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### Title

A Genome-Wide Association Study of Myasthenia Gravis

### Permalink

<https://escholarship.org/uc/item/54z7m98p>

### Journal

JAMA Neurology, 72(4)

### ISSN

2168-6149

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### Publication Date

2015-04-01

### DOI

10.1001/jamaneurol.2014.4103

Peer reviewed



Published in final edited form as:

*JAMA Neurol.* 2015 April ; 72(4): 396–404. doi:10.1001/jamaneurol.2014.4103.

## A Genome-wide Association Study of Myasthenia Gravis

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### Abstract

**IMPORTANCE**—Myasthenia gravis is a chronic, autoimmune, neuromuscular disease characterized by fluctuating weakness of voluntary muscle groups. Although genetic factors are known to play a role in this neuroimmunological condition, the genetic etiology underlying myasthenia gravis is not well understood.

**OBJECTIVE**—To identify genetic variants that alter susceptibility to myasthenia gravis, we performed a genome-wide association study.

**DESIGN, SETTING, AND PARTICIPANTS**—DNA was obtained from 1032 white individuals from North America diagnosed as having acetylcholine receptor antibody–positive myasthenia gravis and 1998 race/ethnicity-matched control individuals from January 2010 to January 2011. These samples were genotyped on Illumina OmniExpress single-nucleotide polymorphism arrays. An independent cohort of 423 Italian cases and 467 Italian control individuals were used for replication.

**MAIN OUTCOMES AND MEASURES**—We calculated *P* values for association between 8114394 genotyped and imputed variants across the genome and risk for developing myasthenia gravis using logistic regression modeling. A threshold *P* value of  $5.0 \times 10^{-8}$  was set for genome-wide significance after Bonferroni correction for multiple testing.

**RESULTS**—In the over all case-control cohort, we identified association signals at *CTLA4* (rs231770;  $P = 3.98 \times 10^{-8}$ ; odds ratio, 1.37; 95% CI, 1.25–1.49), *HLA-DQA1* (rs9271871;  $P = 1.08 \times 10^{-8}$ ; odds ratio, 2.31; 95% CI, 2.02 – 2.60), and *TNFRSF11A* (rs4263037;  $P = 1.60 \times 10^{-9}$ ; odds ratio, 1.41; 95% CI, 1.29–1.53). These findings replicated for *CTLA4* and *HLA-DQA1* in an independent cohort of Italian cases and control individuals. Further analysis revealed distinct,

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**Author Contributions:** Drs Drachman and Traynor had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Drs Renton, Provenzano, Evoli, Ricciardi, Drachman, and Traynor, and Ms Pliner contributed equally to this work.

**Study concept and design:** Drachman, Traynor. **Acquisition, analysis, or interpretation of data:** All authors. **Drafting of the manuscript:** Drachman, Traynor.

**Critical revision of the manuscript for important intellectual content:** All authors. **Statistical analysis:** Renton, Nalls, Gibbs, Drachman, Traynor. **Obtained funding:** Drachman, Traynor. **Administrative, technical, or material support:** Provenzano, Evoli, Ricciardi, Hernandez, Bartoccioni, Scuderì, Maestri, Errichiello, Macek, Scholz, Corse, Benatar, Barohn, Pasnoor, Rowin, Kissel, Massey, Chopra, Koopman, Nicolle, Pestronk, Wulf, Florence, Soloway, Wolfe, Jiwa, Oger, Drachman, Traynor. **Study supervision:** Drachman, Traynor.

**Conflict of Interest Disclosures:** None reported.

**Additional Contributions:** We thank the patients and research participants who contributed samples for this study. This study used the high-performance computational capabilities of the Biowulf Linux cluster at the National Institutes of Health, Bethesda, Maryland (<http://biowulf.nih.gov>).

but overlapping, disease-associated loci for early- and late-onset forms of myasthenia gravis. In the late-onset cases, we identified 2 association peaks: one was located in *TNFRSF11A* (rs4263037;  $P = 1.32 \times 10^{-12}$ ; odds ratio, 1.56; 95% CI, 1.44–1.68) and the other was detected in the major histocompatibility complex on chromosome 6p21 (*HLA-DQA1*; rs9271871;  $P = 7.02 \times 10^{-18}$ ; odds ratio, 4.27; 95% CI, 3.92–4.62). Association within the major histocompatibility complex region was also observed in early-onset cases (*HLA-DQA1*; rs601006;  $P = 2.52 \times 10^{-11}$ ; odds ratio, 4.0; 95% CI, 3.57–4.43), although the set of single-nucleotide polymorphisms was different from that implicated among late-onset cases.

**CONCLUSIONS AND RELEVANCE**—Our genetic data provide insights into aberrant cellular mechanisms responsible for this prototypical autoimmune disorder. They also suggest that clinical trials of immunomodulatory drugs related to CTLA4 and that are already Food and Drug Administration approved as therapies for other autoimmune diseases could be considered for patients with refractory disease.

Autoimmune myasthenia gravis is a disorder of neuro-muscular transmission clinically characterized by muscle fatigability manifested by diplopia, ptosis, and bulbar and limb weakness.<sup>1,2</sup> The disorder is typically mediated by antibodies against nicotinic acetylcholine receptors (AChRs) or against related proteins located at the neuromuscular junction such as muscle-specific tyrosine kinase (MuSK), lipoprotein receptor-related protein 4, and agrin.<sup>1–4</sup> Although myasthenia gravis is relatively uncommon, the apparent incidence has increased in the white population over time owing, at least in part, to improved recognition of the disorder among elderly individuals.<sup>5</sup> Acute respiratory failure requiring mechanical ventilation (myasthenic crisis) occurs in up to 20% of patients and is associated with significant morbidity and mortality.<sup>6,7</sup>

There is increasing recognition that myasthenia gravis is not a monolithic disease.<sup>8,9</sup> Epidemiological studies have shown a bimodal pattern of incidence, with early-onset cases (defined as initial symptoms occurring before age 40 years) being predominantly women and late-onset patients being mostly men.<sup>9–11</sup> Advanced age is associated with an increased response to autoantigens, although the implications of the age- and sex-specific frequency distribution of myasthenia gravis regarding pathogenesis remain unclear.<sup>10,12</sup>

Genetic factors contribute to the susceptibility to develop myasthenia gravis. Identified more than 30 years ago, the human leukocyte antigen (HLA) locus remains the most strongly associated risk factor for the disease.<sup>9,13,14</sup> A genome-wide association study (GWAS) involving 649 early-onset cases drawn from the Scandinavian, British, French, Dutch, German, and American populations identified variants in the major histocompatibility complex (MHC) class II locus, protein tyrosine phosphatase nonreceptor type 22 (*PTPN22*), and TNFAIP3 interacting protein 1 (*TNIP1*).<sup>15</sup> The cytotoxic T-lymphocyte-associated protein 4 gene (*CTLA4*) has also been previously suggested as a susceptibility factor for myasthenia gravis based on candidate gene studies.<sup>16</sup> Furthermore, patients with myasthenia gravis frequently have a personal or family history of other autoimmune disorders, most notably autoimmune thyroid disease, rheumatoid arthritis, and type 1 diabetes mellitus,<sup>17,18</sup> although the genetic basis underlying this predisposition to autoimmunity is unknown.

Finally, approximately 5% of patients report a family history of myasthenia gravis that usually follows an autosomal dominant pattern of inheritance.<sup>14,19</sup>

To identify susceptibility loci operating in AChR antibody–positive myasthenia gravis, we undertook a GWAS of 1032 patients diagnosed as having myasthenia gravis (case cohort) and 1998 neurologically normal individuals (control cohort) using Illumina HumanOmniExpress Beadchips.

## Methods

### Samples

From January 2010 to January 2011, DNA was collected from patients attending myasthenia gravis clinics at 14 centers throughout North America using Oragene DNA Saliva Collection kits (DNA Genotek Inc). All patients included in the case cohort had been diagnosed by a neurologist specializing in myasthenia gravis, had onset of symptoms after age 18 years, and were of non-Hispanic white race/ethnicity (eTable 1 in the Supplement). The diagnosis of myasthenia gravis was based on standard clinical criteria of characteristic weakness, fatigue, and electrophysiological and/or pharmacological abnormalities,<sup>1</sup> and it was confirmed by the presence of anti-AChR antibodies.<sup>20</sup> Patients with positive test results for antibodies to muscle-specific kinase (anti-MuSK) were excluded from enrollment.<sup>21</sup> For the control cohort, we downloaded genotype data from dbGAP (<http://www.ncbi.nlm.nih.gov/gap;phs000196.v2.p1>) for 1998 US neurologically normal individuals. The control cohort was matched to the case cohort for race/ethnic group but not for age or sex.

The replication cohort consisted of DNA obtained from 423 Italian patients diagnosed as having AChR-positive myasthenia gravis and 467 Italian neurologically normal control individuals (eTable 1 in the Supplement). Blood samples were collected from January 2010 to January 2011 at the Catholic University Rome and at Cisanello Hospital, Pisa, Italy. The control cohort was matched to the case cohort for race/ethnic group but not for age or sex.

Written consent was obtained from all patients enrolled in this study. Institutional review board approval was obtained at all participating institutions including Johns Hopkins University and the National Institute on Aging.

### Genome-wide Genotyping

The case cohort was genotyped in the Laboratory of Neurogenetics, National Institute on Aging, using HumanOmniExpress BeadChips (Illumina), which assay 730 525 single nucleotide polymorphisms (SNPs) across the genome. Individual-level genotypes for the myasthenia gravis case cohort are available on the dbGAP web portal (phs000726.v1.p1). The control cohort had been previously genotyped at the Center for Inherited Disease Research at Johns Hopkins University on HumanOmni1-Quad BeadChips (Illumina) as part of a GWAS of Parkinson disease.<sup>22</sup> Analyses were confined to the 677 673 autosomal SNPs that were common to both chips.

## Genotyping Assays in the Replication Cohort

Rs231770, rs4263037, and rs9270986 were analyzed using Taqman genotyping assays and scanned on an ABI 7900HT Real-Time polymerase chain reaction system (Applied Biosystems) according to the manufacturer's protocol. Rs601006 and rs9271850 in the MHC region were analyzed by sequencing using the Big-Dye Terminator version 3.1 sequencing kit (Applied Biosystems), run on an ABI 3730xl DNA analyzer, and analyzed with Sequencher software (version 4.2; Gene Codes) and Mutation Surveyor (version 4.0.9; Softgenetics). Primers and polymerase chain reaction conditions are listed in eTable 2 in the Supplement.

Genomic DNA from the Italian replication cohort was used for the Taqman assays. Owing to low quantities, the remaining genomic DNA was amplified using the REPLI-g kit according to the manufacturer's protocol (Qiagen), and the amplified DNA was used in the Sanger sequencing experiments.

## Statistical Analysis

### Genome-wide Association

Statistical analyses were performed using R statistical software (version 2.15.2).<sup>23</sup> We applied standard quality-control procedures to the data: exclusion of samples with SNP call rates of less than 95%, non-European ancestry, or cryptic relatedness defined as identity-by-descent proportion of inheritance ( $\pi_{\text{hat}}$  from the PLINK software toolset version 1.06<sup>24</sup>) greater than 0.1, as well as exclusion of SNPs with call rates of less than 95%, minor allele frequency less than 0.01 in the control cohort, and Hardy-Weinberg equilibrium  $P < .001$  in the control cohort. The cryptic-relatedness threshold led to the exclusion of individuals who shared more than 10% of their genome, which meant that related individuals down to third- or fourth-degree relatives were not included in the final analysis. The index individual whose sample had the better call rate from each related pair was excluded from the analysis (17 related pairs).

After quality control, we used a Markov chain-based Haplotyper (version 1.0.16) to impute genotypes for all participants.<sup>25</sup> A total of 8 114 394 SNPs (consisting of 513 081 genotyped SNPs and 7 601 313 imputed SNPs) were available for analysis.

We calculated  $P$  values using logistic regression modeling incorporating the first 2 principal components as covariates to compensate for any residual population stratification. Principal components were generated using Genome-wide Complex Trait Analysis software package implementation of eigenstrat.<sup>26</sup> A threshold of  $5.0 \times 10^{-8}$  was set for genome-wide significance after Bonferroni correction for multiple testing.<sup>27</sup>

### Probability Analysis and Heritability Estimates

We used density estimation to generate posterior probabilities of developing myasthenia gravis based on age and sex.

The Genome-wide Complex Trait Analysis package uses covariance matrices and mixed modeling to estimate the heritability of a trait in an ostensibly outbred population.<sup>26,28,29</sup> We

applied this method to compare each case series (all cases, early-onset cases, and late-onset cases) to all control individuals. For each model, we compared 2 separate subsets of SNPs, one inclusive of all genotyped SNPs and another including only SNPs within  $\pm 1$  MB from the loci identified as genome-wide significant in the discovery phase of analyses (Table 1). Only SNPs and samples passing quality control were used to evaluate the heritability and all analyses were adjusted for population substructure by using C1 and C2 from principle component analysis as covariates. Further details are provided in the eAppendix in the Supplement.

## Results

### GWAS Identifies Loci Influencing Risk for Myasthenia Gravis

After imputation and quality-control measures, 8 114 394 genotyped and imputed SNPs from 972 North American patients diagnosed as having AChR antibody–positive myasthenia gravis and 1977 North American control individuals were available for analysis. Quartile-quartile plots did not show evidence of significant population stratification (Figure 1A; genomic inflation factor  $\lambda = 1.036$ ).

In addition to the previously reported MHC class II region signal near the MHC class II DQ  $\alpha 1$  (*HLA-DQA1*) gene,<sup>30</sup> we observed a strong association signal on chromosome 2q33 in the *CTLA4* locus with a *P* value that reached genome-wide significance (rs231770; *P* =  $3.98 \times 10^{-8}$ ; odds ratio [OR], 1.37; 95% CI, 1.25–1.49; Figure 1B). We also identified a strong association peak on chromosome 18q21.33 within the tumor necrosis factor receptor 4 superfamily, member 11a, NF $\kappa$ B activator (*TNFRSF11A*) gene (rs4263037; *P* =  $1.60 \times 10^{-9}$ ; OR, 1.41; 95% CI, 1.29–1.53). Markers that achieved genome-wide significance in the analysis of the whole cohort are shown in Table 1, and SNPs associated at  $P < 1.0 \times 10^{-6}$  are listed in eTable 3 in the Supplement.

### Early- and Late-Onset Myasthenia Gravis Possess Distinct Genetic

Architecture Density estimate plots confirmed a bimodal sex distribution within our case cohort, with myasthenia gravis being more common in younger women and older men (Figure 1C). To elucidate the genetic architecture underlying this age difference, we performed GWAS of early-onset cases (235 cases with onset at age  $< 40$  years and 1977 control individuals) and late-onset cases (737 cases with onset at age  $> 40$  years and 1977 control individuals). The *TNFRSF11A* and *HLA-DQA1* association signals were significantly enhanced among late-onset cases (rs4263037, *P* =  $1.32 \times 10^{-12}$ ; OR, 1.56; 95% CI, 1.44–1.68 and rs9271871, *P* =  $7.02 \times 10^{-18}$ ; OR, 4.27; 95% CI, 3.92–4.62, respectively; Figure 1D; Table 1). In contrast, there was no evidence of association within *TNFRSF11A* in the young-onset cases (rs4263037; *P* = .83; Figure 1E). Furthermore, although an association signal was present near *HLA-DQA1* in the younger patients, the most significantly associated SNPs were different compared with those observed in the late-onset cohort (rs601006; *P* =  $2.52 \times 10^{-11}$ ; OR, 4.0; 95% CI, 3.57–4.43; eTables 4 and 5 in the Supplement).

Myasthenia Gravis Risk Loci Replicated in an Independent Cohort We selected 3 SNPs from the risk loci identified on chromosomes 2, 6, and 18 in the overall cohort for genotyping in an independent replication collection consisting of 423 Italian AChR antibody-positive myasthenia gravis cases and 467 Italian control individuals. We found the strongest signals at rs9270986 in the intergenic region between *HLA-DRB1* and *HLA-DQA1* and at rs231770 located 3.3 kb upstream of *CTLA4* (Figure 2A and B). In a combined analysis, we integrated the US GWAS and the Italian replication data using fixed effects modeling. Two loci (*CTLA4* and *HLA-DQA1*) were associated with myasthenia gravis at genome-wide significance ( $P = 9.25 \times 10^{-11}$  and  $6.17 \times 10^{-8}$ , respectively; Table 1).

We also attempted to replicate the most highly associated SNPs observed in the early- and late-onset cases using the same Italian replication collection. Rs601006 near *HLA-DQA1* was significantly associated among early-onset cases (169 Italian myasthenia gravis cases and 467 control individuals;  $P = 5.53 \times 10^{-5}$ ; Table 1; Figure 2C). This SNP was not associated among late-onset cases (250 cases and 467 control individuals;  $P = .22$ ), recapitulating the pattern observed in the discovery cohort.

Similarly, we replicated the SNPs observed near *HLA-DQA1* and within *TNFRSF11A* in the late-onset cases. Rs9271850 near *HLA-DQA1* was significantly associated in the late-onset Italian cohort ( $P = 8.15 \times 10^{-4}$ ; Table 1; Figure 2D) but was not associated among the early-onset cases ( $P = .53$ ). Rs4263037 located on chromosome 18 showed a trend toward significance ( $P$  for patients aged 40 years at symptom onset = .09; Figure 2E). This SNP became progressively more associated with increasing age of the replication cohort ( $P$  for patients aged 50 years at symptom onset = .04;  $P$  for patients aged 70 years =  $8.75 \times 10^{-3}$ ).

### Myasthenia Gravis Is Highly Heritable

The relative importance of genetic factors in a complex disease like myasthenia gravis can be estimated by quantifying heritability. We generated heritability estimates for AChR antibody-positive myasthenia gravis using a sophisticated algorithm (Genome-wide Complex Trait Analysis) that analyzes all of the genome-wide SNPs simultaneously (Table 2). Our genotype data accounted for 25.6% (95% CI, 18.6–32.6) of the phenotype variance observed in all myasthenia gravis cases. Analysis of more precise phenotypes (early- and late-onset cases) generated even higher estimates of heritability (37.9%; 95% CI, 16.8–59.0 and 35.3%; 95% CI, 27.1–43.5, respectively). These estimates are substantially higher than heritability identified using genome-wide-associated SNPs alone (1%–3%), indicating that additional risk loci remain to be identified.

## DISCUSSION

Our GWAS of AChR antibody-positive myasthenia gravis identified several new loci that drive susceptibility to develop disease. We also provide strong evidence that early- and late-onset myasthenia gravis have distinct, but overlapping, genetic architecture, thereby confirming previous epidemiological and clinical data suggesting a division between these 2 forms of disease. Genetic variation within the *TNFRSF11A* locus drives susceptibility to disease only among older cases, whereas different haplotypes across the same HLA region

on chromosome 6 were identified in early- and late-onset cases. *CTLA4* exerts a significant effect regardless of age at symptom onset, suggesting that it plays a central role in generating the aberrant autoimmune response that leads to neuromuscular junction dysfunction.

CTLA4 is a 45-kD immunoglobulin protein expressed by activated T cells and sharing significant sequence identity with CD28 (Figure 2F). CTLA4 increases T-cell motility and reduces contact periods between T cells and antigen-presenting cells leading to decreased cytokine production and proliferation. In this way, CTLA4 is thought to down-regulate T-cell activation, terminate T-cell responses, and protect against autoimmunity.<sup>31</sup> CTLA4-deficient mice have high serum immunoglobulin concentrations and develop massive lymphocyte infiltration of lymph nodes and spleen, suggesting that CTLA4 plays an inhibitory role in regulating lymphocyte expansion.<sup>32</sup> The mechanism of CTLA4's regulatory action is controversial; however, evidence suggests that cells expressing CTLA4 avidly endocytose the co-stimulatory factors CD80 and CD86 on antigen-presenting cells and degrade them.<sup>33</sup> Genetic variant in the *CTLA4* locus has been implicated in other autoimmune disorders including celiac disease,<sup>34</sup> type 1 diabetes mellitus,<sup>35</sup> thyroiditis,<sup>36</sup> and rheumatoid arthritis.<sup>37</sup> Indeed, abatacept and belatacept are commercially available proteins consisting of CTLA4 rendered soluble by fusion to antibodies (CTLA4-IgG) approved for use in the treatment of refractory rheumatoid arthritis<sup>38</sup> and as first-line immunosuppressants for renal transplant patients immune to Epstein-Barr virus.<sup>39</sup> Clinical trials of these agents are also under way in inflammatory bowel disease, systemic lupus erythematosus, and other autoimmune diseases. They have not previously been used therapeutically in myasthenia gravis, although our experimental studies demonstrated the effectiveness of CTLA4-Ig in the treatment of rats with experimental autoimmune myasthenia gravis.<sup>40,41</sup>

Genetic analysis of additional case-control cohorts will be required to confirm the association observed for the *TNFRSF11A* locus. Nevertheless, the pattern of association uncovered for this locus, with a large effect in late-onset disease that is absent in younger patients, led us to speculate that age-related changes in local gene expression predispose toward an aberrant immune response to autoantigens. *TNFRSF11A* encodes the 4.5-kDa receptor activator of nuclear factor- $\kappa$  B expressed on the surface of antigen-presenting dendritic cells.<sup>42</sup> This receptor is an important regulator of the interaction between T cells and dendritic cells that is essential for immune surveillance and the regulation of specific immunity.<sup>42</sup> Furthermore, TNFRSF11A is critical for lymph node organogenesis and osteoclast differentiation.<sup>43</sup> Mutations in this gene are responsible for a form of familial Paget disease of the bone<sup>44</sup> and for autosomal recessive osteopetrosis associated with defective immunoglobulin production.<sup>45</sup> Receptor activator of nuclear factor- $\kappa$  B knockout mice also have profound osteopetrosis resulting from a complete lack of osteoclasts, as well as near total absence of peripheral lymph nodes.<sup>43,45</sup>

The association signal that we identified in the MHC region was distinct from that reported by Gregersen et al<sup>15</sup> to increase susceptibility for myasthenia gravis. Furthermore, our study did not show any evidence of association in the *PTPN22* or *TNIP1* loci found by the same study (eTable 6 in the Supplement).<sup>15</sup> These disparities likely arise from differences in the



populations studied. In their study, patients and control individuals from 7 countries were included.<sup>15</sup> Such population stratification impedes the identification of true association and increases the rate of false-positive association<sup>46</sup> particularly in the HLA region that is known to be highly divergent across races/ethnicities.<sup>47</sup> In contrast, we attempted to reduce clinical heterogeneity by selecting only cases from North America for the discovery cohort and from Italy for the replication cohort.

We considered the possibility that our study may have had population stratification, especially because we used an outbred American population as our original discovery cohort. The lack of age and sex matching of control samples to cases also may have introduced artifacts into our analysis. To avoid this, we applied strict filters to exclude outlying samples and incorporated principal components generated from the genome-wide data into the analysis model to compensate for residual population stratification between case and control samples. Furthermore, we replicated our results in a case-control cohort drawn from a distinct single population and found identical patterns of association among early- and late-onset cases.

Heritability can be considered the proportion of a disease that arises from genetics. Prior studies have estimated that only between 3.8% and 7.1% of individuals who receive a diagnosis of myasthenia gravis have a family history of disease.<sup>14,48</sup> The genome-wide nature of our data set yielded a higher estimate of the heritability associated with myasthenia gravis risk by capturing more of the polygenic variance. This pattern is consistent with other neurological diseases, such as amyotrophic lateral sclerosis and Parkinson disease, where heritability estimates are significantly higher than the rate of familial disease.<sup>49,50</sup> Furthermore, our heritability estimates suggest that there are genetic factors contributing to the disease that have yet to be identified and that GWASs involving larger cohorts will yield additional loci. To that end, we have made our raw genotype data publicly available on dbGaP to facilitate meta-analysis with existing and future studies.

A strength of our study was that genetic analysis was confined to patients diagnosed as having AChR antibody–positive myasthenia gravis. This approach decreased disease heterogeneity and improved the power of the study to identify association signals. Approximately 85% of patients with myasthenia gravis have detectable AChR antibodies and approximately 40% of the remainder are MuSK antibody positive.<sup>2,51,52</sup> Furthermore, AChR antibody–negative myasthenia gravis is less common among elderly individuals,<sup>52</sup> and MuSK-positive patients are more like to be women.<sup>53</sup> In light of this, care should be taken in generalizing our results to the AChR antibody–negative myasthenia gravis population. Nevertheless, our data support the existence of distinct, but overlapping, genetic profiles for patients with early- and late-onset myasthenia gravis with detectable AChR antibodies.

## Conclusions

Our GWAS identified susceptibility loci for AChR antibody–positive myasthenia gravis at *CTLA4*, *HLA-DRB1/HLA-DQA1*, and *TNFRSF11A*, and it showed distinct, but overlapping, genetic risk factors underlying early- and late-onset disease. Although future

studies involving deep resequencing of these loci will be required to identify the functional variants underlying these association signals, our data also suggest that clinical trials of CTLA4 immunomodulatory therapies could be considered in patients with myasthenia gravis with refractory disease failing to respond to standard therapies.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Acknowledgments

**Funding/Support:** The work was supported by the Myasthenia Gravis Foundation (Drs Drachman and Traynor), a generous bequest by Geraldine Weinrib, and a gift from Philip Swift. This work was also supported in part by the Intramural Research Programs of the National Institutes of Health National Institute on Aging (Z01-AG000949-02).

**Role of the Funder/Sponsor:** The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

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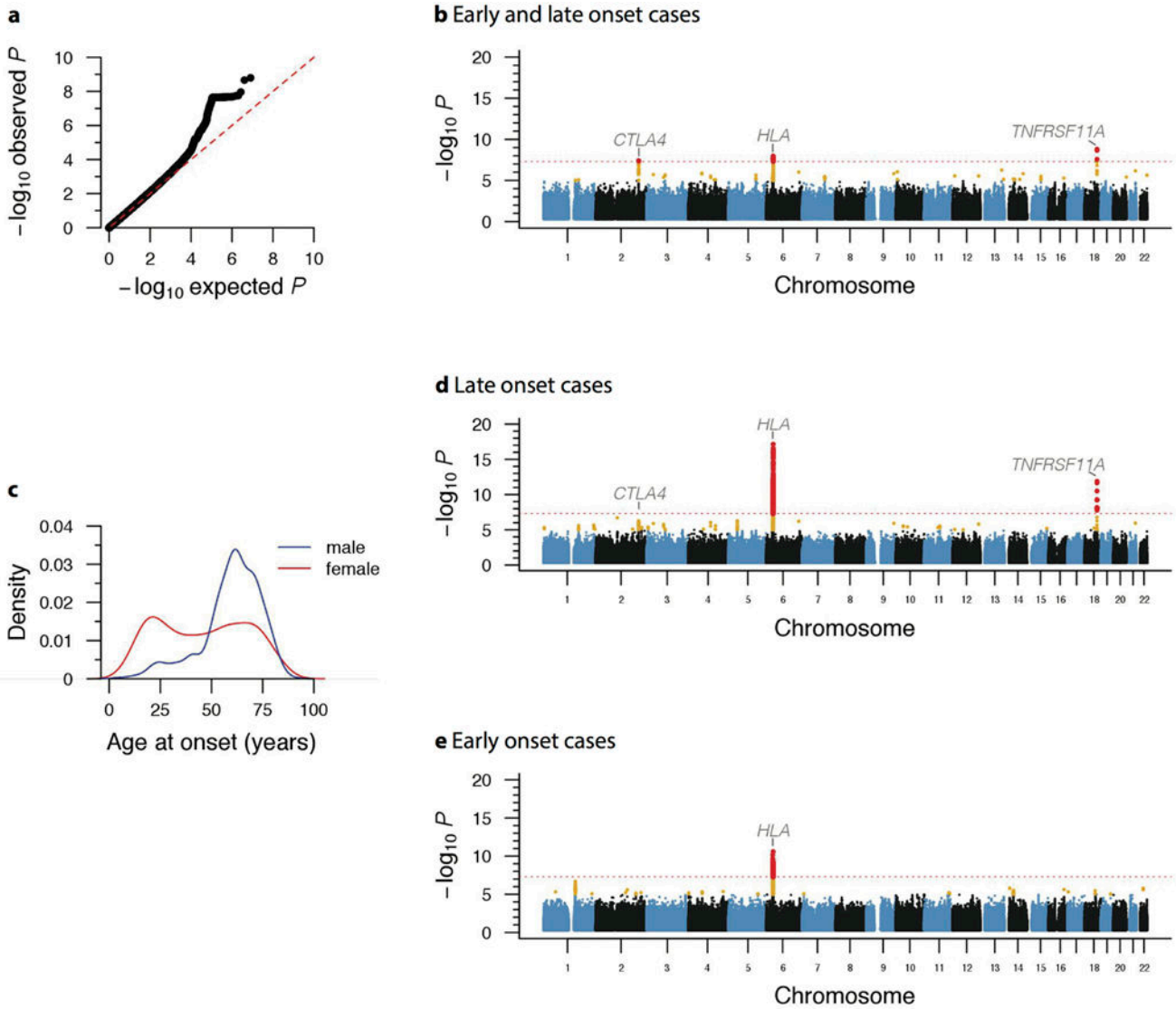
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**Figure 1. Myasthenia Gravis Cases and P Values From the Genome-Wide Association Study of Myasthenia Gravis**

A, Quartile-quartile plot showing the distribution of expected vs observed P values for the US discovery cohort (972 myasthenia gravis cases and 1977 control individuals;  $\lambda = 1.036$ ). B, Manhattan plot of genome-wide association results for all myasthenia gravis cases (972 cases and 1977 control individuals). C, Density plot of myasthenia gravis cases (n = 972) showing bimodal frequency distribution with disease being more common in younger women and older men. D, Plot of genome-wide association results for late-onset myasthenia gravis cases (737 cases and 1977 control individuals). E, Plot of genome-wide association results for early-onset myasthenia gravis cases (235 cases and 1977 control individuals). CTLA4 indicates cytotoxic T-lymphocyte-associated protein 4; HLA, human leukocyte antigen; and TNFRSF11A, tumor necrosis factor receptor 4 superfamily, member 11a, NFKB activator gene. The orange horizontal line depicts the Bonferroni-adjusted significance threshold ( $5.0 \times 10^{-8}$ ). Single-nucleotide polymorphisms exceeding this

threshold are indicated with red dots, whereas single-nucleotide polymorphisms with a  $P < 10^{-4}$  and  $>5.0 \times 10^{-8}$  are indicated with yellow dots.

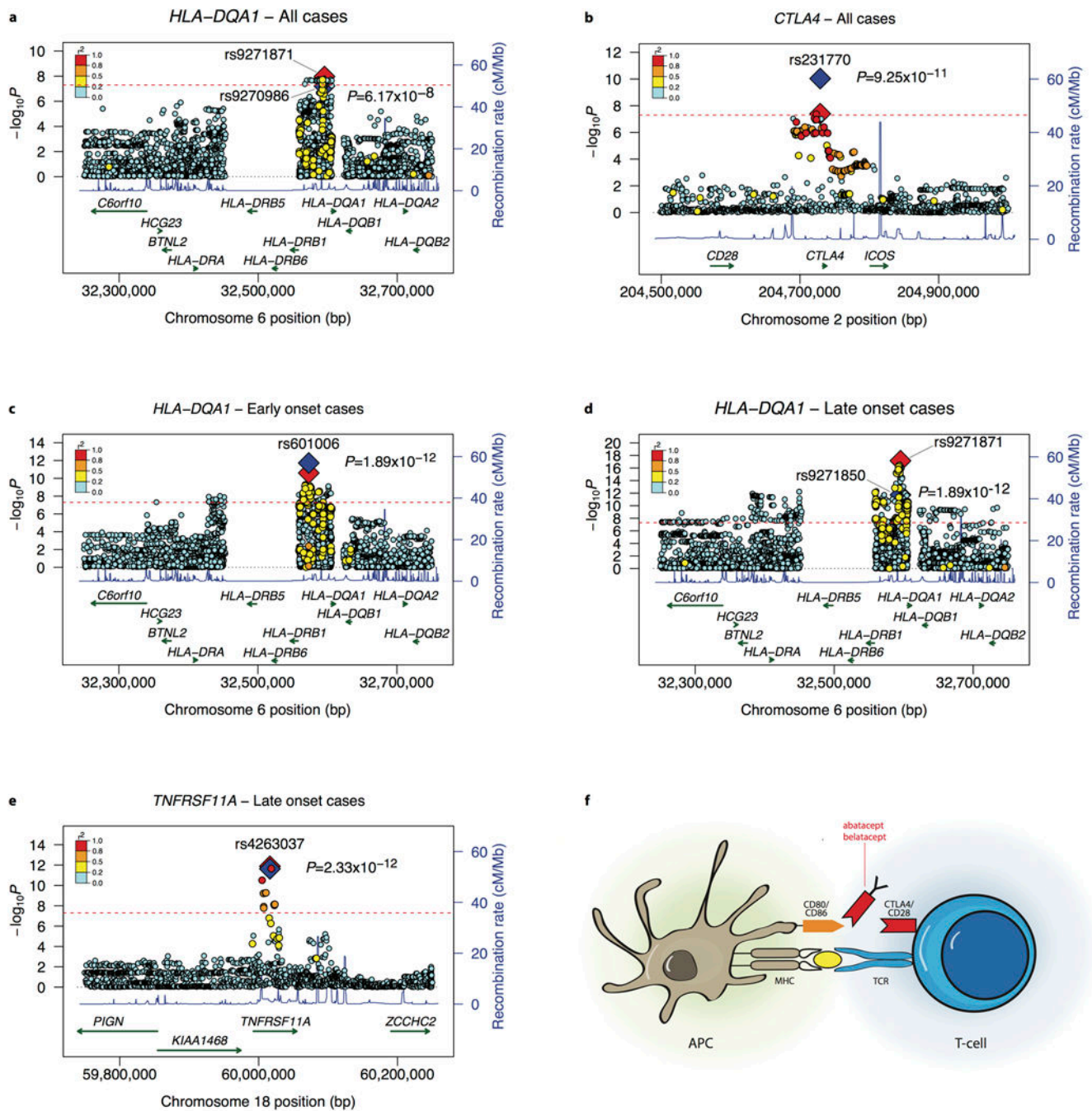
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**Figure 2. Regional Association Plots Across Loci Identified by the Genome-Wide Association Study of Myasthenia Gravis**

Regional association plots of the signals at the major histocompatibility complex (MHC) region (A) and CTLA4 (B) in all myasthenia gravis cases (972 cases and 1977 control individuals), as well as the MHC region in early-onset myasthenia gravis cases (C; 235 cases and 1977 control individuals), the MHC region in late-onset cases (D), and TNFRSF11A in late-onset myasthenia gravis cases (E; 737 cases and 1977 control individuals). Single-nucleotide polymorphisms are colored on the basis of their linkage disequilibrium with the labeled hit single-nucleotide polymorphism. Recombination rates estimated from the CEU

HapMap population are represented by the blue line and genes are marked as arrows. Red diamonds represent the most associated single-nucleotide polymorphism in the discovery cohort and blue diamonds, P values for the combined discovery and replication cohorts. F, CTLA4 regulates T-cell activation by antigen-presenting cells (APCs). TCR indicates T-cell receptor.

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Table 1

Loci showing genome-wide association with myasthenia gravis.

| Locus                    | SNP                      | Chr | Position (bp) | Nearest gene(s)           | Effect allele | EAF   |          |       | Stage 1                |      |     | Stage 2               |      |       | Stage 1 + 2            |      |   |
|--------------------------|--------------------------|-----|---------------|---------------------------|---------------|-------|----------|-------|------------------------|------|-----|-----------------------|------|-------|------------------------|------|---|
|                          |                          |     |               |                           |               | Cases | Controls | N     | P                      | OR   | N   | P                     | OR   | N     | P                      | OR   | N |
| <b>All cases</b>         |                          |     |               |                           |               |       |          |       |                        |      |     |                       |      |       |                        |      |   |
| 1                        | rs231770 <sup>a</sup>    | 2   | 204,729,153   | <i>CTLA4</i>              | T             | 0.45  | 0.38     | 2,949 | 3.98×10 <sup>-8</sup>  | 1.37 | 890 | 5.27×10 <sup>-4</sup> | 1.48 | 3,839 | 9.25×10 <sup>-11</sup> | 1.39 |   |
| 2                        | rs9271871 <sup>a,b</sup> | 6   | 32,595,383    | <i>HLA-DRB1, HLA-DQA1</i> | T             | 0.87  | 0.85     | 2,949 | 1.08×10 <sup>-8</sup>  | 2.31 | -   | -                     | -    | -     | -                      | -    | - |
| 2                        | rs9270986 <sup>b</sup>   | 6   | 32,574,060    | <i>HLA-DRB1, HLA-DQA1</i> | A             | 0.19  | 0.15     | 2,949 | 1.80×10 <sup>-6</sup>  | 1.43 | 890 | 0.01                  | 1.43 | 3,839 | 6.17×10 <sup>-8</sup>  | 1.43 |   |
| 3                        | rs4263037                | 18  | 60,016,233    | <i>TNFRSF11A</i>          | G             | 0.46  | 0.38     | 2,949 | 1.60×10 <sup>-9</sup>  | 1.41 | 890 | 0.34                  | 1.10 | 3,839 | 1.40×10 <sup>-8</sup>  | 1.26 |   |
| <b>Early-onset cases</b> |                          |     |               |                           |               |       |          |       |                        |      |     |                       |      |       |                        |      |   |
| 1                        | rs231770 <sup>a</sup>    | 2   | 204,729,153   | <i>CTLA4</i>              | T             | 0.46  | 0.38     | 2,212 | 1.77×10 <sup>-3</sup>  | 1.38 | -   | -                     | -    | -     | -                      | -    | - |
| 2                        | rs601006 <sup>a</sup>    | 6   | 32,573,173    | <i>HLA-DRB1, HLA-DQA1</i> | G             | 0.89  | 0.77     | 2,212 | 2.52×10 <sup>-11</sup> | 4.00 | 636 | 5.53×10 <sup>-5</sup> | 1.98 | 2,889 | 1.89×10 <sup>-12</sup> | 2.79 |   |
| 3                        | rs4263037                | 18  | 60,016,233    | <i>TNFRSF11A</i>          | G             | 0.38  | 0.38     | 2,212 | 0.83                   | 1.02 | -   | -                     | -    | -     | -                      | -    | - |
| <b>Late-onset cases</b>  |                          |     |               |                           |               |       |          |       |                        |      |     |                       |      |       |                        |      |   |
| 1                        | rs231770 <sup>a</sup>    | 2   | 204,729,153   | <i>CTLA4</i>              | T             | 0.44  | 0.38     | 2,714 | 5.89×10 <sup>-7</sup>  | 1.37 | -   | -                     | -    | -     | -                      | -    | - |
| 2                        | rs9271871 <sup>a,c</sup> | 6   | 32,595,383    | <i>HLA-DRB1, HLA-DQA1</i> | T             | 0.90  | 0.85     | 2,714 | 7.02×10 <sup>-18</sup> | 4.27 | -   | -                     | -    | -     | -                      | -    | - |
| 2                        | rs9271850 <sup>a,c</sup> | 6   | 32,595,060    | <i>HLA-DRB1, HLA-DQA1</i> | A             | 0.79  | 0.67     | 2,714 | 3.92×10 <sup>-17</sup> | 1.99 | 717 | 8.15×10 <sup>-4</sup> | 1.43 | 3,395 | 3.48×10 <sup>-17</sup> | 1.70 |   |
| 3                        | rs4263037                | 18  | 60,016,233    | <i>TNFRSF11A</i>          | G             | 0.49  | 0.38     | 2,714 | 1.32×10 <sup>-12</sup> | 1.56 | 717 | 0.09                  | 1.61 | 3,372 | 2.33×10 <sup>-12</sup> | 1.41 |   |

<sup>a</sup> variants that were imputed;<sup>b</sup> rs9270986 was chosen for replication, as it was the most associated genotyped SNP in that locus based on the “all cases” analysis and the Taqman genotyping assay for rs9271871 failed quality control;<sup>c</sup> rs9271850 was chosen for replication, as it was the second most associated SNP in that locus based on the “late-onset cases” analysis and the Taqman genotyping assay for rs9271871 failed quality control; chr, chromosome; EAF, effect allele frequency; OR, odds ratio.

**Table 2**

Heritability estimates based on the discovery cohort of myasthenia gravis

|                   | All SNPs              |             |                         | Significant loci only |           |                        |
|-------------------|-----------------------|-------------|-------------------------|-----------------------|-----------|------------------------|
|                   | Heritability estimate | 95% CI      | P                       | Heritability estimate | 95% CI    | P                      |
| All cases         | 25.6%                 | 18.6 – 32.6 | $8.79 \times 10^{-14}$  | 1.8%                  | 0.8 – 2.7 | $5.42 \times 10^{-9}$  |
| Early-onset cases | 37.9%                 | 16.8 – 59.0 | $5.61 \times 10^{-5}$   | 2.8%                  | 0.7 – 4.9 | $1.55 \times 10^{-7}$  |
| Late-onset cases  | 35.3%                 | 27.1 – 43.5 | $<1.00 \times 10^{-20}$ | 2.1%                  | 0.9 – 3.2 | $1.73 \times 10^{-13}$ |

95% CI, 95% confidence interval.