Identification of genetic risk loci and prioritization of genes and pathways for myasthenia gravis: a genome-wide association study

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Myasthenia gravis is a chronic autoimmune disease characterized by autoantibody-mediated interference of signal transmission across the neuromuscular junction. We performed a genome-wide association study (GWAS) involving 1,873 patients diagnosed with acetylcholine receptor antibody-positive myasthenia gravis and 36,370 healthy individuals to identify disease-associated genetic risk loci. Replication of the discovered loci was attempted in an independent cohort from the UK Biobank. We also performed a transcriptome-wide association study (TWAS) using expression data from skeletal muscle, whole blood, and tibial nerve to test the effects of disease-associated polymorphisms on gene expression. We discovered two signals in the genes encoding acetylcholine receptor subunits that are the most common antigenic target of the autoantibodies: a GWAS signal within the cholinergic receptor nicotinic alpha 1 subunit (CHRNA1) gene and a TWAS association with the cholinergic receptor nicotinic beta 1 subunit (CHRONBI) gene in normal skeletal muscle. Two other loci were discovered on 10p14 and 11q21, and the previous association signals at PTPN22, HLA-DQA1/HLA-B, and TNFRSF11A were confirmed. Subgroup analyses demonstrate that early- and late-onset cases have different genetic risk factors. Genetic correlation analysis confirmed a genetic link between myasthenia gravis and other autoimmune diseases, such as hypothyroidism, rheumatoid arthritis, multiple sclerosis, and type 1 diabetes. Finally, we applied Priority Index analysis to identify potentially druggable genes/proteins and pathways. This study provides insight into the genetic architecture underlying myasthenia gravis and demonstrates that genetic factors within the loci encoding acetylcholine receptor subunits contribute to its pathogenesis.

Myasthenia gravis manifests as ocular, bulbar, and limb weakness with muscle fatigability (1, 2). In severe cases, respiratory muscles are affected, leading to acute respiratory failure that can be fatal (myasthenic crisis) (1). This archetypal neuromuscular disease is relatively rare, affecting ~77 persons per million of the population, though the actual rate may be rising due to population aging in Western societies (2). Epidemiological studies distinguish two incidence peaks, with the first occurring before 40 and predominantly affecting women (3). The second peak occurs at the age of 60 and is mainly observed in men (3).

The discovery of autoantibodies has been central to advancing our understanding of myasthenia gravis (4). Myasthenic patients have autoantibodies against proteins in the neuromuscular junction involving the nicotinic acetylcholine receptor, muscle-specific kinase, and lipoprotein receptor-related protein 4, among others (5). These antibodies are responsible for an autoimmune attack on the neuromuscular junction that interferes with neuromuscular transmission (6). Current treatment strategies focus on decreasing the antibody levels using immunosuppressive medications, such as corticosteroids and azathioprine, or plasma exchange (5).

Despite their relevance to the pathogenesis of myasthenia gravis, the exact mechanisms by which autoantibodies develop and what nonimmunologic biology may modify disease onset and progression are poorly understood. More in-depth knowledge of this process may provide additional targets for drug development and guide therapeutic agent selection to treat the disease. Here, we performed a genome-wide association study (GWAS) and a transcriptome-wide association study (TWAS) to identify the genetic risks and candidate genes involved in disease etiology to address this knowledge gap (Fig. 1). This effort increased the sample size from 972 cases and 1,977 controls in the previous GWAS (7) to 1,873 cases and 36,370 controls, expanding our power to detect genetic risk factors associated with myasthenia gravis. We then used the genetic data to identify diseases linked to myasthenia gravis and prioritize genes and pathways that could be potentially targeted for disease-modifying therapy.

Significance

Our study, involving 1,873 patients and 36,370 healthy individuals, is an extensive genome-wide study of myasthenia gravis. Our genome-wide association and transcriptome-wide association analyses identified two signals, namely CHRNA1 and CHRONBI, encoding acetylcholine receptor subunits, which were replicated in an independent cohort obtained from the UK Biobank. Identifying these genes confirms the potential utility of using genetics to identify proteins that are the antigenic targets of autoantibodies. We confirmed that the genetic abnormalities underlying early-onset and late-onset myasthenia gravis are different. Our data offer a broader insight into the genetic architecture underlying the pathophysiology of myasthenia gravis.

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A complete list of the International Myasthenia Gravis Genomics Consortium is available in the SI Appendix.

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Results

GWAS Implicates Loci in Myasthenia Gravis. We performed a GWAS involving ~24 million single nucleotide polymorphisms (SNPs) across a cohort of 1,873 patients diagnosed with myasthenia gravis and 36,370 healthy individuals. We focused on acetylcholine receptor antibody-positive cases, as these antibodies are found in ~90% of patients with generalized myasthenia gravis. In addition to the previously reported signals in PTPN22, HLA-DQA1/ HLA-B, and TNFRSF11A (7, 8), we observed association signals on chromosomes 2q31.1, 10p14, and 11q21 (see Fig. 2A for the Manhattan plot and Table 1 and SI Appendix, Table S1 for details of the associated SNPs). SI Appendix, Figs. S1 and S2 show the forest and regional association plots for each index variant.

The locus on chromosome 2 lies in a promoter region of the cholinergic receptor nicotinic alpha 1 subunit (CHRNA1) gene (rs35274388, \( P = 3.07 \times 10^{-8} \), odds ratio [OR] = 1.57), which encodes a glycoprotein subunit of the nicotinic acetylcholine receptor on the forward strand, and in the noncoding transcript of AC010894.2, which is an antisense gene to CHRNA1. The two other signals were found within the first intron of the Scm-like with four mbt domains 2 (SFMBT2) gene on chromosome 10 (rs2245569, \( P = 1.66 \times 10^{-11} \), OR = 1.27) and downstream of the family with sequence similarity 76 member B (FAM76B) gene on chromosome 11 (rs4409785, \( P = 1.54 \times 10^{-8} \), OR = 1.29). Within the locus on chromosome 6, we identified two association signals near the HLA-B gene (rs9266277, \( P = 3.31 \times 10^{-11} \), OR = 1.29) and the HLA-DQA1 gene (rs76815088, \( P = 1.58 \times 10^{-14} \), OR = 0.42). These variants were located ~1.3 million base pairs apart, and conditional analysis confirmed that the loci were independent of each other (SI Appendix, Fig. S3). Fine-mapping at the HLA region showed that rs2962777 was highly correlated with amino acids 45 and 74 in the HLA-B protein, whereas rs76815088 was tagging the HLA-DRB1*13:01:02 and HLA-DQB1*06:03:02 alleles (SI Appendix, Figs. S4A and S5A). Only CHRNA1 showed association in the UK Biobank replication cohort (\( P = 0.012, \) OR = 1.60; see Table 1), likely reflecting the small number of cases and age distribution within this cohort (\( n = 354 \) cases and 7,058 controls). Variant rs2476601 (PTPN22, \( P = 5.11 \times 10^{-13} \),
In early-onset cases, we performed genome-wide association testing separately.

The index variant tagging the HLA-B*08:01:02 and HLA-C*07:01 alleles.

OR = 1.36) was replicated in the UK Biobank dataset, but variants rs2245569 (SFMBT2, P = 0.487, OR = 1.07) and rs4409785 (FAM76B, P = 0.965, OR = 1.00) did not.

**Early-Onset and Late-Onset Myasthenia Gravis.** Because of the age-dependent genetic heterogeneity known to exist in myasthenia gravis, we performed genome-wide association testing separately in early-onset cases (n = 595 cases and 2,718 controls, aged 40 y or younger) and late-onset cases (n = 1,278 cases and 33,652 controls).

We found distinct differences in the genetic architecture among these cohorts (Fig. 2 B and C). An association on chromosome 11q23.3 was also identified downstream of a long intergenic noncoding RNA, LINC02151 (rs73007767, P = 4.36 \times 10^{-3}, OR = 1.85). However, the genetic risk of early-onset (predominantly female) myasthenia gravis cases was driven primarily by the Major Histocompatibility Complex (MHC) on chromosome 6 (Fig. 2 B and Table 2).

The MHC locus was also strongly associated with an increased risk of late-onset myasthenia gravis. However, the risk-associated variants were distinct from those observed among the early-onset cases (Table 3). Furthermore, the PTPN22, CHRNA1, and TNFRSF11A loci were more prominently associated with disease in the late-onset myasthenia gravis cases than the early-onset cases (Table 3). These findings show that genetic factors other than those in the MHC play a prominent role in the occurrence of myasthenia gravis in older, predominantly male patients. A correlation plot comparing the effect sizes among early-onset and late-onset cases further confirmed their distinct patterns of genetic predispositions to myasthenia gravis (SI Appendix, Fig. S7).

**TWAS Implicates Loci in Myasthenia Gravis.** Next, we performed a TWAS of myasthenia gravis to identify additional genes relevant to the disease’s pathogenesis. This analytical approach correlates genotypic and expression to phenotypic traits to identify the cis-genetic component of gene expression relevant to the trait (9).

To do this, we integrated our myasthenia gravis GWAS results to the disease’s pathogenesis. This analytical approach correlates genotypic and expression to phenotypic traits to identify the cis-genetic component of gene expression relevant to the trait (9).
subunit, namely the cholinergic receptor nicotinic beta 1 subunit (CHRNA1; rs415112, 3 = 19.81, pFDR adjusted = 0.44, pFDR adjusted = 0.04), type 1 diabetes (rs2, 1 = 1.04, pFDR adjusted = 0.04), ulcerative colitis (rs2, 1 = 0.84, pFDR adjusted = 0.05), and hypothyroidism (rs2, 1 = 0.28, pFDR adjusted = 0.41 × 10^-3). There was no significant correlation with any of the tested neurological disorders (SI Appendix, Table S5). Of note, the genetic correlation was present after excluding chromosome 6, confirming that the HLA locus was not the only source of the observed correlation (SI Appendix, Table S5). Different genetic overlaps were also observed for the early-onset and late-onset myasthenia gravis cohorts (SI Appendix, Table S5).

Identifying Targets and Pathways for Myasthenia Gravis Based on Genetic Data. Finally, we performed a target prioritization analysis using the Priority Index pipeline to gain insights into the pathways involved in myasthenia gravis and identify potentially druggable targets (14). This analysis integrated the genetic information from our GWAS with functional genomic data and immune-related annotation data to nominate the most relevant genes (Fig. 4A). Enrichment analysis of the top 1% genes with the highest Priority

Table 1. Genome-wide significant association signals in the myasthenia gravis GWAS

| Chr | Position (SNPid) | Nearest gene | EA/OA | Cohort | EAF | OR (95% CI) | P | Meta-analysis |
|-----|------------------|--------------|-------|--------|-----|------------|---|--------------|
| 1   | 11383496A (rs2476601) | PPAN22 | A/G | All | 0.101/0.093 | 1.49 (1.32–1.67) | 7.95 × 10^-11 | 0.141/0.107 | 1.36 (1.10–1.69) | 5.11 × 10^-3 | 1.85 × 10^-12 |
| 2   | 174764492 | CHRNA1 | A/G | All | 0.089/0.093 | 1.34 (1.12–1.59) | 3.15 × 10^-3 | 0.147/0.107 | 1.41 (1.05–1.90) | 2.38 × 10^-2 | 1.15 × 10^-4 |
| 6   | 31358836 | HLA-B | A/G | All | 0.063/0.036 | 1.77 (1.46–2.15) | 8.06 × 10^-9 | 0.066/0.03 | 2.34 (1.52–3.60) | 1.13 × 10^-4 | 7.19 × 10^-12 |

Significant hits were stratified by sex to determine the contribution of sex and are highlighted in bold. Positions were in build hg38; Chr, chromosome; EA/OA, effect allele/other allele; EAF, effect allele frequency in cases and controls; discovery, meta-analysis from the US and Italian cohorts (1,873 cases [989 male, 884 female] and 33,370 controls); replication, cohort from the UK Biobank where controls were age- and sex-matched (354 cases [181 male, 173 female] and 7,078 controls); rs9266277 is an independent variant identified from conditional analysis on rs7681508. Results in the table are from the unconditional analysis. Discovery phase conditional statistics for rs9266277 (95% CI) = 1.28 (1.19–1.38), P = 1.92 × 10^-10. The replication significance threshold was set at P = 0.017 = 0.0143 (Bonferroni correction for seven loci with the same effect direction).

Table 2. Genome-wide significant association signals in early-onset myasthenia gravis

| Chr | Position (SNPid) | Nearest gene | EA/OA | Cohort | EAF | OR (95% CI) | P |
|-----|------------------|--------------|-------|--------|-----|------------|---|
| 6   | 31442774 (rs3093958) | HCP5/MICA | G/A | All | 0.245/0.094 | 4.49 (3.62–5.57) | 3.97 × 10^-42 |
| 11  | 116158033 (rs73007767) | LINCO02151 | G/T | All | 0.129/0.087 | 1.85 (1.48–2.30) | 4.36 × 10^-6 |

Positions are in build hg38; Chr, chromosome; EA/OA, effect allele/other allele; EAF, effect allele frequency in cases and controls; meta-analysis is based on early-onset myasthenia gravis cases from the US and Italian cohorts (95 cases and 2,718 controls).
Index score identified a set of enriched pathways involved in the adaptive immune response, cytokine signaling in the immune system, and receptor tyrosine kinase signaling (Fig. 4B).

Discussion

We performed a GWAS in a large cohort of patients diagnosed with myasthenia gravis and attempted to replicate our findings in an independent cohort. Our data confirmed that early-onset and late-onset myasthenia gravis have distinct genetic bases. We also identified several susceptibility loci as having a role in the disease’s pathogenesis. Most interesting among these, we implicated genetic variants in two genes encoding nicotinic acetylcholine receptor subunits as relevant to the disease. We used our data to show a genetic overlap between myasthenia gravis and other autoimmune diseases, most notably thyroid abnormalities, rheumatoid arthritis, and multiple sclerosis. Building on those observations, we nominated genes and pathways that may contribute to the underlying disease pathobiology and are worthy of additional study as therapeutic targets.

The adult acetylcholine receptor is a complex heteropentamer consisting of alpha, beta, epsilon, and delta subunits (α2βεδ). Our GWAS and TWAS analyses identified association signals in the genes encoding two of these subunits (CHRNA1 and CHRN1). These nicotinic acetylcholine receptor subunits are targets of autoantibodies in myasthenia gravis (15, 16). Furthermore, coding mutations in CHRNA1 and CHRN1 are known causes of congenital myasthenia gravis, a condition characterized by a markedly diminished number of functioning acetylcholine receptors on the postsynaptic membrane (17). Based on what is already known about myasthenia gravis, there are several mechanisms by which the genetic signals identified in our study could lead to disease. For example, altered expression of CHRNA1 in the thymus or other immune cells may interfere with the development of immunological tolerance to the acetylcholine receptor (18) or cause intrathymic immunization (19).

Alternatively, the associated variants appear to reduce the expression of their respective acetylcholine receptor subunits. The disease-associated SNP that we identified on chromosome 2q31.1 is located within the CCAAT-Enhancer-Binding Protein-Beta transcription factor binding site of CHRNA1 (20) and within the antisense gene to CHRNA1 (AC010894.2) on the reverse strand, suggesting that it may decrease CHRNA1 expression. The allele showing subsignificant association with myasthenia gravis in CHRN1 similarly reduces expression of that subunit. The reduced expression of these subunits could, in turn, decrease the number of functional acetylcholine receptors within the neuromuscular synapse, similar to what is observed with autoantibody-mediated myasthenia gravis and congenital myasthenic syndrome. Altered subunit composition may also alter how the immune system perceives the acetylcholine receptors on the neuromuscular synapse, leading to an abnormal response.

Our data will be a seeding point for future research into myasthenia gravis. This will include functional studies to confirm the

**Table 3. Genome-wide significant association signals in late-onset myasthenia gravis**

| Chr | Position (SNPId) | Nearest gene | EA/OA | Cohort | Discovery | Replication | Meta-analysis |
|-----|------------------|--------------|-------|--------|-----------|-------------|--------------|
|     |                  |              | EA/OA | OR (95% CI) | P   | EA/OA | OR (95% CI) | P   |
| 2   | 174764692 (rs35274388) | CHRNA1 | A/G | 0.065/0.036 | 1.86 (1.56–2.23) | 1.53 × 10⁻³ | 0.049/0.03 | 1.69 (1.15–2.48) | 7.32 × 10⁻³ | 1.40 × 10⁻³ |
| 6   | 32603181 (rs679242) | HLA-DRB1 | A/G | 0.062/0.036 | 1.73 (1.29–2.32) | 2.87 × 10⁻⁴ | 0.022/0.03 | 0.72 (0.32–1.64) | 0.44 | 1.02 × 10⁻³ |
| 18  | 62343215 (rs4369774) | TNFRSF11A | A/G | 0.66/0.036 | 0.26 (0.11–0.65) | 0.005 | 0.212/0.189 | 1.18 (0.97–1.45) | 0.11 | 1.87 × 10⁻² |

Significant hits were stratified by sex to determine the contribution of sex and are highlighted in bold. Positions are in build hg38; EA/OA, effect allele/other allele; EAF, effect allele frequency in cases and controls; discovery, meta-analysis from the US and Italian cohorts (1,278 cases [835 male, 443 female] and 33,652 controls); replication, a cohort from the UK Biobank where controls were age- and sex-matched (308 cases [168 male, 140 female] and 7,056 controls); *rs679242 is an independent variant identified from conditional analysis on rs9271375. The results in the table are from the unconditioned analysis. Discovery phase conditional statistics for rs679242: OR (95% CI) = 1.72 (1.49–2.00), P = 4.42 × 10⁻¹³. The replication significance threshold was set at P = 0.1/3 = 0.0333 (Bonferroni correction for three loci identified on chromosomes 2, 6, and 18 with the same effect direction).

Fig. 3. TWAS in myasthenia gravis. Manhattan plots depicting the TWAS results of the overall discovery cohort (n = 1,873 myasthenia gravis cases and 36,370 control individuals) using expression data for skeletal muscle obtained from GTEx. The x-axis denotes the chromosomal position for the autosomes in hg38, and the y-axis indicates the association P values on a −log₁₀ scale. Each dot represents a variant, where red dots denote variants that reached genome-wide significance and permutation P values < 0.05. The significance threshold for skeletal muscle was 6.8 × 10⁻⁶ and is shown with the dashed line. The orange dots denote variants that are one log-fold lower than the significant threshold. The dots with a black diamond outline are variants with colocalization posterior prior probability H4 (PPH4) > 0.75.

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molecular mechanisms by which CHRNA1 and CHRNBI expression changes lead to disease. Our genomic data already provide clues to this mechanism; patients who were homozygous for the CHRNA1 risk alleles had a four times higher risk of developing myasthenia gravis than patients who carried only one risk allele (rs35274388, OR in homozygotes = 5.86 and OR in heterozygous carriers = 1.45). A similar, though smaller, trend was observed for CHRNBI (rs4151121, OR for homozygous carriers = 1.42 and OR in heterozygous carriers = 1.10). Skeletal muscle expression data from GTEx also showed that homozygous carriers of the rs4151121 risk allele had an 8-fold reduced expression compared to heterozygous carriers (https://gtexportal.org/home/snp/rs4151121).

Existing autoimmune acquired mouse models of myasthenia gravis are unsuitable for modeling the implicated genetic loci, meaning that transgenic lines will have to be developed. Patient-orientated studies would require tissue samples collected from

Table 4. Causal genes associated with myasthenia gravis based on TWAS of skeletal muscle

| Chr | ID     | MAF    | eQTL Z | TWAS Z | P       | PERM.P | PPH4 |
|-----|--------|--------|--------|--------|---------|--------|------|
|     | Overall|        |        |        |         |        |      |
| 17  | rs4151121 | 0.433  | 0.372  | -11.79 | 4.39    | -4.67  | 3.01 \times 10^{-6} | 2.03 \times 10^{-4} | 0.98   |
| 17  | rs2102928 | 0.353  | 0.322  | -3.42  | 2.16    | -5.00  | 5.63 \times 10^{-7} | 7.99 \times 10^{-4} | 0.18   |
|     | ERBB2  |        |        |        |         |        |      |
| 6   | rs3101018 | 0.225  | 0.089  | 4.55   | 12.72   | 10.90  | 7.32 \times 10^{-28} | 3.76 \times 10^{-3} | 0.98   |
| 6   | rs35404844 | 0.061  | 0.042  | 7.12   | 3.58    | 7.60   | 2.88 \times 10^{-14} | 1.13 \times 10^{-3} | 0.89   |
|     | Early-onset|       |        |        |         |        |      |
| 6   | CYP21A2 | 0.180  | 0.180  | -3.97  | 4.51    | -6.80  | 1.04 \times 10^{-11} | 1.23 \times 10^{-3} | 0.71   |
| 6   | HLA-DMA | 0.180  | 0.158  | 16.95  | 5.46    | 7.68   | 1.65 \times 10^{-14} | 5.05 \times 10^{-3} | 0.00   |
|     | Late-onset|       |        |        |         |        |      |
| 6   | C2     | 0.180  | 0.180  | -3.97  | 4.51    | -6.80  | 1.04 \times 10^{-11} | 1.23 \times 10^{-3} | 0.71   |
| 6   | HLA-DRB5 | 0.180  | 0.158  | 16.95  | 5.46    | 7.68   | 1.65 \times 10^{-14} | 5.05 \times 10^{-3} | 0.00   |

Chr, chromosome; ID, rs number of the best eQTL locus in the locus; MAF, minor allele frequency; cont., controls; eQTL Z, Z-score of the best eQTL in the locus; GWAS.Z, GWAS Z-score for the eQTL; PERM.P, TWAS permutation P values; PPH4, Bayesian derived posterior probability for colocalization of the eQTL and the GWAS variant.

Fig. 4. Prioritization of immunological targets in myasthenia gravis based on Priority Index and druggability. (A) Autosomal genes were scored based on the gene-predictor matrix generated from the Priority Index pipeline. The x-axis denotes the chromosomal position in hg38, and the y-axis indicates the Priority Index rating on a scale of 0 to 5. Each dot represents a gene, and the red dots indicate the top 30 genes. Dots with a black diamond outline are druggable genes based on a Pocketome analysis. The red dashed line shows the threshold for the top 30 genes (scale > 3.9). (B) An enrichment analysis shows the prioritized target pathways in the immune and the signal transduction modules. The x-axis shows the REACTOME pathways that were significantly overrepresented by the top genes. The y-axis denotes the strength of the enrichment quantified by the OR on a log2 scale. The 95% CIs are represented by lines flanking each dot. Enrichment significance was measured by calculating the false discovery rate (FDR) from a one-sided Fisher's exact test.
individuals diagnosed with myasthenia gravis, and our work argues in favor of establishing a public biobank to facilitate this work.

Identifying the ERBB2 gene in our TWAS analysis further supports the central importance of the acetylcholine receptor in the pathogenesis of myasthenia gravis. The encoded ERBB2 protein is highly expressed at the neuromuscular junction in skeletal muscle (21), where it regulates the expression of acetylcholine receptor subunits via MAP kinase activation (22). Synapses were less efficient in mutant mice with reduced Erbb2 muscle expression, and there were reduced levels of acetylcholine receptors at the motor endplates in these animals (4). The risk conferred by ERBB2 in our study appears to be mediated by its low expression, which would affect the formation of the functional acetylcholine receptor complex.

As with previous GWASes of myasthenia gravis (7, 8), our study identified the HLA locus as a susceptibility locus for this autoimmune disease. Despite the individual-level and population-level variability intrinsic to this locus, our results agreed with a recent study of HLA Class I and II effects on early-onset and late-onset myasthenia gravis risk in the Norwegian, Swedish, and Italian populations (23). This served as an additional replication cohort and underscored the veracity of our results.

Identifying pathogenic autoantibodies is a critical step in defining any autoimmune disease (24). In addition to providing insight into the antigenic target, the ability to screen patients for the relevant autoantibody allows for a more precise diagnosis of specific syndromes and disease subtypes (25). The field’s central issue is that the current methods used to discover novel autoantibodies are time-consuming, involving immunoblotting or precipitation of the antigen by the patient’s serum or binding of antibodies in cell-based assays (26). Our analyses identified association signals in the CHRNA1 and CHRNB1 loci that encode the proteins that are the antigenic targets of some acetylcholine receptor antibodies. We propose that the corollary approach is also valid in some cases: GWAS can be used as a screening tool to narrow the search scope for autoantibodies. Thus, genes located within associated loci and encoding proteins expressed within the tissue damaged in patients with a particular autoimmune disease are candidates for more directed autoantibody discovery efforts using enzyme-linked immunosorbent assays.

We found genetic evidence linking myasthenia gravis with other autoimmune diseases, including rheumatoid arthritis, multiple sclerosis, type 1 diabetes, and ulcerative colitis. Epidemiological data have extensively reported that patients with myasthenia gravis and their family members are at increased risk of other autoimmune diseases (27). Nonetheless, using genetic data to map these commonalities represents a powerful approach in neurologic disease, and our results point to the molecular basis for this overlap. An intriguing possibility is that medications used to treat one of the linked autoimmune diseases may also help patients with myasthenia gravis. In contrast, we found only weak evidence of overlap with neurological disorders.

Over 90% of drugs fail during clinical development, driving up drug development costs and, ultimately, healthcare expenditures (28). An emerging theme in the pharmaceutical industry is that drugs targeting proteins and pathways with genetic evidence are more likely to succeed (5). Based on this, we used our genetic data to prioritize gene targets and pathways that may be amenable to therapeutic intervention (14). Additional experimental evidence in preclinical disease models is required to assess their relevance for treating myasthenia gravis. However, the present scope of research in myasthenia gravis is limited to studies involving the destruction of acetylcholine receptors by autoantibodies. Our results serve as an alternative starting point for generating data-driven hypotheses that can be tested for their ability to modify disease risk.

Our study has limitations. Several loci identified in the discovery cohorts failed to replicate in our follow-up cohort, likely reflecting our replication cohort’s small size and limited power to detect an association. Furthermore, the CTLA4 locus identified in our previous GWAS (7) was subsignificant in our new analysis (rs231770, \( P = 2.46 \times 10^{-3} \); OR = 1.12, 95% CI = 1.04–1.21). The acetylcholine receptor antibody status of the UK Biobank samples used in the replication cohort was not available, further adding to the heterogeneity of this group and perhaps accounting for this failure of replication. Our initial nomination of CTLA4 may have been a false-positive finding, or it may reflect variability in the alleles influencing myasthenia gravis risk across different populations. Although these loci remain biologically plausible, more extensive studies are required to confirm their association.

Another constraint is the difficulty in identifying a single gene responsible for disease within the HLA locus, leading us to rely instead on the association between haplotypes and disease susceptibility (29). By extension, TWAS analysis also underestimates the complexity of the linkage disequilibrium landscape in the HLA region. A full exploration of this locus will likely require long-range sequencing to resolve this repetitive and GC-rich genomic region and determine which variants and genes within HLA are driving the disease association (30).

Finally, our study focused on European ancestry individuals, as this is the only population in which extensive collections of myasthenia gravis patients were available. As myasthenia gravis is found worldwide, future research should focus on recruiting patients and healthy controls from diverse, non-European individuals and patients with anti-MuSK and other autoantibodies.

In conclusion, we identified loci relevant to the pathogenesis of myasthenia gravis and confirmed the different genetic architectures among early-onset and late-onset cases. Among these, the discovery of risk variants within the CHRNA1 and CHRNB1 genes that encode subunits of the acetylcholine receptor is remarkable, and it provides insights into the mechanisms that may trigger the disease.

Materials and Methods

Participants and Study Design. Myasthenia gravis samples were collected from collaborators in the United States and Italy. The US samples were collected from January 2010 to January 2011 from patients attending myasthenia gravis clinics at 14 centers throughout North America (7). Our group previously published a GWAS analysis based on 972 of the US cases and 1,977 of the US control subjects (7). These genotype and phenotype data are available on the dbGaP database of Genotype and Phenotype (dbGaP) web portal (accession number phs000726). Blood samples were collected from Italian patients at the Catholic University Rome and Cisanello Hospital, Pisa. The diagnosis of myasthenia gravis was based on standard clinical criteria of characteristic fatigable weakness and electrophysiological and/or pharmacological abnormalities and confirmed by the presence of anti-acetylcholine receptor antibodies (7). Patients with positive test results for antibodies to muscle-specific kinase (anti-MuSK) were excluded from enrollment. We used publicly available genotype data from the dbGaP web portal for the US and Italian neurologically normal individuals for the control cohort. Written consent was obtained from all subjects enrolled in this study. This study was approved by the institutional review boards of all participating institutions, including Johns Hopkins University, the National Institute on Aging (protocol 03-AG-N329), the University of Pisa, and the Catholic University of Rome.

For replication, we used myasthenia gravis cases identified within the UK Biobank based on the ICD10 code G70.0 (31). Only samples identified as European and White British were included in the analysis. The control subjects were selected from a cohort of over 400,000 in a ratio of 20 controls for each case, using the Matchit (version 3.0.2) R library. The control subjects were matched to cases using the “nearest” method based on age, gender, and uniform manifold approximation and projection (UMAP) components 1 and 2 to account for population structure.

Procedures. The US and Italian myasthenia gravis cases were genotyped on HumanOmniExpress arrays (Illumina Inc.) at the National Institute on Aging. The UK Biobank samples were genotyped on UK BILEVE Axiom or UK Biobank
Axiom arrays (Affymetrix, Thermofisher Scientific Inc.). The Illumina and Affymetrix arrays assayed over 730,000 and 850,000 SNPs.

Genome-Wide Association Analysis. The current study analyzed data from 1,873 myasthenia gravis cases and 36,370 controls. This cohort included 972 cases and 1,977 controls used in a previous GWAS of myasthenia gravis (7). Genotype data from the US and Italian samples were processed using a standard quality control pipeline (32). The pipeline avoids P value inflation due to imputation, but one pair member was removed before the association testing. Plotting of the principal components demonstrated that the study samples were centered around the European cohort of HapMap3, indicating the absence of population stratification (SI Appendix, Fig. S10).

Variants were excluded from the analysis for the following reasons: 1) monomorphic SNPs; 2) palindromic SNPs; 3) variants that showed nonrandom missingness between cases and controls (P value ≤ 1.0 × 10−4); 4) variants with haplotype-based nonrandom missingness (P value ≤ 1.0 × 10−4); 5) variants with an overall missingness rate of ≥5.0%; 6) nonautosomal variants (X, Y, and mitochondrial chromosomes); and 7) variants that significantly departed from Hardy-Weinberg equilibrium in the control cohort (P value ≤ 1.0 × 10−16).

Imputation was performed for the US and Italian cohorts against the Trans-omics for Precision Medicine (TopMed) imputation reference panel (https://topmed.broadinstitute.org). Imputation was run via the Impute2 package (32) and the Imputation Server (33). The US cohort was divided equally into two subsets before imputation. After imputation, variants were included in the analysis if they met the following criteria: 1) imputation quality score (R2) > 0.3 and 2) minor allele frequency > 0.001. The UK Biobank cohort was previously imputed using the Human Reference Consortium, UK10K, and 1000 Genomes reference panels (hg19) (34). After applying quality control filters, there were 5,110,254 variants in 964 US myasthenia gravis cases and 35,580 controls and 919 Italian cases and 2,478 Italian controls. Index variants from the discovery phase analysis were extracted from the UK Biobank dataset. Genomic positions were lifted over to hg38 before the association analysis using the 354 myasthenia cases and 7,080 controls. All samples were of European descent, and only unrelated samples were included in the analysis (SI Appendix, Table S6).

Statistical Analysis. Logistic regression with dosage data was performed for the following cohorts: (i) all samples; (ii) early-onset myasthenia gravis, defined as an age of onset <40 y; and (iii) late-onset myasthenia gravis with onset age ≥40 y. Results were additionally stratified by sex in the early-onset and late-onset cohorts. The covariates were adjusted in each analysis with the optimum combination (age, sex, and principal components to account for population structure). Genotype imputation quality was assessed using Plink (version 1.9) and a quality control pipeline (32). The pipeline avoids P value inflation due to imputation, but one pair member was removed before the association testing. Plotting of the principal components demonstrated that the study samples were centered around the European cohort of HapMap3, indicating the absence of population stratification (SI Appendix, Fig. S10).

Priority Index analysis (R package R commander) was applied to identify targets that may be amenable to therapy (i.e., druggable) for myasthenia gravis (14). Summary statistics from the overall GWAS were filtered to retain variants with minor allele frequency > 0.05 and P values > 0.05. Enrichment analysis of the prioritized genes was then performed using the REACTOME database (https://www.reactome.org) to identify the immune and signal transduction pathways overrepresented by these genes (14).

Data Availability. Summary GWAS statistics and the programming code used for analysis are deposited as Jupyter notebooks on https://github.com/pjurschina/MyastheniaGravis_AnalysisCode. Genotype data have been deposited in dbGAP (phs000726). Anonymized summary statistics data have been deposited in GWAS catalog (GCT90009306). Previously published data were used for this work (A. E. Renton et al., A genome-wide association study of myasthenia gravis. JAMA Neurol. 72, 396–404 (2015)).

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Genealogical Correlation Analysis. The shared genetic risks between myasthenia gravis and other diseases were estimated using the Linkage Disequilibrium Score Regression method (13) using the LDSC tool (available on https://github.com/bulik/lodsc). Default parameters were used for analysis and using the precomputed linkage disequilibrium (LD) scores derived from the 1000 Genomes European dataset. We selected GWASes for each disease based on the availability of the corresponding summary statistics in the GWAS catalog (https://www.ncbi.nlm.nih.gov/gwas) and HATK tools (36). HLA alleles were imputed against the SNP2HLA preformatted 1000 Genomes European reference panel mapped against the IMGT-HLA database.

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