IJHSR
ijhighschoolresearch.org
RESEARCH ARTICLE

A Meta-Analysis of Genes Associated with Myasthenia Gravis

Caleb Tsai

El Segundo High School, 640 Main St., El Segundo, CA, 90245, USA; ctsai771@gmail.com

ABSTRACT: Myasthenia Gravis (MG) is a neuromuscular autoimmune disorder characterized by decreased muscle strength caused by the antibodies targeting proteins on the neuromuscular junction. While many genes have been attributed to MG susceptibility and pathogenesis, the disease's complexity means current research on MG only adds to a provisional theory, leaving its molecular basis still widely unexplored. This study aimed to perform a meta-analysis on 517 genes with functional associations to MG through gene ontology and enrichment analysis. Results revealed enriched terms generally correlating to autoimmunity and immune signaling pathways, focusing on cytokine signaling. Additional enriched terms of interest were related to Kaposi sarcoma-associated herpesvirus, the spleen, and NF-κB. This analysis uncovers the role these enriched terms may play in the condition's development and discusses their possible implications for future MG research.

KEYWORDS: Biomedical and Health Sciences, Immunology, Rare Diseases, Autoimmune Disorders, Myasthenia Gravis, Cytokine Signaling.

Introduction

Myasthenia Gravis (MG) is a rare neuromuscular autoimmune disorder, typically affecting 150-250 per million individuals globally, with approximately 8-10 new cases per million each year.[1] The disease is primarily characterized by decreased muscle strength that worsens over frequent use, with 15% of MG patients experiencing weakness isolated to the extraocular muscles (small voluntary muscles controlling the movement of the eyeball within the eye socket), causing common symptoms such as the asymmetrical drooping of the eyelid (ptosis) and double vision (diplopia). Although ocular muscle weakness is still frequent, the other 85% of MG patients will experience a more generalized form of MG, finding additional muscle weakness in reduced facial expression, speech difficulties, or reduced neck movement.[2,3] In 15-20% of MG patients, respiratory muscle failure can occur, causing airways to collapse in a life-threatening situation, referred to as a myasthenic crisis.[4]

For most populations, MG follows a bimodal pattern of a low peak of onset at 30 years of age and a high peak at 70-80 years of age, which can be used as approximate reference points for categorization of early-onset (EOMG) or late-onset MG (LOMG).[5] However, the typical age of onset can vary greatly by region, which is especially exemplified by a uniquely common development of Juvenile MG (up to 50% under 15 years of age) in East Asian countries, such as China.[6] Furthermore, the condition appears to correlate with sex, as males are more likely to develop LOMG with a sex ratio of 3:2, and females are significantly more likely to develop EOMG (3:1), which can be attributed to preferential X chromosome inactivation.[7] According to available data, the general prevalence of MG is higher in modern times, likely due to the availability of newer testing methods with increased sensitivity to detect MG biomarkers rather than an actual increase in disease occurrence.[3]

Myasthenia Gravis has been proven to be greatly influenced by genetic and epigenetic factors, as is supported by its concordance of 35% in monozygotic twins and 5% in heterozygous twins.[8] The disruption of normal molecular processes in MG patients can be attributed to antibodies that target three main proteins located on the neuromuscular junction: the acetylcholine receptor (AChR), muscle-specific kinase (MuSK), and low-density lipoprotein receptor-related protein 4 (LRP4) (Figure 1).[9] Anti-AChR antibodies are found in 80% of MG patients and act as competitive inhibitors of the neurotransmitter, acetylcholine, by binding to the AChR, blocking typical ACh-AChR binding.[10,11] Through their attachments, they may also increase AChR internalization and degradation, resulting in the receptor losing complete functionality.[12] Although only present in a minority of patients, anti-MuSK and anti-LRP4 antibodies cover binding sites and disrupt a signaling pathway stimulated by a release of agrin from the motor neuron.[13] At an unaffected neuromuscular junction, after the agrin binds to LRP4 and MuSK amplifies a signal, the AChR begins to cluster with the aid of rapsyn, which is essential for the efficient reception of neurotransmitters.[14] Individuals with anti-MuSK antibodies generally experience a more severe form of MG than those with anti-AChR or anti-LRP4 antibodies alone.[15]

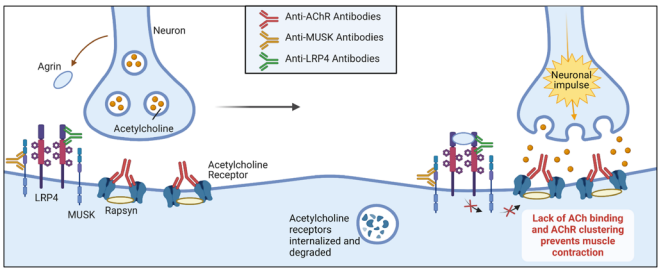

Figure 1: Schematic illustration of the autoantibodies disrupting efficient ACh reception at the neuromuscular junction. It was created with Biorender.

DOI: 10.36838/v7i1.14

Given that MG is a multifactorial disorder, many studies have uncovered various biomarkers and factors that may influence the development of MG. Although not directly related to the development of symptoms, as previously mentioned, antibodies targeting Titin, Rapsyn, Agrin, Collagen Q, and Cortactin all have associations with the condition and are viable genetic markers.16 Cytokines such as IL-17A, IL-21, IL-10, IL-14, IL-2, and IL-20 and increased κFLC (kappa-free light chain) levels, all acting as various signaling molecules in the immune system also possibly play a role in the mechanisms of the disease.[16,17] Epigenetic factors of MG include the dysregulation of long non-coding RNAs (lncRNAs) such as XLOC_003810, SNHG16, IFNG-AS1, and MALAT-1. Other epigenetic factors include microRNAs (miRNAs) such as miR-150-5p, miR-155, miR-146a-5p, miR-20b, miR-21-5p, miR-126, let-7a-5p, and let-7f-5.[18] Studies have even begun to investigate the role of environmental factors as well, including one study that has uncovered a relationship between the dysbiosis of gut bacteria and the imbalance of T helper 17 (Th17) cells and regulatory T (Treg) cells that are present in individuals with MG.[19]

Amongst MG biomarkers, the genetic factors that have been most extensively researched are the genes themselves. Unlike most autoimmune diseases, familial autoimmunity of MG is rare. Still, in a study that investigated a unique case of an Italian-American family with four affected siblings, it was found that sequence variants in the ENOX1 gene play a role in the development of EOMG.[20] As expected of autoimmune disorders, other genetic studies have highlighted the human leukocyte antigen (HLA) locus as a possible key contributor to MG susceptibility. In contrast, genome-wide association studies (GWAS) have found numerous additional genes that possibly have associations with MG, including TNIP1 and PTPN22.[21,22] However, even amidst all comprehensive studies, the disease's complexity means this research only adds to a provisional theory, leaving its genetic and molecular basis still widely unexplored.[23] Therefore, this meta-analysis seeks to investigate the relationship between the genes and the functional associations to MG to gain a more holistic understanding of the disease's development.

Methods

A list of genes with associations to MG was first curated through the compilation of seven independent gene sets from Harmonizome[24] (https://maayanlab.cloud/Harmonizome/), a collection of processed datasets from over 70 primary online resources that are organized based on the functional associations between genes/proteins and their attributes.[24] Among all datasets, various methods were employed to obtain MG-related genes, including genome-wide association studies (GWAS), text-mining, and the manual curation of lists from the relevant scientific literature. Categorized through Harmonizome by their relative functional associations, no individual gene set exceeded 300 total genes, and a final list of 517 unique genes was produced for analysis (Table 1).

Table 1: Shows various MG datasets, their sources, and gene numbers. This study used unique genes obtained from this dataset to analyze.

Dataset Name	Resource	No. of genes	URL
CTD Gene-Disease Associations	Comparative Toxicogenomics Database	46	https://maayanlab.cloud/Harmonizome/gene_set/Myasthenia+Gravis/CTD+Gene-Disease+Associations
DISEASES Text-mining Gene-Disease Association Evidence Scores	DISEASES	188	https://maayanlab.cloud/Harmonizome/gene_set/myasthenia+gravis/DISEASES+Text-mining+Gene-Disease+Assocation+Evidence+Scores
GWASdb SNP-Disease Association	GWASdb	91	https://maayanlab.cloud/Harmonizome/gene_set/myasthenia+gravis/GWASdb+SNP-Disease+Associations
GAD Gene-Disease Associations	Genetic Association Database	23	https://maayanlab.cloud/Harmonizome/gene_set/myasthenia+gravis/GAD+Gene-Disease+Associations
GWAS Catalog SNP-Phenotype Associations	GWAS Catalog	2	https://maayanlab.cloud/Harmonizome/dataset/GWAS+Catalog+SNP-Phenotype+Associations
HuGE Navigator Gene-Phenotype Associations	HuGE Navigator	45	https://maayanlab.cloud/Harmonizome/gene_set/Myasthenia+Gravis/HuGE+Navigator+Gene-Phenotype+Associations
DisGeNET Gene-Disease Associations	DisGeNET	299	https://maayanlab.cloud/Harmonizome/gene_set/Myasthenia+Gravis/DisGeNET+Gene-Disease+Associations

To perform this analysis, 517 genes were inputted into Enrichr (https://maayanlab.cloud/Enrichr/), a web-based application containing 35 common gene-set libraries that provide a wide variety of visualizations for gene set enrichment analysis (GSEA).[25] The utilization of GSEA uncovers patterns of common biological functions, chromosomal locations, and regulations in the gene lists of multiple independent studies, highlighting top enriched terms correlated to the genes from many published studies.[26] The gene-set libraries in this analysis were found under the "Ontologies" and "Pathways" categories. One particularly notable resource used is the Gene Ontology Consortium (GO), an evolving yet standardized set of vocabulary containing over 40,000 terms.[27] The three web-based ontologies currently available revealed significant terms in the MG-associated gene list belonging to one of three categories: the overall biological goal to which the genes contribute (biological process), the specific biochemical activities carried out by the gene products (molecular function), and the locations in the cell where the gene products are active (cellular component).[28]

Additional software to Enrichr used on the same 517 genes during this analysis was Metascape (https://metascape.org/gp/index.html#/main/step1), a comprehensive web-based portal centered on functional enrichment, gene annotation, interactome analysis, and membership search.[29] Analysis through Metascape provided an alternative perspective of the MG-associated genes by displaying how the genes and their gene products interact. These genes were entered under "express analysis" isolated to *Homo sapien* genes. Drawing from a list of databases including Reactome, Kegg, Mouse Genome Informatics (MGI) Mammalian Phenotype, STRING, BioGrid, OmniPath, InWeb_IM, and PaGenBase, the produced visualizations collected from both Enrichr and Metascape are discussed in the Results section of this study.

Results

MG-associated genes are enriched in the cytokine signaling pathway:

Using Enrichr, the genes were first analyzed through GO to identify the most enriched terms relating to molecular function (MF), biological process (BP), and cellular component (CC). The GO analysis for biological processes generally revealed enriched terms relating to the cytokine signaling pathway and activity (Figure 2A). Other enriched terms correlate with reg

DOI: 10.36838/v7i1.14

ulating additional signaling pathways involved in the immune response. These are similar to the top enriched terms displayed in the GO analysis for molecular function with general results related to cytokine signaling and chemokines, which are subsets of chemokine molecules involved in the migration of white blood cells (Figure 2B). Enriched terms that differentiated from those found in the previous analysis (biological function) were related to cell proliferation and Major Histocompatibility Complex (MHC) Class II molecules, which are essential for the adaptive immune response and self-recognition. It should be noted that although potentially presenting itself as a term of interest, it does not have a clear connection to the immune system. "Growth Factor Activity" is significantly less enriched than the top enriched terms, as is supported by the term's higher p-value.

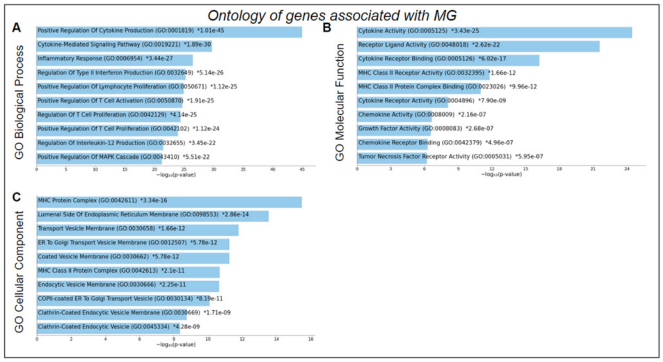

Figure 2: Gene ontology of 517 genes associated with MG. Bar graph of ten most enriched terms for Biological Process (A), Molecular Function (B), and Cellular Component (C). The GO terms are sorted by p-value. The top enriched terms found are most associated with cytokine signaling and production.

The ten most enriched terms from the GO analysis for cellular components relate to the MHC Protein Complex and Vesicle Transport (Figure 2C). The terms pertaining to vesicle transport would logically support BP and MF results that displayed the top term relating to cytokine signaling, as cytokine signaling often relies upon vesicles for transportation inside and out of the cell.[30] However, as the second most enriched term and with no explicit correlation to the common theme of autoimmunity present in other enriched terms, the "lumenal side of the endoplasmic reticulum" should be noted as a unique term for further investigations (Figure 2C).

Next, the MG-associated genes were analyzed through phenotype ontologies to investigate the potential outcomes of the disease's development. The Mouse Genome Informatics (MGI) Mammalian Phenotype ontology provided an additional point of reference that could be used to compare gene-phenotype relationships in those of humans to those of animal models, specifically mice. Alternatively, the human phenotype ontology analysis provided results that were more exclusive to phenotypes observed in humans, making enriched terms more compatible with phenotypes expected from MG patients.

The most enriched terms from the mammalian phenotype ontology revealed terms relating to abnormal immune response processes and susceptibility to infection, excluding premature death (Figure 3A). Once again, the enriched term with the lowest p-value, cytokine secretion, is correlated with cytokine activity. One particularly notable result is that both increased and decreased interferon-gamma signaling are present in the list of the ten most enriched terms from the analysis, given the contrasting nature of both terms. Furthermore, the Human Phenotype Ontology analysis revealed enriched terms related to symptoms of various autoimmune conditions and fatigue (Figure 3B). Abnormality of the neuromuscular junction and fatigue are directly related to the development of the disease. Still, it should be noted that all other enriched terms are not symptoms of the disease. The term "autosomal recessive inheritance" is also intriguing, given that MG is not heritable.

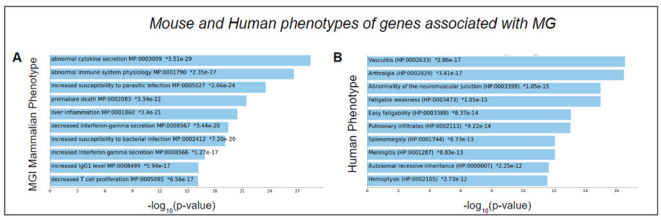

Figure 3: Mouse and Human phenotype ontology of genes associated with MG. Bar graph of ten most enriched terms from MGI_Mammalian_Phenotype_Level_4_2021 (A) and Human_Phenotype_Ontology (B) gene set libraries. The GO terms are sorted by p-value. The top enriched terms found are most associated with immune system irregularities.

Contribution of additional immune signaling pathways in Myasthenia Gravis:

The genes were also investigated using two pathway analysis gene sets available through Enrichr: Reactome_2022 and Kegg_2021. The Reactome analysis revealed top enriched terms correlated with cytokine, interferon, and interleukin signaling, with the most enriched term being cytokine signaling in the immune system (Figure 4A). Additionally, the KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis revealed enriched terms related to conditions originating from immune responses and pathogen infection, with the top enriched term being cytokine-cytokine receptor interaction (Figure 4B). These terms reflect the common theme of cytokine signaling and autoimmune dysfunction present in the enriched terms of other analysis ontologies. One point of differentiation between the two results lies in their variability, as the p-values of the three most enriched terms in the Reactome set are significantly lower when compared to all other terms. Different terms seem to have much closer p-values in the KEGG analysis, where various terms are much closer in distance to the top three most enriched terms.

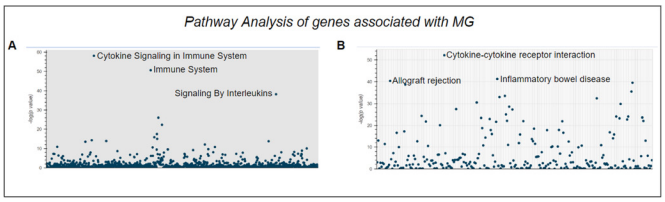

Figure 4: Pathway analysis of genes associated with MG. Manhattan plot of enriched terms from Reactome_2022 (A) and Kegg_2021_Human (B) gene sets. The top enriched terms found are most associated with autoimmune conditions and cytokine signaling.

The genes were then inputted into a pathway and process enrichment analysis through Metascape that utilized a combination of ontology sources: KEGG Pathway, GO Biological Processes, Reactome Gene Sets, Canonical Pathways, CORUM, WikiPathways, and PANTHER Pathway. Significantly enriched terms were grouped into clusters, and the top 20 clusters were displayed in a color-coded cluster network, highlighting the relationships between the genes. The top three enriched genes, as scored by p-value, were Cytokine Signaling in the Immune System, Allograft rejection, and the positive regulation of cytokine production, with 24.12%, 10.51%, and 18.29% of genes of the list that were found under the given ontology term, respectively. With the thickness of the lines representing the strength of the relationship between the clusters, these results would appear to align with the cluster network, as gene nodes correlating to the top three most enriched terms were found to be in the center of the diagram, demonstrating the key role that they may play in affecting multiple of the mechanisms listed (Figure 5A). Except for a singular gene node under the term "JAK-STAT signaling pathway" and a group of nodes correlating with the adaptive immune response, the overall lack of clearly isolated node groups indicates an interconnectedness amongst multiple factors contributing to the development of MG.

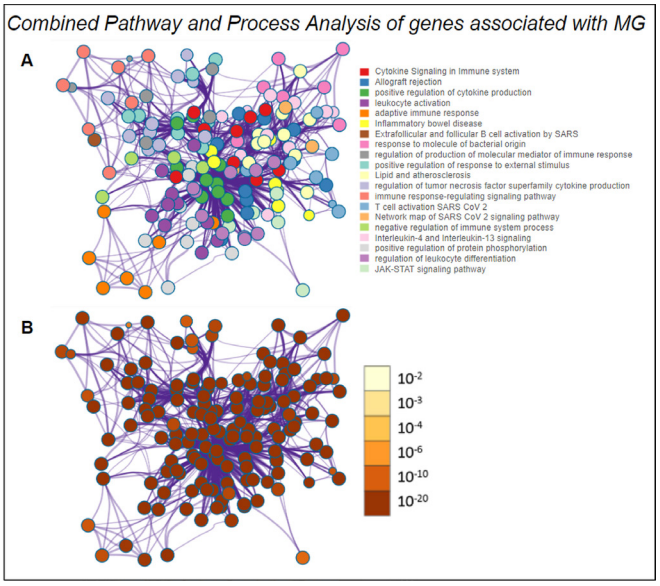

Figure 5: Combined Pathway and Process Analysis of MG-associated genes. Networks of enriched terms colored by cluster ID (A), where nodes that share the same cluster ID are typically close to each other, or colored by p-value (B), where terms containing more genes tend to have a more significant p-value. Almost all nodes appear to be significantly enriched.

The most unusual aspect of the pathway network may be the p-values associated with each node depicted in the network. Despite being found under differing cluster IDs, the majority of nodes are significantly enriched, having p-values less than 10-20, as indicated by their dark red color (Figure 5B). This would seem to reveal the relevance of the many different signaling pathways and immune response cells in MG, confirming the complexity of its genetic origins.

To understand the protein-protein interaction (PPI) nature of MG genes, STRING, BioGrid, OmniPath, and InWeb_IM databases were used for the analysis. Only physical interactions between the proteins were included. A network was created showing densely connected network components, as identified by the Molecular Complex Detection (MCODE) algorithm (Figure 6). The pathway and process enrichment analysis applied revealed MCODE components correlating to cytokine signaling, muscle development, antigen processing, G-protein signaling, interleukin signaling, growth factors, and other signaling pathways. The MCODE components with the highest statistical significance were related to cytokine signaling (MCODE_1), antigen processing (MCODE_3), and ligand-binding signaling pathways (MCODE_4), which are related to cytokine signaling, antigen processing, and ligand-binding signaling pathways, respectively (Table 2). This can be illustrated by their uniquely compact appearance as a network on the diagram (Figure 6). While still reflecting similarities in past results, three new enriched terms with statistical significance (log10(P) values less than -20) that have been provided as a description of the MCODE components include Kaposi sarcoma-associated herpesvirus infection, G alpha (i) signaling events, and GPCR ligand binding.

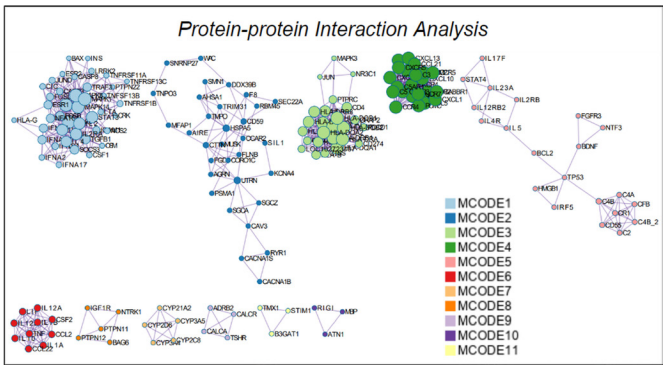

Figure 6: Protein-protein interaction networks of MG-associated genes. Networks are arranged into separate, densely connected MCODE network components.

Table 2 shows the results of the top three highest-scoring terms by p-value after pathway and process enrichment analysis is applied to each MCODE component. The MCODE clusters that were most enriched related to cytokine signaling and various other signaling pathways.

Table 2: Shows the results of the top three highest-scoring terms by p-value after pathway and process enrichment analysis is applied to each MCODE component.

Color	MCODE	GO	Description	log10(P)
	MCODE_1	R-HSA-1280215	Cytokine Signaling in Immune system	-39.9
	MCODE_1	GO:0071345	cellular response to cytokine stimulus	-31.1
	MCODE_1	hsa05167	Kaposi sarcoma-associated herpesvirus infection	-28.3
	MCODE_2	hsa05020	Prion disease	-6.4
	MCODE_2	GO:0007517	muscle organ development	-6.1
	MCODE_2	GO:0061061	muscle structure development	-6
	MCODE_3	GO:0048002	antigen processing and presentation of peptide antigen	-36.7
	MCODE_3	hsa05330	Allograft rejection	-35.2
	MCODE_3	GO:0002478	antigen processing and presentation of exogenous peptide antigen	-34.6
	MCODE_4	R-HSA-418594	G alpha (i) signalling events	-43.9
	MCODE_4	R-HSA-375276	Peptide ligand-binding receptors	-41.6
	MCODE_4	R-HSA-500792	GPCR ligand binding	-40.1
	MCODE_5	R-HSA-977606	Regulation of Complement cascade	-14.8
	MCODE_5	GO:0002252	immune effector process	-14.6
	MCODE_5	R-HSA-174577	Activation of C3 and C5	-14.3
	MCODE_6	R-HSA-6783783	Interleukin-10 signaling	-28.5
	MCODE_6	WP5095	Overview of proinflammatory and profibrotic mediators	-23.9
	MCODE_6	R-HSA-6785807	Interleukin-4 and Interleukin-13 signaling	-21.2
	MCODE_7	R-HSA-211897	Cytochrome P450 - arranged by substrate type	-13.4
	MCODE_7	GO:0070989	oxidative demethylation	-12.7
	MCODE_7	R-HSA-211945	Phase I - Functionalization of compounds	-12.3

DOI: 10.36838/v7i1.14

	MCODE_8	M100	PID SHP2 PATHWAY	-7.2
	MCODE_8	GO:0071363	cellular response to growth factor stimulus	-6.5
	MCODE_8	GO:0070848	response to growth factor	-6.4
	MCODE_9	GO:0007189	adenylate cyclase-activating G protein-coupled receptor signaling pathway	-9.3
	MCODE_9	R-HSA-418555	G alpha (s) signalling events	-9.1
	MCODE_9	GO:0007188	adenylate cyclase-modulating G protein-coupled receptor signaling pathway	-8.5

Additional enrichment analysis was carried out using PaGenBase (A Pattern Gene Database for the Global and Dynamic Understanding of Gene Function) to identify enriched terms correlated to tissue- or cell-specific pattern genes within the list of MG-associated genes (Figure 7A). This ontology revealed top enriched terms related to the spleen, lung, thymus, and bone marrow, with 11%, 7.8%, 4.7%, and 4.1% of the genes, respectively. It should be noted that the top enriched term, tissue-specific: spleen, is significantly more enriched than all other terms (which can be seen by its red color), highlighting itself as a future point of interest.

Because transcription factors drive large gene expression changes, an enrichment analysis was conducted to identify possible transcription factors associated with MG (Figure 7B). The most significantly enriched terms were all related to the transcription factor, Nuclear factor-kappa B (NF-κB). It should be mentioned, however, that these terms had higher p-values than top enriched terms of previous analysis, and all terms contained less than 6% of the genes found in the total gene list inputted, which indicates that they may be less enriched.

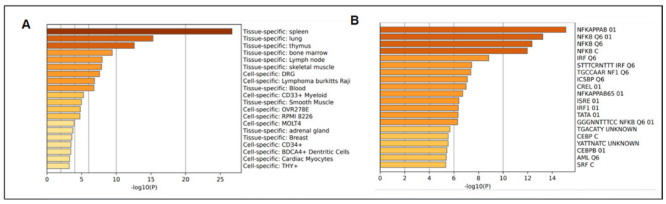

Figure 7: Summary of enrichment analysis in PaGenBase (A) and Transcription Factor Targets (B). The top enriched terms found are most associated with the spleen and transcription factor NF-kB.

Discussion

With the complex genetic origins of Myasthenia Gravis, this study sought to explore the key genes implicated in the disorder's development through a meta-analysis. The results confirm immune system irregularities as key contributors to MG development. With the most significantly enriched terms amongst all datasets converging into cytokine signaling, allograft rejection, susceptibility to infection, T cell activity, and inflammation, the analysis might suggest that the body's reduced ability to correctly distinguish between harmful foreign invaders and normal proteins from the body characterizes a major part of the condition's pathogenesis. Indeed, some top enriched terms have a direct link to the aforementioned common understanding of MG as described in the "Introduction" section of this paper, including "Abnormality of the Neuromuscular Junction," "Fatigable Weakness," "Easy Fatigability," "G alpha (i) signaling events," and "GPCR [(G protein-coupled receptor)] ligand binding" (Figure 2B). Even when not directly linked to the symptoms of MG, top enriched terms "vasculitis" (inflammation of the joint), "arthralgia" (pain in a joint), and "pulmonary infiltrates" (dense substances in the lung) can all originate from diseases characterized by immunological dysfunction associated with Myasthenia Gravis (Figure 2B).[31-34] Therefore, there seems to be clear evidence supporting MG's classification as an autoimmune disorder.

While the data presented in this study generally attributes MG with autoimmunity, it explicitly highlights terms relating to the cytokine signaling pathway as the most significantly enriched biological process. Uzawa et al. investigated the overall role these cytokines may play in MG and found that the IL-17, IL-21, IL-10, BAFF, APRIL, IL-6, and IL-23 were all generally up-regulated in samples of MG patients. This would create a logical connection between these terms and MG, as all of these up-regulated cytokines interact with a variety of T cells that contribute to MG by promoting chronic inflammation (Th17), autoantibody production (Tfh), and an overstimulated immune response (Treg) through their dysfunction.[35] Additionally, while first appearing to have no explicit link to MG, the lumenal side of the endoplasmic reticulum (ER) folds and prepares proteins for the process of protein secretion through vesicle transport, playing a similar role in cytokine secretion as all other enriched cellular components (Figure 1C).[36] Through their consistent appearance in enriched terms with the lowest p-values in multiple forms of analysis, cytokines seem to present themselves as an essential aspect of MG's pathogenesis, and further research investigating the IL loci may provide a deeper understanding of the genetic origins underlying the condition's development.

The large number of statistically significant terms and the lack of isolated nodes within the process and pathway analysis node network justify the consideration and possible significance of other enriched terms as well (Figure 4). Kaposi Sarcoma (KS) is a lymphoproliferative disorder correlated with human herpesvirus 8 (HHV-8), resulting in purpuric lesions on many different body parts. Interestingly, in a case study of two MG patients who developed KS, it was found that the lesions only developed after taking prednisone and azathioprine, two immunosuppressants used to treat MG.[37] While this side effect of KS from corticosteroid treatment is rare, and there are very few cases where both conditions are developed, the enriched term "Kaposi sarcoma-associated herpesvirus" (Table 2) prompts further investigation into the way MG increases the susceptibility of conditions that develop from taking its treatment.

The spleen is an essential organ of the immune system, as it stores white blood cells needed for combating pathogens in the body. Recent studies investigating the spleen's role in MG have typically highlighted methods of improving the condition through replenishing spleen "qi," the force flowing through all living things, as defined by traditional Chinese medicine (TCM).[38] However, in a 1985 case study of five MG patients who underwent a splenectomy, it was found that three patients saw significant improvement, and one patient saw moderate improvement. The last patient saw no improvement in reducing muscle weakness.[39] Therefore, given the lack of modern studies investigating this relationship beyond "qi" and its potential applications for MG treatment, the top enriched term "spleen" (Figure 6A) highlights the need for novel

research in this vastly unexplored area that applies rigorous scientific methodology to understand the relationship between the spleen and MG in humans.

The top enriched terms relating to NF-κB (Figure 6B) would again support a focus on autoimmunity, as the transcription factor has been extensively researched for its effect on various immune processes. However, an additional point of consideration may lie in its further influence on cancer development, as its activation can induce cell proliferation, suppression of antitumor immunity, invasion, and metastasis.[40] Thymoma and MG have been associated with one another, as 30-50% of thymoma patients have MG, and 10-20% of MG patients have thymoma. However, the exact mechanism linking the two is still not fully understood.[41] Further examination of NF-κB in MG may help uncover the causative factors behind this relationship and aid in developing medical interventions treating those afflicted by both conditions.

One limitation of this study may be found in the analysis resources themselves. Gene ontology, as a whole, has often been criticized for its incomplete evaluation of the genome, as the current set of annotations can be considered shallow or even noisy when accounting for the lack of "NOT" annotations that can clarify negative associations.[42,43] However, although the results provided by gene set ontology and enrichment analysis cannot be used to reach definite conclusions of causation, the top enriched terms produced from the various libraries still potentially inspire further studies. Future research stemming from this study should evaluate the possible significance of the intriguing, enriched terms previously mentioned to shed light on unknown autoimmune pathways and processes contributing to the complex nature of MG's pathogenesis.

Acknowledgments

I would like to thank Dr. Kif Liakath-Ali for his guidance throughout this study. His consistent support and insights have been pivotal to completing this research. I would also like to thank all reviewers for their constructive feedback.

References

1. Carr, A. S.; Cardwell, C. R.; McCarron, P. O.; McConville, J. A Systematic Review of Population Based Epidemiological Studies in Myasthenia Gravis. *BMC Neurol.* **2010**, *10*, 46. https://doi.org/10.1186/1471-2377-10-46.
2. Kerty, E.; Elsais, A.; Argov, Z.; Evoli, A.; Gilhus, N. E. EFNS/ENS Guidelines for the Treatment of Ocular Myasthenia. *Eur. J. Neurol.* **2014**, *21* (5), 687–693. https://doi.org/10.1111/ene.12359.
3. Gilhus, N. E.; Tzartos, S.; Evoli, A.; Palace, J.; Burns, T. M.; Verschuuren, J. J. G. M. Myasthenia Gravis. *Nat. Rev. Dis. Primer* **2019**, *5* (1), 1–19. https://doi.org/10.1038/s41572-019-0079-y.
4. Claytor, B.; Cho, S.-M.; Li, Y. Myasthenic Crisis. *Muscle Nerve* **2023**, *68* (1), 8–19. https://doi.org/10.1002/mus.27832.
5. Andersen, J. B.; Heldal, A. T.; Engeland, A.; Gilhus, N. E. Myasthenia Gravis Epidemiology in a National Cohort; Combining Multiple Disease Registries. *Acta Neurol. Scand.* **2014**, *129* (s198), 26-31. https://doi.org/10.1111/ane.12233.
6. Zhang, X.; Yang, M.; Xu, J.; Zhang, M.; Lang, B.; Wang, W.; Vincent, A. Clinical and Serological Study of Myasthenia Gravis in Hubei Province, China. J. Neurol. Neurosurg. *Psychiatry* **2007**, *78* (4), 386–390. https://doi.org/10.1136/jnnp.2006.100545.
7. Nicoli, V.; Tabano, S. M.; Colapietro, P.; Maestri, M.; Ricciardi, R.; Stoccoro, A.; Fontana, L.; Guida, M.; Miozzo, M.; Coppedè, F.; Migliore, L. Preferential X Chromosome Inactivation as a Mechanism to Explain Female Preponderance in Myasthenia Gravis. *Genes* **2022**, *13* (4), 696. https://doi.org/10.3390/genes13040696.
8. Ramanujam, R.; Pirskanen, R.; Ramanujam, S.; Hammarström, L. Utilizing Twins Concordance Rates to Infer the Predisposition to Myasthenia Gravis. Twin Res. *Hum. Genet.* **2011**, *14* (2), 129–136. https://doi.org/10.1375/twin.14.2.129.
9. Gilhus, N. E.; Verschuuren, J. J. Myasthenia Gravis: Subgroup Classification and Therapeutic Strategies. *Lancet Neurol.* **2015**, *14* (10), 1023–1036. https://doi.org/10.1016/S1474-4422(15)00145-3.
10. Gilhus, N. E. Myasthenia Gravis. *N. Engl. J. Med.* **2016**, *375* (26), 2570–2581. https://doi.org/10.1056/NEJMra1602678.
11. Gilhus, N. E.; Skeie, G. O.; Romi, F.; Lazaridis, K.; Zisimopoulou, P.; Tzartos, S. Myasthenia Gravis - Autoantibody Characteristics and Their Implications for Therapy. *Nat. Rev. Neurol.* **2016**, *12* (5), 259–268. https://doi.org/10.1038/nrneurol.2016.44.
12. Kordas, G.; Lagoumintzis, G.; Sideris, S.; Poulas, K.; Tzartos, S. J. Direct Proof of the In Vivo Pathogenic Role of the AChR Autoantibodies from Myasthenia Gravis Patients. *PLoS ONE* **2014**, *9* (9), e108327. https://doi.org/10.1371/journal.pone.0108327.
13. Huijbers, M. G.; Zhang, W.; Klooster, R.; Niks, E. H.; Friese, M. B.; Straasheijm, K. R.; Thijssen, P. E.; Vrolijk, H.; Plomp, J. J.; Vogels, P.; Losen, M.; Van der Maarel, S. M.; Burden, S. J.; Verschuuren, J. J. MuSK IgG4 Autoantibodies Cause Myasthenia Gravis by Inhibiting Binding between MuSK and Lrp4. Proc. *Natl. Acad. Sci. U. S. A.* **2013**, *110* (51), 20783–20788. https://doi.org/10.1073/pnas.1313944110.
14. Zong, Y.; Jin, R. Structural Mechanisms of the Agrin–LRP4–MuSK Signaling Pathway in Neuromuscular Junction Differentiation. *Cell. Mol. Life Sci.* **2013**, *70* (17), 3077–3088. https://doi.org/10.1007/s00018-012-1209-9.
15. Andersen, J. B.; Gilhus, N. E.; Sanders, D. B. Factors Affecting Outcome in Myasthenia Gravis. *Muscle Nerve* **2016**, *54* (6), 1041–1049. https://doi.org/10.1002/mus.25205.
16. Afrashteh, F.; Rajabi, R. Overview of Biomarkers in Myasthenia Gravis. Explor. *Neuroprotective Ther.* **2022**, 210–225. https://doi.org/10.37349/ent.2022.00029.
17. Gambino, C. M.; Agnello, L.; Lo Sasso, B.; Giglio, R. V.; Di Stefano, V.; Candore, G.; Pappalardo, E. M.; Ciaccio, A. M.; Brighina, F.; Vidali, M.; Ciaccio, M. The Role of Serum Free Light Chain as Biomarker of Myasthenia Gravis. *Clin. Chim. Acta* **2022**, *528*, 29-33. https://doi.org/10.1016/j.cca.2022.01.004.
18. Ghafouri-Fard, S.; Azimi, T.; Hussen, B. M.; Taheri, M.; Jalili Khoshnoud, R. A Review on the Role of Non-Coding Rnas in the Pathogenesis of Myasthenia Gravis. *Int. J. Mol. Sci.* **2021**, *22* (23), 12964. https://doi.org/10.3390/ijms222312964.
19. Chen, P.; Tang, X. Gut Microbiota as Regulators of Th17/Treg Balance in Patients With Myasthenia Gravis. *Front. Immunol.* **2021**, *12*, 803101. https://doi.org/10.3389/fimmu.2021.803101.
20. Landouré, G.; Knight, M. A.; Stanescu, H.; Taye, A. A.; Shi, Y.; Diallo, O.; Johnson, J. O.; Hernandez, D.; Traynor, B. J.; Biesecker, L. G.; NIH Intramural Sequencing Center; Elkahloun, A.; Rinaldi, C.; Vincent, A.; Willcox, N.; Kleta, R.; Fischbeck, K. H.; Burnett, B. G. A Candidate Gene for Autoimmune Myasthenia Gravis. *Neurology* **2012**, *79* (4), 342–347. https://doi.org/10.1212/WNL.0b013e318260cbd0.
21. Gregersen, P. K.; Kosoy, R.; Lee, A. T.; Lamb, J.; Sussman, J.; McKee, D.; Simpfendorfer, K. R.; Pirskanen-Matell, R.; Piehl, F.; Pan-Hammarstrom, Q.; Verschuuren, J. J. G. M.; Titulaer, M. J.; Niks, E. H.; Marx, A.; Ströbel, P.; Tackenberg, B.; Pütz, M.; Maniaol, A.; Elsais, A.; Tallaksen, C.; Harbo, Hanne. F.; Lie, B. A.; Raychaudhuri, S.; de Bakker, P. I. W.; Melms, A.; Garchon, H.-J.; Willcox, N.; Hammarstrom, L.; Seldin, M. F. Risk for Myasthenia Gravis Maps to 151Pro→ Ala Change in TNIP1 and to HLA-B*08. *An*

DOI: 10.36838/v7i1.14

n. Neurol. **2012**, *72* (6), 927–935. https://doi.org/10.1002/ana.23691.
22. Vandiedonck, C.; Beaurain, G.; Giraud, M.; Hue-Beauvais, C.; Eymard, B.; Tranchant, C.; Gajdos, P.; Dausset, J.; Garchon, H.-J. Pleiotropic Effects of the 8.1 HLA Haplotype in Patients with Autoimmune Myasthenia Gravis and Thymus Hyperplasia. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101* (43), 15464–15469. https://doi.org/10.1073/pnas.0406756101.
23. Avidan, N.; Le Panse, R.; Berrih-Aknin, S.; Miller, A. Genetic Basis of Myasthenia Gravis - a Comprehensive Review. *J. Autoimmun.* **2014**, *52*, 146–153. https://doi.org/10.1016/j.jaut.2013.12.001.
24. Rouillard, A. D.; Gundersen, G. W.; Fernandez, N. F.; Wang, Z.; Monteiro, C. D.; McDermott, M. G.; Ma'ayan, A. The Harmonizome: A Collection of Processed Datasets Gathered to Serve and Mine Knowledge about Genes and Proteins. *Database* **2016**, *2016*, baw100. https://doi.org/10.1093/database/baw100.
25. Chen, E. Y.; Tan, C. M.; Kou, Y.; Duan, Q.; Wang, Z.; Meirelles, G. V.; Clark, N. R.; Ma'ayan, A. Enrichr: Interactive and Collaborative HTML5 Gene List Enrichment Analysis Tool. *BMC Bioinformatics* **2013**, *14*, 128. https://doi.org/10.1186/1471-2105-14-128.
26. Subramanian, A.; Tamayo, P.; Mootha, V. K.; Mukherjee, S.; Ebert, B. L.; Gillette, M. A.; Paulovich, A.; Pomeroy, S. L.; Golub, T. R.; Lander, E. S.; Mesirov, J. P. Gene Set Enrichment Analysis: A Knowledge-Based Approach for Interpreting Genome-Wide Expression Profiles. *Proc. Natl. Acad. Sci.* **2005**, *102* (43), 15545–15550. https://doi.org/10.1073/pnas.0506580102.
27. Aleksander, S. A.; Balhoff, J.; Carbon, S.; Cherry, J. M.; Drabkin, H. J.; Ebert, D.; Feuermann, M.; Gaudet, P.; Harris, N. L.; Hill, D. P.; Lee, R.; Mi, H.; Moxon, S.; Mungall, C. J.; Muruganugan, A.; Mushayahama, T.; Sternberg, P. W.; Thomas, P. D.; Van Auken, K.; Ramsey, J.; Siegele, D. A.; Chisholm, R. L.; Fey, P.; Aspromonte, M. C.; Nugnes, M. V.; Quaglia, F.; Tosatto, S.; Giglio, M.; Nadendla, S.; Antonazzo, G.; Attrill, H.; dos Santos, G.; Marygold, S.; Strelets, V.; Tabone, C. J.; Thurmond, J.; Zhou, P.; Ahmed, S. H.; Asanitthong, P.; Luna Buitrago, D.; Erdol, M. N.; Gage, M. C.; Ali Kadhum, M.; Li, K. Y. C.; Long, M.; Michalak, A.; Pesala, A.; Pritazahra, A.; Saverimuttu, S. C. C.; Su, R.; Thurlow, K. E.; Lovering, R. C.; Logie, C.; Oliferenko, S.; Blake, J.; Christie, K.; Corbani, L.; Dolan, M. E.; Drabkin, H. J.; Hill, D. P.; Ni, L.; Sitnikov, D.; Smith, C.; Cuzick, A.; Seager, J.; Cooper, L.; Elser, J.; Jaiswal, P.; Gupta, P.; Jaiswal, P.; Naithani, S.; Lera-Ramirez, M.; Rutherford, K.; Wood, V.; De Pons, J. L.; Dwinell, M. R.; Hayman, G. T.; Kaldunski, M. L.; Kwitek, A. E.; Laulederkind, S. J. F.; Tutaj, M. A.; Vedi, M.; Wang, S.-J.; D'Eustachio, P.; Aimo, L.; Axelsen, K.; Bridge, A.; Hyka-Nouspikel, N.; Morgat, A.; Aleksander, S. A.; Cherry, J. M.; Engel, S. R.; Karra, K.; Miyasato, S. R.; Nash, R. S.; Skrzypek, M. S.; Weng, S.; Wong, E. D.; Bakker, E.; Berardini, T. Z.; Reiser, L.; Auchincloss, A.; Axelsen, K.; Argoud-Puy, G.; Blatter, M.-C.; Boutet, E.; Breuza, L.; Bridge, A.; Casals-Casas, C.; Coudert, E.; Estreicher, A.; Livia Famiglietti, M.; Feuermann, M.; Gos, A.; Gruaz-Gumowski, N.; Hulo, C.; Hyka-Nouspikel, N.; Jungo, F.; Le Mercier, P.; Lieberherr, D.; Masson, P.; Morgat, A.; Pedruzzi, I.; Pourcel, L.; Poux, S.; Rivoire, C.; Sundaram, S.; Bateman, A.; Bowler-Barnett, E.; Bye-A-Jee, H.; Denny, P.; Ignatchenko, A.; Ishtiaq, R.; Lock, A.; Lussi, Y.; Magrane, M.; Martin, M. J.; Orchard, S.; Raposo, P.; Speretta, E.; Tyagi, N.; Warner, K.; Zaru, R.; Diehl, A. D.; Lee, R.; Chan, J.; Diamantakis, S.; Raciti, D.; Zarowiecki, M.; Fisher, M.; James-Zorn, C.; Ponferrada, V.; Zorn, A.; Ramachandran, S.; Ruzicka, L.; Westerfield, M. The Gene Ontology Knowledgebase in 2023. *Genetics* **2023**, *224* (1), iyad031. https://doi.org/10.1093/genetics/iyad031.
28. Ashburner, M.; Ball, C. A.; Blake, J. A.; Botstein, D.; Butler, H.; Cherry, J. M.; Davis, A. P.; Dolinski, K.; Dwight, S. S.; Eppig, J. T.; Harris, M. A.; Hill, D. P.; Issel-Tarver, L.; Kasarskis, A.; Lewis, S.; Matese, J. C.; Richardson, J. E.; Ringwald, M.; Rubin, G. M.; Sherlock, G. Gene Ontology: Tool for the Unification of Biology. *Nat. Genet.* **2000**, *25* (1), 25–29. https://doi.org/10.1038/75556.
29. Zhou, Y.; Zhou, B.; Pache, L.; Chang, M.; Khodabakhshi, A. H.; Tanaseichuk, O.; Benner, C.; Chanda, S. K. Metascape Provides a Biologist-Oriented Resource for the Analysis of Systems-Level Datasets. *Nat. Commun.* **2019**, *10* (1), 1523. https://doi.org/10.1038/s41467-019-09234-6.
30. Fitzgerald, W.; Freeman, M. L.; Lederman, M. M.; Vasilieva, E.; Romero, R.; Margolis, L. A System of Cytokines Encapsulated in Extracellular Vesicles. *Sci Rep* **2018**, *8* (1), 8973. https://doi.org/10.1038/s41598-018-27190-x.
31. Politei, J.; Remondino, G.; Heguilen, R.; Wallace, E.; Durand, C.; Schenone, A. When Arthralgia Is Not Arthritis. *Eur. J. Rheumatol.* **2016**, *3* (4), 182–184. https://doi.org/10.5152/eurjrheum.2016.15073.
32. COJOCARU, M.; COJOCARU, I. M.; SILOSI, I.; VRABIE, C. D. Pulmonary Manifestations of Systemic Autoimmune Diseases. *Mædica* **2011**, *6* (3), 224–229.
33. Moretti, M.; Treppo, E.; Monti, S.; La Rocca, G.; Del Frate, G.; Delvino, P.; Italiano, N.; Di Cianni, F.; D'Alessandro, F.; Talarico, R.; Ferro, F.; Quartuccio, L.; Baldini, C. Systemic Vasculitis: One Year in Review 2023. *Clin. Exp. Rheumatol.* **2023**, *41* (4), 765-773. https://doi.org/10.55563/clinexprheumatol/zf4daj.
34. Tanovska, N.; Novotni, G.; Sazdova-Burneska, S.; Kuzmanovski, I.; Boshkovski, B.; Kondov, G.; Jovanovski-Srceva, M.; Kokareva, A.; Isjanovska, R. Myasthenia Gravis and Associated Diseases. *Open Access Maced. J. Med. Sci.* **2018**, *6* (3), 472–478. https://doi.org/10.3889/oamjms.2018.110.
35. Uzawa, A.; Kuwabara, S.; Suzuki, S.; Imai, T.; Murai, H.; Ozawa, Y.; Yasuda, M.; Nagane, Y.; Utsugisawa, K. Roles of Cytokines and T Cells in the Pathogenesis of Myasthenia Gravis. *Clin. Exp. Immunol.* **2021**, *203* (3), 366–374. https://doi.org/10.1111/cei.13546.
36. Oakes, S. A.; Papa, F. R. The Role of Endoplasmic Reticulum Stress in Human Pathology. *Annu. Rev. Pathol.* **2015**, *10*, 173–194. https://doi.org/10.1146/annurev-pathol-012513-104649.
37. Frangiamore, R.; Giossi, R.; Vanoli, F.; Tourlaki, A.; Brambilla, L.; Maggi, L.; Mantegazza, R. Iatrogenic Kaposi's Sarcoma in Myasthenia Gravis: Learnings from Two Case Reports. *Neurol. Sci.* **2021**, *42* (5), 2081–2083. https://doi.org/10.1007/s10072-020-04971-9.
38. Jiang, L.; Xu, P.; Zhang, D.; Lu, J.; Chang, T.; Zhang, Y.; Wang, J. Treatment of Myasthenia Gravis with the Method of Tonifying Spleen and Replenishing Qi in Traditional Chinese Medicine. *Medicine (Baltimore)* **2022**, *101* (3), e28530. https://doi.org/10.1097/MD.0000000000028530.
39. Hofmann, W. E.; Reuther, P.; Schalke, B.; Mertens, H. G. Splenectomy in Myasthenia Gravis: A Therapeutic Concept? *J. Neurol.* **1985**, *232* (4), 215–218. https://doi.org/10.1007/BF00313782.
40. Taniguchi, K.; Karin, M. NF-κB, Inflammation, Immunity and Cancer: Coming of Age. *Nat. Rev. Immunol.* **2018**, *18* (5), 309–324. https://doi.org/10.1038/nri.2017.142.
41. Cacho-Díaz, B.; Salmerón-Moreno, K.; Lorenzana-Mendoza, N. A.; Texcocano, J.; Arrieta, O. Myasthenia Gravis as a Prognostic Marker in Patients with Thymoma. *J. Thorac. Dis.* **2018**, *10* (5), 2842–2848. https://doi.org/10.21037/jtd.2018.04.95.
42. Zhao, Y.; Wang, J.; Chen, J.; Zhang, X.; Guo, M.; Yu, G. A Literature Review of Gene Function Prediction by Modeling Gene Ontology. *Front. Genet.* **2020**, *11*.
43. Yon Rhee, S.; Wood, V.; Dolinski, K.; Draghici, S. Use and Misuse of the Gene Ontology Annotations. *Nat. Rev. Genet.* **2008**, *9* (7), 509–515. https://doi.org/10.1038/nrg2363.

Author

Caleb Tsai is a Junior at El Segundo High School. His passions for both music and medicine have drawn him to neuroscience, a field continually revealing new intersections between the two disciplines. In future research, he hopes to continue exploring the underlying processes of neurological disorders and their possible applications to the discovery of novel treatments through neuromusicology.

DOI: 10.36838/v7i1.14