gravis was obtained from myasthenia gravis clinics at fourteen centers across North America between January 2010 and January 2011.[10] The numbers of myasthenia gravis patients attending each of these clinics was not available for this study. Patients were diagnosed by neurologists specializing in myasthenia gravis. Each myasthenia gravis diagnosis was based on standard clinical criteria that included, but was not limited to, weakness, fatigability, and electrophysiological, pharmacological (edrophonium test) and/or serological abnormalities. Inclusion criteria for this study were as follows: confirmed diagnosis of myasthenia gravis, non-Hispanic white ethnicity, and presence of anti-

AChR antibodies. Patients with anti-MuSK antibodies were excluded from the study. The LRP4 antibody was discovered after the collection of the cohort was complete. Thus, the LRP4 antibody status of the patients was not known. Family histories of myasthenia gravis and other autoimmune diseases were systematically obtained for each subject using a simple structured questionnaire (Table S1). A positive family history was defined as having a first-degree (~50% of DNA in common), second-degree (~25% of DNA in common), or third-degree (~12.5% of DNA in common) relative with the disease. DNA samples were collected from each subject and used for genetic analyses as previously reported.[10] Patients with genetic forms of myasthenia gravis were not explicitly excluded, though none of the cohort was known to have such a mutation.

Literature review methodology

To find studies about the epidemiology of familial myasthenia gravis, the PubMed and Medline databases were searched using permutations of search terms: 'epidemiology of familial myasthenia gravis', 'epidemiology of myasthenia gravis', and 'heritability of myasthenia gravis'. After reviewing thirty-one epidemiology studies between the years of 1950 through 2018, only ten studies explicitly referenced the family history of myasthenic patients. Five of those papers provided metrics about family members with myasthenia gravis.[12-16]

Patient and public involvement statement

No patients or members of the public were actively involved with co-producing the

research presented in this article.

Statement of ethics approval

Written informed consent was obtained from all patients who participated in this study. Institution review board (IRB) approval was obtained at all participating institutions. Lead institutes for IRB = National Institutes of Health, protocol 03-AG-N329, https://clinicaltrials.gov/ct2/show/NCT02014246

Data availability

The data, consisting of patient family history of myasthenia gravis and other autoimmune diseases in addition to patient personal history of other autoimmune diseases, are not publicly available because of patient privacy concerns but de-identified participant data are available upon request by contacting the corresponding author. In the interests of scientific rigor, the code used for analysis of the dataset is available on GitHub: https://github.com/neurogenetics/Familial-Myasthenia-Gravis.

RESULTS

North American cohort of myasthenia gravis patients

Clinical data were collected from a total of 1,032 patients across fourteen centers in North America and were analyzed in this study. All of the patients had positive anti-AChR antibodies. The median age of symptom onset in this clinic-based cohort was 58 years of age (range = 4-91; median onset age for females = 46; median onset age for males = 62). The female-to-male ratio was 1:1.27. Early-onset disease (< 40 years of

age) was observed among 248 (24.0%) of the cohort. Consistent with other reports, nearly one third of the patients in our North American study cohort (305, 29.6%) had undergone thymectomy.

Fifty-eight (5.6%) patients had a family history of myasthenia gravis. Sibling-sibling (31.0%) and parent-child (32.8%) were the most common type of familial relationship, followed by uncle/aunt-nephew/niece (13.8%), cousin-cousin (12.1%) and grandparent-grandchild (5.2%) relationships. Of note, the indicated percentage of parent-child cases might be inflated because neonatal myasthenia gravis cases (which are not believed to be genetic) could not be discerned from non-neonatal cases. Age at symptom onset was similar among patients with a family history compared to patients without a family history (57.5 years of age, range = 8-80 years versus 58.5 years, range = 4-91 years, p-value = 0.183, Wilcoxon rank sum test, figure 1, table 1).

Table 1. Comparison of familial and sporadic cases among a cohort of patients diagnosed with myasthenia gravis (n = 1,032)

	Familial	Sporadic	*P*-value
Number of myasthenia gravis cases (percent)	58 (5.6)	974 (94.4)	-
Median age of disease onset (years) (range)	57.5 (8-80)	58.5 (4-91)	0.183
Number of patients with early-onset disease (< 40 years) (percent)	15 (25.9)	233 (23.9)	0.86
Number of females (percent)	25 (43.1)	429 (44.0)	0.997

A personal history of an autoimmune disease other than myasthenia gravis was reported in 275 (26.6%) of study participants. A total of 293 (28.4%) subjects had a family history of an autoimmune disease. The prevalence of autoimmunity in the general population is ~3-9%, indicating that the rates observed among our myasthenia gravis subjects are significantly increased.[17] A breakdown of the specific autoimmune diseases

for both personal history and family history of disease is shown in figure 2A and figure 2B.

The most common autoimmune diseases present concomitantly in myasthenia gravis patients were thyroid disease (n = 118, 11.4%), hematological disease (n = 33, 3.2% consisting of autoimmune hemolytic anemia and autoimmune thrombocytopenic purpura), and rheumatoid arthritis (n=28, 2.7%). This was similar to the rates previously reported by Mao et al (figure 2C).[18]

The three most common autoimmune diseases present in the families of myasthenia gravis patients were thyroid disease (n=98, 9.5%), rheumatoid arthritis (n=68, 6.6%), and type 1 diabetes (n=36, 3.5%).

Literature review

Literature review concerning the epidemiology of familial myasthenia gravis identified five studies that discussed the patient's family history of myasthenia gravis with sample sizes that ranged from 264 to 6,638 (table 2).[12-16] The frequency of familial myasthenia gravis ranged from 0.2% to 7.2% across studies, the upper limit having been reported based on a Finnish cohort.[15] Among these, three studies reported that familial myasthenia gravis cases had an earlier symptom onset age than the sporadic cases.[12, 14, 15] Only three studies reported on the patient history of other autoimmune diseases and/or on the family history of other autoimmune diseases.[12, 14, 15] Among these, thyroid disease, rheumatoid arthritis, systemic lupus erythematosus, and Sjogren's syndrome were the most common other autoimmune diseases reported in patients' personal and/or family histories. [12, 14, 15]

Table 2. Studies reporting the rate of familial disease in myasthenia gravis.

Source	Origin	Sample size	Familial rate	Study type
12	Taiwan	6,638	0.2%	Population
13	Japan	3,141	0.7%	Population
14	Spain	462	3.5%	Clinic
15	Finland	264	7.2%	Population
16	United States	702	3.8%	Clinic

DISCUSSION

We found the prevalence of familial myasthenia gravis in our North American cohort to be 5.6%. This is comparable to the prevalence reported in other studies, where the frequency ranged from 0.2% to 7.2% of myasthenia gravis patients having a family history of myasthenia gravis depending on the cohort studied.[12-16] Although the vast majority of myasthenia gravis cases are still non-familial, a prevalence of 5.6% represents a several hundred-fold increase for a disease with an overall prevalence of 1 in 5-10,000.[5] The two studies of myasthenia gravis based on Asian cohorts reported substantially lower rates of familial disease compared to our North American cohort and

other European cohorts. For example, two studies of Taiwanese (n = 6,638) and Japanese (n = 3,141) cohorts reported rates of familial myasthenia gravis at 0.2% and 0.7%, respectively.[12-13] Three studies of Spanish, American, and Finnish cohorts reported rates of 3.5%, 3.8%, and 7.2% (table 2).[14-16] The familial rates reported in our cohort of over 1,000 patients were, as expected, closer in value to the rates reported among European and American cohorts. Overall, these data suggest that there is population variation in the inheritance of myasthenia gravis that warrants further study to identify the genetic contribution to disease risk.

A notable feature of our cohort is that males had a slightly higher prevalence of familial myasthenia gravis compared to the females (1:1.32), suggesting that there might be sex-specific differences in the occurrence of familial versus sporadic disease. This observation may reflect the different age distribution of myasthenia gravis cases in males versus females observed across all myasthenia gravis cases. The reason for this is unclear, though we note that our previous genome-wide association study of myasthenia gravis indicated that the genetic architecture was different among younger and older age groups.[10]

Myasthenia gravis is an immunological disorder and, generally speaking, autoimmune diseases are known to have heritable components.[19] Likewise, approximately one-third of our cohort had a personal history and/or a family history of another autoimmune disease, which is much higher than the prevalence of 3-9%, which has been historically reported in the general population.[17] We found that 19% (11/58) of the familial myasthenia gravis cases also had a personal history of autoimmune diseases; this was more than the sporadic myasthenia gravis cases in which only 10%

(98/974) had a personal history of autoimmune diseases. Similar to previous reports, we found thyroid disease, rheumatoid arthritis, systemic lupus erythematous, and type 1 diabetes to be the most commonly identified comorbidities (figure 2C).[18, 19] Interestingly, the frequency of thyroid disease in this cohort (11.4%) is similar to the frequency range of thyroid disease reported in a study by Kiessling et al.[20] The increased prevalence of familial myasthenia gravis and increased prevalence of other autoimmune disorders both suggest that these autoimmune diseases may share a common predisposition that may be genetic in origin. Future studies focusing on specific genes and genomic variants encountered in patients with familial myasthenia gravis offer the promise to more precisely identify any genetic contributions to the disease.

While our study has some notable strengths, it also has an inherent limitation related to its reliance on self-reported family histories, which could have over- or underestimated the prevalence of the diseases studied. For example, it is plausible that a patient could self-report as not having a family history of myasthenia gravis because the family member was never clinically diagnosed with the disease or did not live long enough for the disease to manifest. Similarly, other studies have found an overestimation of some autoimmune diseases, especially thyroid disease and rheumatoid arthritis, related to self-reporting.[21]

Our analyses provide evidence of a genetic contribution to myasthenia gravis based on the higher than expected rate of familial disease observed among our North American patient cohort, as well as the co-occurrence of autoimmune diseases known to have a genetic basis among this population. More work needs to be done to further elucidate the genetic etiology of this archetypal autoimmune disease.

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Competing interests

Mr. Green none declared.

Dr. Barohn served as a consultant for NuFactor and Momenta Pharmaceutical and receives research support from PTC Therapeutics, Ra Pharma, Orphazyme, Sanofi Genzyme, FDA OOPD, NIH, and PCORI.

Dr. Bartoccion none declared.

Dr. Benetar reports grant support from Muscular Dystrophy Association, ALS Association, ALS Recovery Fund, Kimmelman Estate, Target ALS, Eli Lilly & Company, and the National Institutes of Health (NIH) during the conduct of the study. He also

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Mr. Blackmore none declared

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Dr. Corse none declared.

Dr. Dimachkie serves or recently served as a consultant for ArgenX, Catalyst, CSL-Behring, Kezar, Momenta, NuFactor, RMS Medical, Sanofi Genzyme, Shire Takeda, and Spark Therapeutics. Dr. Dimachkie received grants from Alexion, Alnylam Pharmaceuticals, Amicus, Biomarin, Bristol-Myers Squibb, Catalyst, CSL-Behring, FDA/OOPD, GlaxoSmithKline, Genentech, Grifols, Mitsubishi Tanabe Pharma, MDA, NIH, Novartis, Sanofi Genzyme, Octapharma, Orphazyme, Sarepta Therapeutics, Shire Takeda, Spark, UCB Biopharma,Viromed & TMA.

Dr. Evoli was a member of the advisory board for Alexion, a scientific award jury member for Grifols and safety data monitor for UCB.

Dr. Florence none declared.

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Ms. Koopman none declared.

Ms. Lipscomb none declared.

Dr. Maestri none declared.

Dr. Marino none declared.

Dr. Massey none declared

Dr. McVey none declared.

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Dr. Muppidi has served on advisory board meetings for Alexion and argenx.

Dr. Nicolle none declared.

Dr. Oger none declared.

Dr. Pascuzzi none declared.

Dr. Pasnoor served on advisory board for CSL Behring, Alexion pharmaceuticals, Argenx Pharmaceuticals and has been consultant for Momenta Pharmaceuticals.

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Dr. Sanders none declared.

Dr. Siddiqi none declared.

Ms. Soloway none declared

Dr. Wolfe none declared.

Dr. Wulf none declared.

Dr. Drachman none declared.

Dr. Traynor holds an American and European Union patent on the clinical testing and therapeutic intervention for the hexanucleotide repeat expansion of C9orf72. Has

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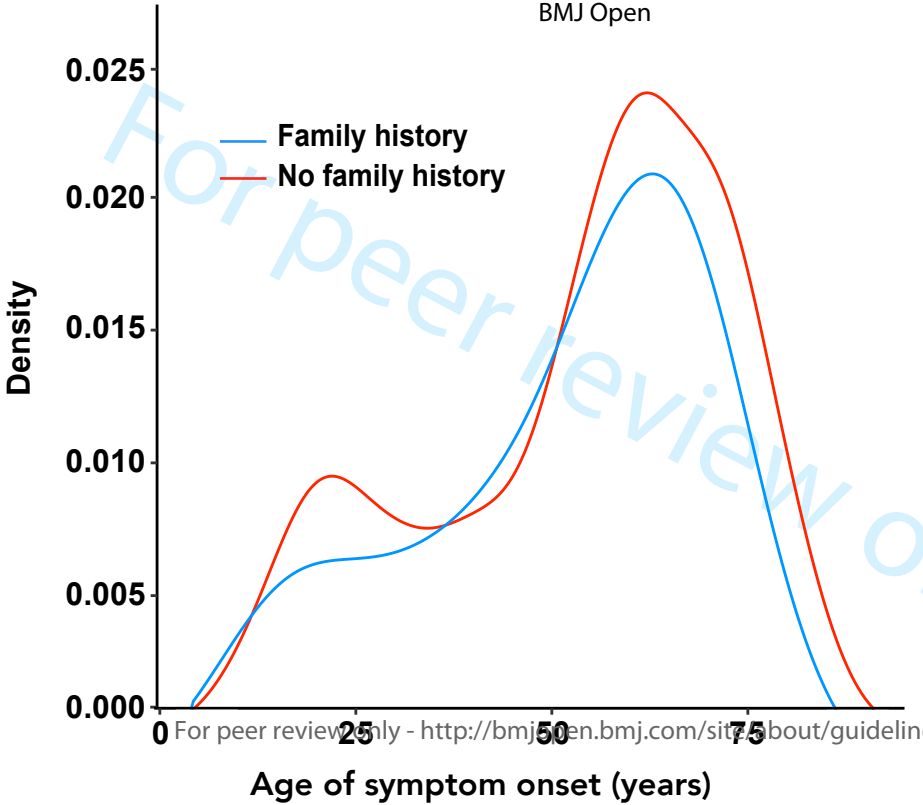
Figures

Figure 1.

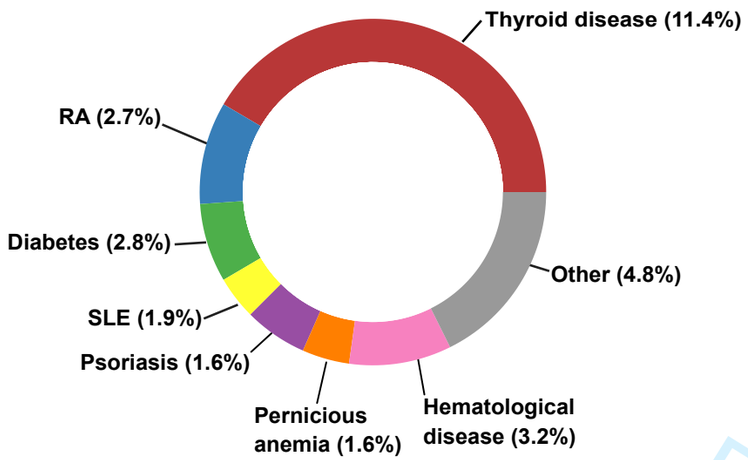
Symptom-onset age distribution of sporadic and familial cases. The density represents the relative probability of myasthenia gravis at each age point.

Figure 2.

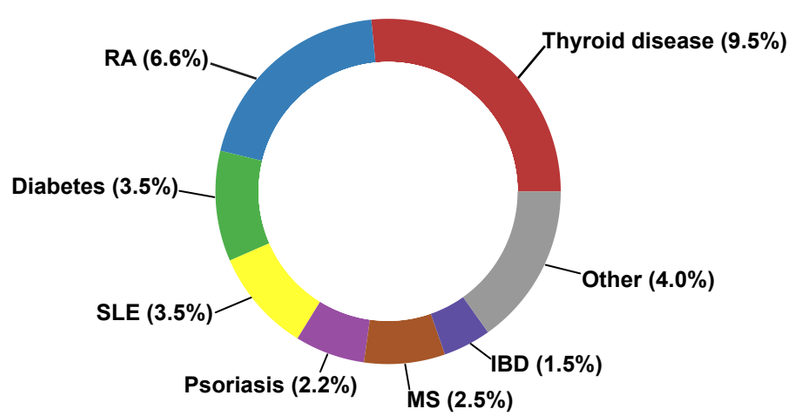
Autoimmune diseases in a cohort of 1,032 patients with myasthenia gravis. (A) Occurrence of autoimmune diseases among patients with myasthenia (n = 275). (B) Occurrence of autoimmune diseases among familial relatives of patients with myasthenia gravis (n = 293). (C) Comparisons of autoimmune diseases among patients with myasthenia gravis and among relatives. Size of circles represent the percentage previously reported by Mao et al.[18]



A. Personal history of autoimmune diseases



B. Family history of autoimmune diseases



C. Relationships between personal and family histories of autoimmune diseases

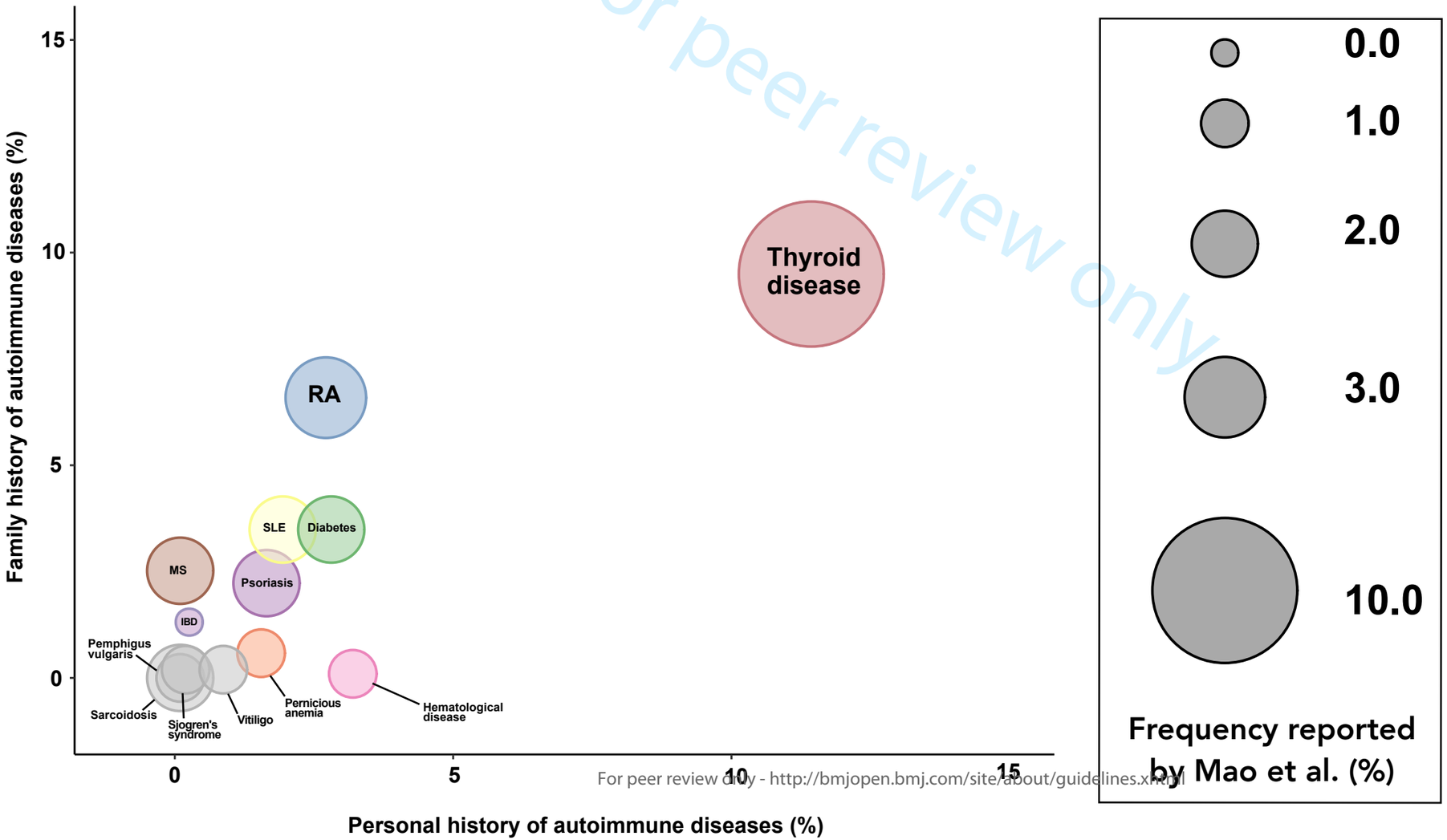


Table S1. Questionnaire used to collect demographic and clinical information from patients diagnosed with myasthenia gravis

Patient Information	
Patient identification number:	String (e.g., JHU1, JHU2)
Institution:	String (e.g., Johns Hopkins, UWO)
Date:	Date
Verified as Caucasian?	Yes/No
AChR titer - Initial or 1st known:	Numeric
Age at symptom onset (years):	Numeric
Gender:	Male/Female
Worst status	
Diplopia or ptosis only?	Yes/No
Mild limb weakness?	Yes/No
Dysphagia dyspnea?	Yes/No
Severe limb weakness?	Yes/No
Hospitalized?	Yes/No
Ventilated?	Yes/No
Feeding tube?	Yes/No
Treatment	
Mestinon?	Yes/No
Benefit: Mestinon:	Ordinal scale [0,1,2]
Prednisone?	Yes/No
Benefit: Prednisone:	Ordinal scale [0,1,2]
CellCept?	Yes/No
Benefit: CellCept?	Ordinal scale [0,1,2]
Cylclosporine?	Yes/No
Benefit: Cyclosporine:	Ordinal scale [0,1,2]
Tacrolimus?	Yes/No
Benefit: Tacrolimus:	Ordinal scale [0,1,2]
Intravenous immunoglobulin?	Yes/No
Benefit: Intravenous immunoglobulin:	Ordinal scale [0,1,2]
Plasmapheresis?	Yes/No
Benefit: Plasmapheresis:	Ordinal scale [0,1,2]
Imuran/Azathioprine?	Yes/No
Benefit: Imuran/Azathioprine:	Ordinal scale [0,1,2]
Other treatment?	Yes/No
Name of other 1:	String (e.g., Mytelase)
Benefit: other 1:	Ordinal scale [0,1,2]
Name of other 2:	String (e.g., Mytelase)
Benefit: other 2:	Ordinal scale [0,1,2]

Best Status	
Back to normal without medication?	Yes/No
Back to normal with medication?	Yes/No
Diplopia or ptosis only?	Yes/No
Mild limb weakness?	Yes/No
Dysphagia/dyspnea?	Yes/No
Severe limb weakness?	Yes/No
Hospitalized?	Yes/No
Ventilated?	Yes/No
Feeding tube:	Yes/No
Other Autoimmune Diseases	
Other autoimmune disease?	Yes/No
Thyroiditis?	Yes/No
Lupus?	Yes/No
Rheumatoid arthritis?	Yes/No
Psoriasis?	Yes/No
Blood disease?	Yes/No
Other Autoimmune Disease?	Yes/No
Other Autoimmune Disease (Name1):	String (e.g., vitiligo, celiac disease)
Other Autoimmune Disease (Name2):	String (e.g., vitiligo, celiac disease)
Other Autoimmune Disease (Name3):	String (e.g., vitiligo, celiac disease)
Family history of myasthenia gravis	
Family history of myasthenia gravis?	Yes/No
Myasthenia gravis (Relationship):	String (e.g., mother, sister)
Family history of other Autoimmune Disease	
Family history of other Autoimmune Disease?	Yes/No
Autoimmune Disease (Relationship):	String (e.g., mother, sister)
Autoimmune Disease (Disease):	String (e.g., polymyalgia, vitiligo)
Family history of other Autoimmune Disease 2nd Member?	Yes/No
Autoimmune Disease (Relationship) 2nd Member:	String (e.g., mother, sister)
Autoimmune Disease (Disease) 2nd Member:	String (e.g., polymyalgia, vitiligo)
Family history of other Autoimmune Disease 3rd Member?	Yes/No
Autoimmune Disease (Relationship) 3rd Member:	String (e.g., mother, sister)
Autoimmune Disease (Disease) 3rd Member:	String (e.g., polymyalgia, vitiligo)
Other relative in study?	Yes/No
Other relative in study id	String (e.g., JHU1, JHU2)
Thymectomy?	Yes/No
Thymoma?	Yes/No
Histology of thymus if known?	String (e.g., Follicular Hyperplasia)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	7-8
Introduction			**10-11**
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	10-11
Objectives	3	State specific objectives, including any prespecified hypotheses	11
Methods			**11-13**
Study design	4	Present key elements of study design early in the paper	11
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	11-13
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	11
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	11-13
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	11-13
Bias	9	Describe any efforts to address potential sources of bias	11-13
Study size	10	Explain how the study size was arrived at	11-13
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	11-13
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	14-15
		(*b*) Describe any methods used to examine subgroups and interactions	14-15
		(*c*) Explain how missing data were addressed	14-15
		(*d*) If applicable, explain how loss to follow-up was addressed	14-15
		(*e*) Describe any sensitivity analyses	NA
Results			13-15

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7,11,14
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	13
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	Report numbers of outcome events or summary measures over time	13-15
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	13-15
		(*b*) Report category boundaries when continuous variables were categorized	13-15
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	13-15
Discussion			16-18
Key results	18	Summarise key results with reference to study objectives	16-18
Limitations			17-18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	17-18
Generalisability	21	Discuss the generalisability (external validity) of the study results	17-18
Other information			NA
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	23

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Epidemiological evidence for a hereditary contribution to myasthenia gravis: a retrospective cohort study of patients from North America

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ABSTRACT

Objectives To approximate the rate of familial myasthenia gravis and the coexistence of other autoimmune disorders in the patients and their families.

Design Retrospective cohort study

Setting Clinics across North America

Participants The study included 1,032 patients diagnosed with acetylcholine receptor antibody (AChR)-positive myasthenia gravis

Methods Phenotype information of 1,032 patients diagnosed with acetylcholine receptor antibody (AChR)-positive myasthenia gravis was obtained from clinics at fourteen centers across North America between January 2010 and January 2011. A critical review of the epidemiological literature on the familial rate of myasthenia gravis was also performed.

Results Among 1,032 patients, 58 (5.6%) reported a family history of myasthenia gravis. A history of autoimmune diseases was present in 26.6% of patients and in 28.4% of their family members.

Discussion The familial rate of myasthenia gravis was higher than would be expected for a sporadic disease. Furthermore, a high proportion of patients had a personal or

family history of autoimmune disease. Taken together, these findings suggest a genetic contribution to the pathogenesis of myasthenia gravis.

ARTICLE SUMMARY

Strengths and limitations of this study

- A strength of this study is that it analyzes a large cohort of myasthenia gravis patients with complete data on each patient, allowing multiple clinical correlations to be made.
- A strength of this study is that standardized criteria were used to diagnose patients with myasthenia gravis, including establishing the specific subtype of the disease for each patient.
- A strength of this study is that the cohort of myasthenia gravis patients was sufficiently large to allow the generation of evidence confirming a genetic contribution to the disease.
- A limitation of this study is the reliance on self-reported family history status for both myasthenia gravis and other autoimmune diseases by the patients.
- A limitation of this study is its retrospective design, which precludes ascertaining additional information from individual patients.

INTRODUCTION

Myasthenia gravis is a rare autoimmune disease that is characterized by antibody-mediated interference with neuromuscular transmission at the neuromuscular junction. Originally, the role for an autoimmune attack against the acetylcholine receptor (AChR) on the postsynaptic side of the neuromuscular junction was recognized.[1] However, over the past decade, additional autoimmune targets have been identified: muscle-specific tyrosine kinase (MuSK), agrin, and lipoprotein receptor-related protein 4 (LRP4).[2-4] All of these proteins play essential roles in maintaining the structure and function of the neuromuscular junction.

Myasthenia gravis manifests clinically with muscle weakness and fatigability. Symptoms of ocular muscle weakness are observed early in 85% of myasthenic patients.[5] These symptoms include diplopia and/or ptosis. Weakness of the bulbar musculature is observed in 60% of myasthenic patients, with symptoms including dysarthria and/or dysphagia.[5]

A recent literature review of thirty-one epidemiological studies suggests that the annual incidence of myasthenia gravis may range between 3 and 30 cases per million people.[6] A common theme among myasthenia gravis epidemiological studies is that there is a significant degree of variability between cohorts. For example, a study of a Hong Kong Chinese cohort reported an incidence of 4 per million people per year, whereas a study of an English cohort reported an incidence of 30 per million people per year.[7, 8] This substantial degree of variability may be the result of inconsistent diagnostic criteria, varying case ascertainment, lack of physician awareness concerning myasthenia gravis, or it may reflect different occurrence across populations.

Historically, adult-onset myasthenia gravis has been regarded as a sporadic disease with only a minimal genetic component.[9] However, genome-wide association studies, fine-mapping studies, and epidemiological studies of myasthenia gravis suggest a genetic contribution to the disease.[10, 11] In fact, studies have described myasthenic patients with a family history of myasthenia gravis and/or a family history of autoimmune diseases.[12-16] In this study, we performed a literature search of the familial rate reported by myasthenia gravis epidemiological studies and, using our cohort of 1,032 North American myasthenia gravis patients, approximated the prevalence of familial myasthenia gravis, compared the characteristics of familial disease with sporadic disease, and assessed the co-morbidity of other autoimmune diseases among patients and among their families.

METHODS

Patient ascertainment

Phenotype information of 1,032 patients diagnosed with myasthenia gravis was obtained from myasthenia gravis clinics at fourteen centers across North America between January 2010 and January 2011.[10] The numbers of myasthenia gravis patients attending each of these clinics was not available for this study. Patients were diagnosed by neurologists specializing in myasthenia gravis. Each myasthenia gravis diagnosis was based on standard clinical criteria that included, but was not limited to, weakness, fatigability, and electrophysiological, pharmacological (edrophonium test) and/or serological abnormalities. Inclusion criteria for this study were as follows: confirmed diagnosis of myasthenia gravis, non-Hispanic white ethnicity, and presence of anti-

AChR antibodies. Patients with anti-MuSK antibodies were excluded from the study. The LRP4 antibody was discovered after the collection of the cohort was complete. Thus, the LRP4 antibody status of the patients was not known. Family histories of myasthenia gravis and other autoimmune diseases were systematically obtained for each subject using a simple structured questionnaire (Table S1). A positive family history was defined as having a first-degree (~50% of DNA in common), second-degree (~25% of DNA in common), or third-degree (~12.5% of DNA in common) relative with the disease. DNA samples were collected from each subject and used for genetic analyses as previously reported.[10] Patients with genetic forms of myasthenia gravis were not explicitly excluded, though none of the cohort was known to have such a mutation.

Literature review methodology

To find studies about the epidemiology of familial myasthenia gravis, the PubMed and Medline databases were searched using permutations of search terms: 'epidemiology of familial myasthenia gravis', 'epidemiology of myasthenia gravis', and 'heritability of myasthenia gravis'. After reviewing thirty-one epidemiology studies between the years of 1950 through 2018, only ten studies explicitly referenced the family history of myasthenic patients. Five of those papers provided metrics about family members with myasthenia gravis.[12-16]

Patient and public involvement statement

No patients or members of the public were actively involved with co-producing the

research presented in this article.

Statement of ethics approval

Written informed consent was obtained from all patients who participated in this study. Institution review board (IRB) approval was obtained at all participating institutions. Lead institutes for IRB = National Institutes of Health, protocol 03-AG-N329, https://clinicaltrials.gov/ct2/show/NCT02014246

Data availability

The data, consisting of patient family history of myasthenia gravis and other autoimmune diseases in addition to patient personal history of other autoimmune diseases, are not publicly available because of patient privacy concerns but de-identified participant data are available upon request by contacting the corresponding author. In the interests of scientific rigor, the code used for analysis of the dataset is available on GitHub: https://github.com/neurogenetics/Familial-Myasthenia-Gravis.

RESULTS

North American cohort of myasthenia gravis patients

Clinical data were collected from a total of 1,032 patients across fourteen centers in North America and were analyzed in this study. All of the patients had positive anti-AChR antibodies. The median age of symptom onset in this clinic-based cohort was 58 years of age (range = 4-91; median onset age for females = 46; median onset age for males = 62). The female-to-male ratio was 1:1.27. Early-onset disease (< 40 years of

age) was observed among 248 (24.0%) of the cohort. Consistent with other reports, nearly one third of the patients in our North American study cohort (305, 29.6%) had undergone thymectomy.

Fifty-eight (5.6%) patients had a family history of myasthenia gravis. Sibling-sibling (31.0%) and parent-child (32.8%) were the most common type of familial relationship, followed by uncle/aunt-nephew/niece (13.8%), cousin-cousin (12.1%) and grandparent-grandchild (5.2%) relationships. Of note, the indicated percentage of parent-child cases might be inflated because neonatal myasthenia gravis cases (which are not believed to be genetic) could not be discerned from non-neonatal cases. Age at symptom onset was similar among patients with a family history compared to patients without a family history (57.5 years of age, range = 8-80 years versus 58.5 years, range = 4-91 years, p-value = 0.183, Wilcoxon rank sum test, figure 1, table 1).

Table 1. Comparison of familial and sporadic cases among a cohort of patients diagnosed with myasthenia gravis (n = 1,032)

	Familial	Sporadic	*P*-value
Number of myasthenia gravis cases (percent)	58 (5.6)	974 (94.4)	-
Median age of disease onset (years) (range)	57.5 (8-80)	58.5 (4-91)	0.183
Number of patients with early-onset disease (< 40 years) (percent)	15 (25.9)	233 (23.9)	0.86
Number of females (percent)	25 (43.1)	429 (44.0)	0.997

A personal history of an autoimmune disease other than myasthenia gravis was reported in 275 (26.6%) of study participants. A total of 293 (28.4%) subjects had a family history of an autoimmune disease. The prevalence of autoimmunity in the general population is ~3-9%, indicating that the rates observed among our myasthenia gravis subjects are significantly increased.[17] A breakdown of the specific autoimmune diseases

for both personal history and family history of disease is shown in figure 2A and figure 2B.

The most common autoimmune diseases present concomitantly in myasthenia gravis patients were thyroid disease (n = 118, 11.4%), hematological disease (n = 33, 3.2% consisting of autoimmune hemolytic anemia and autoimmune thrombocytopenic purpura), and rheumatoid arthritis (n=28, 2.7%). This was similar to the rates previously reported by Mao et al (figure 2C).[18]

The three most common autoimmune diseases present in the families of myasthenia gravis patients were thyroid disease (n=98, 9.5%), rheumatoid arthritis (n=68, 6.6%), and type 1 diabetes (n=36, 3.5%).

Literature review

Literature review concerning the epidemiology of familial myasthenia gravis identified five studies that discussed the patient's family history of myasthenia gravis with sample sizes that ranged from 264 to 6,638 (table 2).[12-16] The frequency of familial myasthenia gravis ranged from 0.2% to 7.2% across studies, the upper limit having been reported based on a Finnish cohort.[15] Among these, three studies reported that familial myasthenia gravis cases had an earlier symptom onset age than the sporadic cases.[12, 14, 15] Only three studies reported on the patient history of other autoimmune diseases and/or on the family history of other autoimmune diseases.[12, 14, 15] Among these, thyroid disease, rheumatoid arthritis, systemic lupus erythematosus, and Sjogren's syndrome were the most common other autoimmune diseases reported in patients' personal and/or family histories. [12, 14, 15]

Table 2. Studies reporting the rate of familial disease in myasthenia gravis.

Source	Origin	Sample size	Familial rate	Study type
12	Taiwan	6,638	0.2%	Population
13	Japan	3,141	0.7%	Population
14	Spain	462	3.5%	Clinic
15	Finland	264	7.2%	Population
16	United States	702	3.8%	Clinic

DISCUSSION

We found the prevalence of familial myasthenia gravis in our North American cohort to be 5.6%. Our previous genetic analysis of this cohort showed the heritability of myasthenia gravis to be 25.5%.[10] This is comparable to the prevalence reported in other studies, where the frequency ranged from 0.2% to 7.2% of myasthenia gravis patients having a family history of myasthenia gravis depending on the cohort studied.[12-16] Although the vast majority of myasthenia gravis cases are still non-familial, a prevalence of 5.6% represents a several hundred-fold increase for a disease with an overall prevalence of 1 in 5-10,000.[5] The two studies of myasthenia gravis based on Asian

cohorts reported substantially lower rates of familial disease compared to our North American cohort and other European cohorts. For example, two studies of Taiwanese (n = 6,638) and Japanese (n = 3,141) cohorts reported rates of familial myasthenia gravis at 0.2% and 0.7%, respectively.[12-13] Three studies of Spanish, American, and Finnish cohorts reported rates of 3.5%, 3.8%, and 7.2% (table 2).[14-16] The familial rates reported in our cohort of over 1,000 patients were, as expected, closer in value to the rates reported among European and American cohorts. Overall, these data suggest that there is population variation in the inheritance of myasthenia gravis that warrants further study to identify the genetic contribution to disease risk.

A notable feature of our cohort is that males had a slightly higher prevalence of familial myasthenia gravis compared to the females (1:1.32), suggesting that there might be sex-specific differences in the occurrence of familial versus sporadic disease. This observation may reflect the different age distribution of myasthenia gravis cases in males versus females observed across all myasthenia gravis cases. The reason for this is unclear, though we note that our previous genome-wide association study of myasthenia gravis indicated that the genetic architecture was different among younger and older age groups.[10]

Myasthenia gravis is an immunological disorder and, generally speaking, autoimmune diseases are known to have heritable components.[19] Likewise, approximately one-third of our cohort had a personal history and/or a family history of another autoimmune disease, which is much higher than the prevalence of 3-9%, which has been historically reported in the general population.[17] We found that 19% (11/58) of the familial myasthenia gravis cases also had a personal history of autoimmune

diseases; this was more than the sporadic myasthenia gravis cases in which only 10% (98/974) had a personal history of autoimmune diseases. Similar to previous reports, we found thyroid disease, rheumatoid arthritis, systemic lupus erythematous, and type 1 diabetes to be the most commonly identified comorbidities (figure 2C).[18, 19] Interestingly, the frequency of thyroid disease in this cohort (11.4%) is similar to the frequency range of thyroid disease reported in a study by Kiessling et al.[20] The increased prevalence of familial myasthenia gravis and increased prevalence of other autoimmune disorders both suggest that these autoimmune diseases may share a common predisposition that may be genetic in origin. We speculate that the link between thyroid disease and myasthenia gravis may be due to a common genetic background or an immunological cross-reactivity against epitopes or auto-antigens shared by the thyroid and other tissues relevant to myasthenia gravis.[21,22] Future studies focusing on specific genes and genomic variants encountered in patients with familial myasthenia gravis offer the promise to more precisely identify any genetic contributions to the disease.

While our study has some notable strengths, it also has an inherent limitation related to its reliance on self-reported family histories, which could have over- or underestimated the prevalence of the diseases studied. For example, it is plausible that a patient could self-report as not having a family history of myasthenia gravis because the family member was never clinically diagnosed with the disease or did not live long enough for the disease to manifest. Similarly, other studies have found an overestimation of some autoimmune diseases, especially thyroid disease and rheumatoid arthritis, related to self-reporting.[23]

Our analyses provide evidence of a genetic contribution to myasthenia gravis

based on the higher than expected rate of familial disease observed among our North American patient cohort, as well as the co-occurrence of autoimmune diseases known to have a genetic basis among this population. More work needs to be done to further elucidate the genetic etiology of this archetypal autoimmune disease.

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Competing interests

Mr. Green none declared.

Dr. Barohn served as a consultant for NuFactor and Momenta Pharmaceutical and receives research support from PTC Therapeutics, Ra Pharma, Orphazyme, Sanofi Genzyme, FDA OOPD, NIH, and PCORI.

Dr. Bartoccion none declared.

Dr. Benetar reports grant support from Muscular Dystrophy Association, ALS Association, ALS Recovery Fund, Kimmelman Estate, Target ALS, Eli Lilly & Company, and the National Institutes of Health (NIH) during the conduct of the study. He also reports grant support from FDA, CDC, and DOD; research support from Alexion Pharmaceuticals, UCB, Cytokinetics, Neuraltus, Biogen and Orphazyme A/S; and personal fees from NMD Pharma, Ra Pharmaceuticals, Mitsubishi-Tanabe, Avexis, UCB and Denali outside the submitted work.

Mr. Blackmore none declared

Dr. Chaudhry served as a consultant for review and expert testimony for the Department of Health and Human Services and the Department of Justice under the Vaccine Injury and Compensation Program. Dr. Chaudhry has received royalty for total neuropathy score (TNS) patented (through Johns Hopkins University) for license of TNS use from AstraZeneca, Genentech, Seattle Genetics, Calithera Biosciences, Inc, Merrimack Pharmaceuticals, Inc., Levicept, and Acetylon Pharmaceuticals, Inc.

Dr. Chopra none declared.

Dr. Corse none declared.

Dr. Dimachkie serves or recently served as a consultant for ArgenX, Catalyst, CSL-Behring, Kezar, Momenta, NuFactor, RMS Medical, Sanofi Genzyme, Shire Takeda, and Spark Therapeutics. Dr. Dimachkie received grants from Alexion, Alnylam Pharmaceuticals, Amicus, Biomarin, Bristol-Myers Squibb, Catalyst, CSL-Behring, FDA/OOPD, GlaxoSmithKline, Genentech, Grifols, Mitsubishi Tanabe Pharma, MDA, NIH, Novartis, Sanofi Genzyme, Octapharma, Orphazyme, Sarepta Therapeutics, Shire

Takeda, Spark, UCB Biopharma,Viromed & TMA.

Dr. Evoli was a member of the advisory board for Alexion, a scientific award jury member for Grifols and safety data monitor for UCB.

Dr. Florence none declared.

Dr. Freimer has received honoraria for serving on advisory boards for ARGNX pharma, Alexion. Dr. Freimer also has research support from Catalyyst, Ra pharma, Amicus, Orphazyme, Alexion, Momenta and Alnylum.

Dr. Howard reports research support and grants from Alexion Pharmaceuticals, argenx BVBA, Centers for Disease Control and Prevention, Muscular Dystrophy Association, NIH (including the National Institute of Neurologic Disorders and Stroke and the National Institute of Arthritis and Musculoskeletal and Skin Disease), PCORI (Patient-Centered Outcomes Research Institute), and Ra Pharmaceuticals; and nonfinancial support from Alexion Pharmaceuticals, argenx BVBA, Ra Pharmaceuticals, and Toleranzia.

Dr. Jiwa none declared.

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Ms. Koopman none declared.

Ms. Lipscomb none declared.

Dr. Maestri none declared.

Dr. Marino none declared.

Dr. Massey none declared

Dr. McVey none declared.

Dr. Mezei has received honoraria as a speaker and/or moderator from Alnylam, Akcea, Pfizer and CSL Behring. She has served on Advisory Boards for Pfizer, Alnylam and Akcea. She serves as an investigator for clinical trials with Alnylam and Biogen.

Dr. Muppidi has served on advisory board meetings for Alexion and argenx.

Dr. Nicolle none declared.

Dr. Oger none declared.

Dr. Pascuzzi none declared.

Dr. Pasnoor served on advisory board for CSL Behring, Alexion pharmaceuticals, Argenx Pharmaceuticals and has been consultant for Momenta Pharmaceuticals.

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Dr. Provenzano none declared.

Dr. Ricciardi none declared.

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Ms. Soloway none declared

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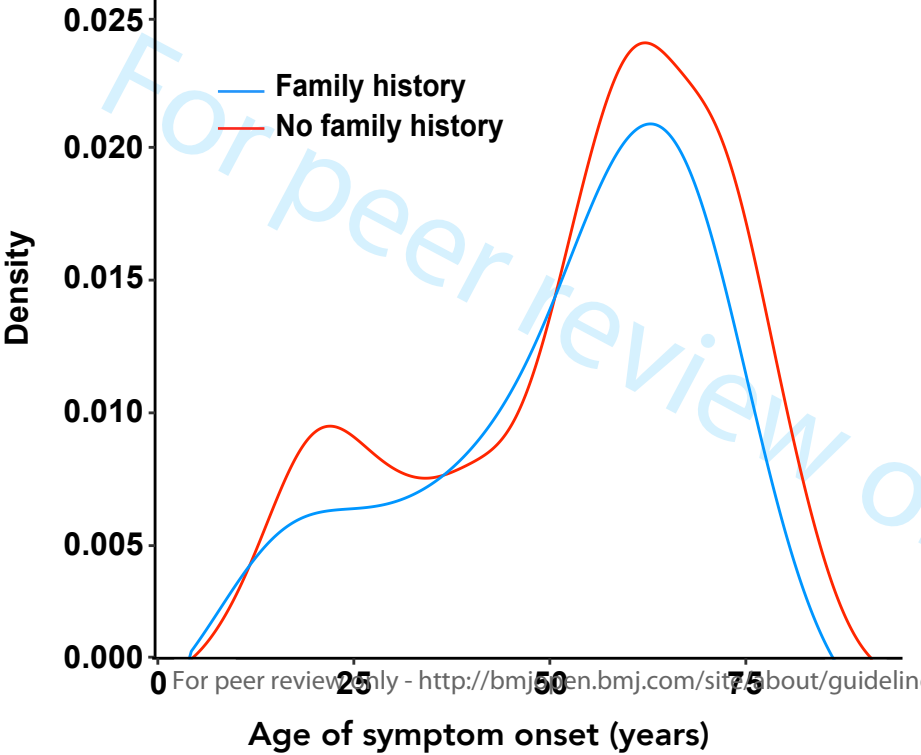
Figures

Figure 1.

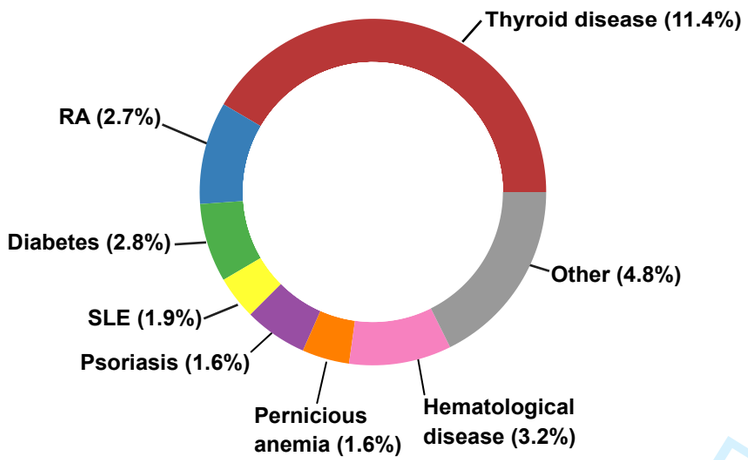
Symptom-onset age distribution of sporadic and familial cases. The density represents the relative probability of myasthenia gravis at each age point.

Figure 2.

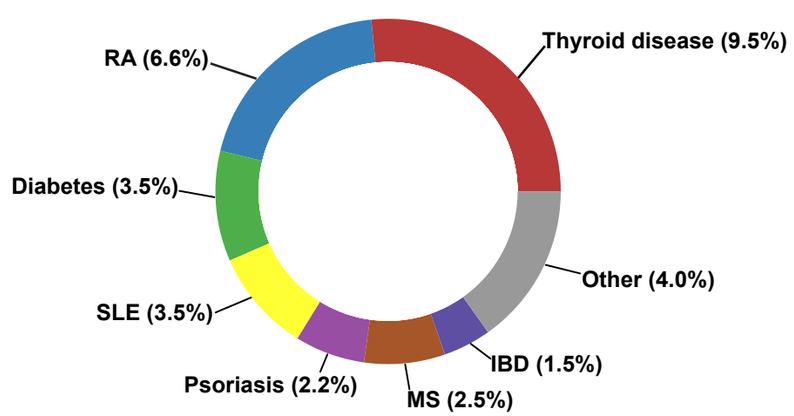
Autoimmune diseases in a cohort of 1,032 patients with myasthenia gravis. (A) Occurrence of autoimmune diseases among patients with myasthenia (n = 275). (B) Occurrence of autoimmune diseases among familial relatives of patients with myasthenia gravis (n = 293). (C) Comparisons of autoimmune diseases among patients with myasthenia gravis and among relatives. Size of circles represent the percentage previously reported by Mao et al.[18]



A. Personal history of autoimmune diseases



B. Family history of autoimmune diseases



C. Relationships between personal and family histories of autoimmune diseases

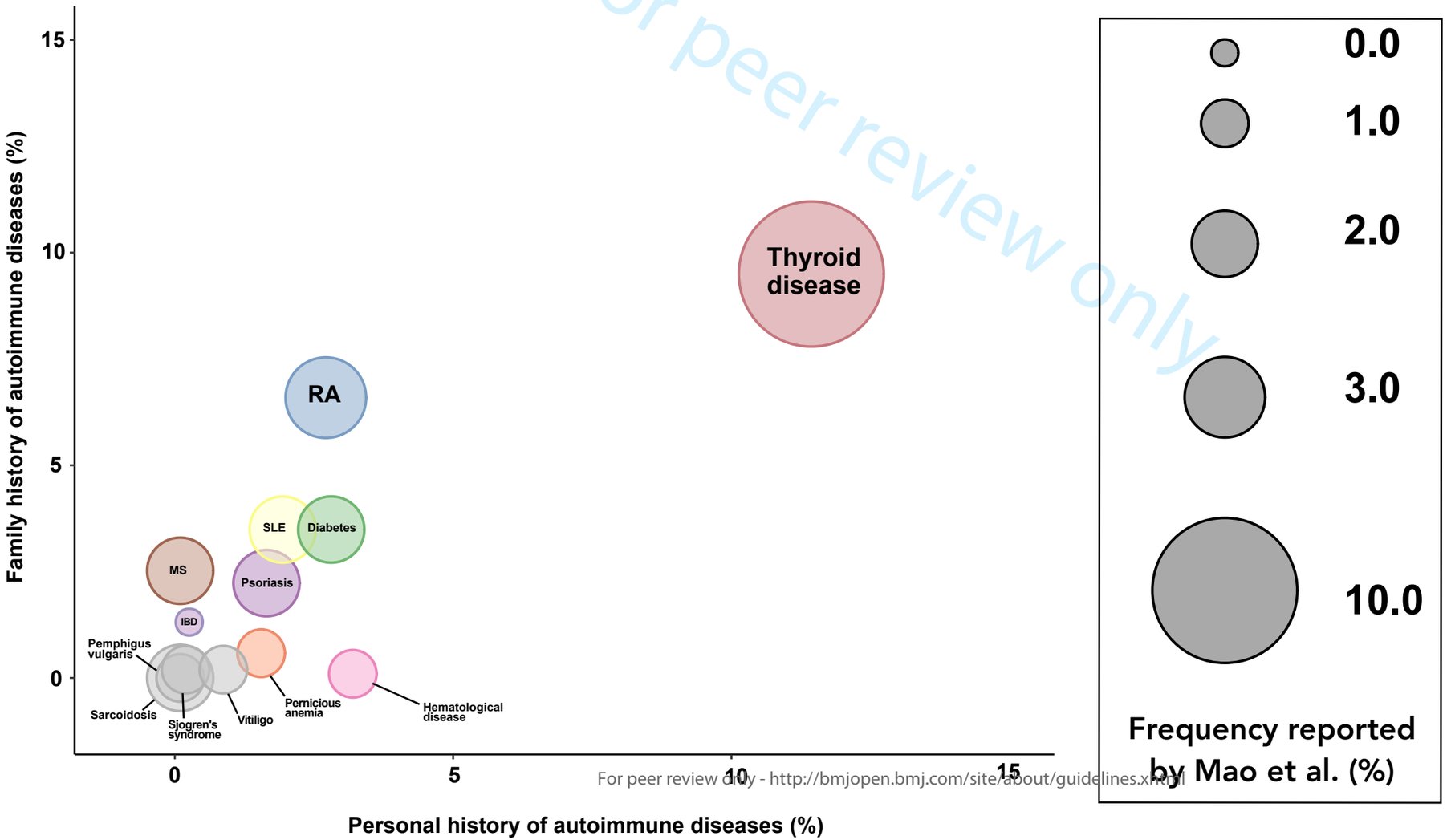


Table S1. Questionnaire used to collect demographic and clinical information from patients diagnosed with myasthenia gravis

Patient Information	
Patient identification number:	String (e.g., JHU1, JHU2)
Institution:	String (e.g., Johns Hopkins, UWO)
Date:	Date
Verified as Caucasian?	Yes/No
AChR titer - Initial or 1st known:	Numeric
Age at symptom onset (years):	Numeric
Gender:	Male/Female
Worst status	
Diplopia or ptosis only?	Yes/No
Mild limb weakness?	Yes/No
Dysphagia dyspnea?	Yes/No
Severe limb weakness?	Yes/No
Hospitalized?	Yes/No
Ventilated?	Yes/No
Feeding tube?	Yes/No
Treatment	
Mestinon?	Yes/No
Benefit: Mestinon:	Ordinal scale [0,1,2]
Prednisone?	Yes/No
Benefit: Prednisone:	Ordinal scale [0,1,2]
CellCept?	Yes/No
Benefit: CellCept?	Ordinal scale [0,1,2]
Cylclosporine?	Yes/No
Benefit: Cyclosporine:	Ordinal scale [0,1,2]
Tacrolimus?	Yes/No
Benefit: Tacrolimus:	Ordinal scale [0,1,2]
Intravenous immunoglobulin?	Yes/No
Benefit: Intravenous immunoglobulin:	Ordinal scale [0,1,2]
Plasmapheresis?	Yes/No
Benefit: Plasmapheresis:	Ordinal scale [0,1,2]
Imuran/Azathioprine?	Yes/No
Benefit: Imuran/Azathioprine:	Ordinal scale [0,1,2]
Other treatment?	Yes/No
Name of other 1:	String (e.g., Mytelase)
Benefit: other 1:	Ordinal scale [0,1,2]
Name of other 2:	String (e.g., Mytelase)
Benefit: other 2:	Ordinal scale [0,1,2]

Best Status	
Back to normal without medication?	Yes/No
Back to normal with medication?	Yes/No
Diplopia or ptosis only?	Yes/No
Mild limb weakness?	Yes/No
Dysphagia/dyspnea?	Yes/No
Severe limb weakness?	Yes/No
Hospitalized?	Yes/No
Ventilated?	Yes/No
Feeding tube:	Yes/No
Other Autoimmune Diseases	
Other autoimmune disease?	Yes/No
Thyroiditis?	Yes/No
Lupus?	Yes/No
Rheumatoid arthritis?	Yes/No
Psoriasis?	Yes/No
Blood disease?	Yes/No
Other Autoimmune Disease?	Yes/No
Other Autoimmune Disease (Name1):	String (e.g., vitiligo, celiac disease)
Other Autoimmune Disease (Name2):	String (e.g., vitiligo, celiac disease)
Other Autoimmune Disease (Name3):	String (e.g., vitiligo, celiac disease)
Family history of myasthenia gravis	
Family history of myasthenia gravis?	Yes/No
Myasthenia gravis (Relationship):	String (e.g., mother, sister)
Family history of other Autoimmune Disease	
Family history of other Autoimmune Disease?	Yes/No
Autoimmune Disease (Relationship):	String (e.g., mother, sister)
Autoimmune Disease (Disease):	String (e.g., polymyalgia, vitiligo)
Family history of other Autoimmune Disease 2nd Member?	Yes/No
Autoimmune Disease (Relationship) 2nd Member:	String (e.g., mother, sister)
Autoimmune Disease (Disease) 2nd Member:	String (e.g., polymyalgia, vitiligo)
Family history of other Autoimmune Disease 3rd Member?	Yes/No
Autoimmune Disease (Relationship) 3rd Member:	String (e.g., mother, sister)
Autoimmune Disease (Disease) 3rd Member:	String (e.g., polymyalgia, vitiligo)
Other relative in study?	Yes/No
Other relative in study id	String (e.g., JHU1, JHU2)
Thymectomy?	Yes/No
Thymoma?	Yes/No
Histology of thymus if known?	String (e.g., Follicular Hyperplasia)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	7-8
Introduction			**10-11**
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	10-11
Objectives	3	State specific objectives, including any prespecified hypotheses	11
Methods			**11-13**
Study design	4	Present key elements of study design early in the paper	11
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	11-13
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	11
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	11-13
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	11-13
Bias	9	Describe any efforts to address potential sources of bias	11-13
Study size	10	Explain how the study size was arrived at	11-13
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	11-13
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	14-15
		(*b*) Describe any methods used to examine subgroups and interactions	14-15
		(*c*) Explain how missing data were addressed	14-15
		(*d*) If applicable, explain how loss to follow-up was addressed	14-15
		(*e*) Describe any sensitivity analyses	NA
Results			13-15

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7,11,14
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	13
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	Report numbers of outcome events or summary measures over time	13-15
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	13-15
		(*b*) Report category boundaries when continuous variables were categorized	13-15
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	13-15
Discussion			16-18
Key results	18	Summarise key results with reference to study objectives	16-18
Limitations			17-18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	17-18
Generalisability	21	Discuss the generalisability (external validity) of the study results	17-18
Other information			NA
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	23

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.