

Integrative Genetic and Pathway Profiling Reveals the Molecular Landscape of Dystonia

Neetu Rani Dhiman

Department of Neurology, Institute of Medical Sciences, Banaras Hindu University

Surbhi Singh

Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University

Deepika Joshi

drdeepikajoshi73@gmail.com

Department of Neurology, Institute of Medical Sciences, Banaras Hindu University

Royana Singh

Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University

Ashish Ashish

Multidisciplinary Research Unit, Institute of Medical Sciences, Banaras Hindu University, Varanasi–221005, Uttar Pradesh, India

Shivani Mishra

Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University

Anand Kumar

Department of Neurology, Institute of Medical Sciences, Banaras Hindu University

Varun Kumar Singh

Department of Neurology, Institute of Medical Sciences, Banaras Hindu University

Research Article

Keywords: Dystonia, Whole-exome sequencing, Genetic variants, Bioinformatics, Gene Ontology, Protein–protein interaction network, Molecular pathways, Precision medicine

Posted Date: August 21st, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10443475/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background

Dystonia is a clinically heterogeneous movement disorder characterized by sustained or intermittent muscle contractions arising from dysfunction of distributed motor networks. Although multiple causative genes have been implicated, the extent to which these genetic alterations converge on common molecular pathways remains poorly understood.

Methods

A total of 28 patients with focal isolated dystonia were recruited, of whom 23 samples passed quality control and were included in the final analysis. Genomic DNA was extracted from peripheral blood samples, and whole-exome sequencing (WES) libraries were prepared using the Agilent Sure Select Human All Exon V8 Kit and sequenced on the Illumina NovaSeq 6000 platform using 150-bp paired-end sequencing. Germline variants were identified, filtered, annotated, and prioritized based on sequencing quality, population allele frequency, pathogenicity, and clinical relevance. Functional enrichment analysis was performed using DAVID, protein–protein interaction networks were constructed using STRING, and downstream data integration and visualization were performed using R software.

Results

Pathogenic and likely pathogenic variants were identified in several dystonia-associated genes, including TOR1A, THAP1, SGCE, SPR, SDHA, SCN2A, and ANK2. The detected variants exhibited heterogeneous distribution and mutation types, with a predominance of loss-of-function alterations. Gene Ontology enrichment analysis revealed significant involvement in mitochondrial metabolism, coenzyme A biosynthesis, ion channel activity, transcriptional regulation, and neurotransmitter-related processes ($P < 0.05$). Protein–protein interaction network analysis demonstrated significant enrichment ($P < 0.01$), identifying key hub genes and strong functional connectivity among pathways regulating neuronal excitability and metabolic homeostasis. Gene–phenotype correlation further indicated overlapping yet distinct clinical associations, reflecting substantial molecular heterogeneity.

Conclusion

Genetically diverse variants associated with dystonia converge on interconnected biological pathways governing mitochondrial function, neurotransmitter synthesis, ion channel activity, and transcriptional regulation. These findings highlight a network-level disease mechanism and support the development of pathway-oriented therapeutic strategies targeting shared molecular processes rather than individual gene defects

Introduction

Dystonia is a clinically and genetically heterogeneous group of movement disorders characterized by sustained or intermittent muscle contractions that lead to abnormal, often repetitive movements and postures(1). These motor manifestations frequently result from dysfunction within distributed neural circuits, particularly involving the basal ganglia, cerebellum, and cortical motor networks(2). Clinically, dystonia can be classified based on etiology into inherited, acquired, or idiopathic forms, and based on anatomical distribution into focal, segmental, multifocal, or generalized types(3). This phenotypic diversity contributes to diagnostic challenges and complicates the development of standardized therapeutic strategies. Despite considerable advances in neuroimaging and clinical characterization, the precise molecular mechanisms underlying dystonia remain incompletely understood, limiting early diagnosis and effective intervention(4). Over the past two decades, significant progress has been made in identifying genetic determinants of dystonia. Several causative genes have been implicated, including *TOR1A* (DYT1), *THAP1* (DYT6), *GNAL* (DYT25), *SGCE* (DYT11), and others involved in diverse cellular processes such as transcriptional regulation, synaptic transmission, and intracellular signaling(5). However, these known genetic variants explain only a proportion of dystonia cases, and many patients remain genetically unresolved. Furthermore, even among individuals harboring mutations in the same gene, there exists substantial variability in age of onset, severity, and clinical presentation(6). This phenotypic heterogeneity suggests that dystonia is not solely a monogenic disorder but rather involves complex genetic architectures, including modifier genes, epigenetic influences, and gene–environment interactions(7). Recent advances in whole-exome sequencing (WES) have accelerated the identification of rare genetic variants associated with dystonia, yet understanding how these variants contribute to disease pathogenesis remains challenging. Increasing evidence suggests that dystonia results from disruptions in interconnected molecular pathways involving mitochondrial function, neurotransmitter synthesis, ion channel activity, and transcriptional regulation rather than isolated gene defects. Integrative bioinformatics approaches, including Gene Ontology (GO), pathway enrichment, and protein–protein interaction (PPI) network analyses, provide a systems-level framework for identifying key molecular mechanisms and convergent biological pathways(8, 9). Therefore, this study combines WES with functional enrichment and network analyses to characterize the molecular landscape of dystonia, identify key regulatory pathways, and provide insights into disease mechanisms that may facilitate biomarker discovery and precision medicine(10).

Materials and Methods

Study Participants

This cross-sectional study included patients clinically diagnosed with focal isolated dystonia who were recruited from the Neurology and Movement Disorders Outpatient Department, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, over a two-year period. Diagnosis was established by an experienced movement disorder specialist according to standard clinical diagnostic criteria for dystonia. Patients with secondary dystonia due to structural brain lesions, metabolic disorders, drug-

induced causes, or neurodegenerative diseases were excluded. Written informed consent was obtained from all participants before enrollment. A total of 28 patients were initially recruited. Following DNA quality assessment and sequencing quality control, 23 samples met all predefined quality criteria and were included in the final analysis. The study cohort comprised 14 females and 9 males with a mean age of 53.56 ± 7.51 years. All enrolled participants presented with focal isolated dystonia. The study protocol was reviewed and approved by the Institutional Ethics Committee, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India Approval No- Dean/2024/EC/7101. All procedures were conducted in accordance with the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from all participants before sample collection.

Genomic DNA Extraction

Peripheral venous blood (3–5 mL) was collected in EDTA-coated vacutainer tubes from each participant. Genomic DNA was extracted from peripheral blood leukocytes using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA concentration and purity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA), while DNA integrity was evaluated by 1% agarose gel electrophoresis. DNA samples with an A260/A280 absorbance ratio between 1.8 and 2.0, a concentration ≥ 50 ng/ μ L, and intact high-molecular-weight DNA were considered suitable for whole-exome library preparation. Only high-quality DNA samples meeting the required concentration and purity criteria were processed for sequencing.

Whole Exome Library Preparation and Sequencing

Whole-exome sequencing (WES) libraries were prepared using the Agilent SureSelect Human All Exon V8 Kit (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions. Briefly, high-quality genomic DNA was fragmented to the desired insert size, followed by end repair, A-tailing, adapter ligation, and PCR amplification. Exonic regions were selectively enriched using Agilent SureSelect Human All Exon V8 biotinylated RNA capture probes. Captured DNA fragments were recovered using streptavidin-coated magnetic beads and purified with AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA). Library quality, fragment size distribution, and concentration were assessed using an Agilent 2100 Bioanalyzer before sequencing. Qualified libraries were pooled in equimolar concentrations and sequenced on the Illumina NovaSeq 6000 platform (Illumina Inc., San Diego, CA, USA) using 150-bp paired-end sequencing chemistry according to the manufacturer's protocol. Sequencing was performed to achieve a mean target coverage depth of $> 100\times$, with an average sequencing output of approximately 8–10 GB per sample, enabling high-confidence detection of coding region variants. Raw sequencing reads were generated in FASTQ format and subjected to downstream bioinformatics analysis(11).

Bioinformatics Analysis

Raw sequencing reads were subjected to quality assessment using FastQC (v0.11.9)(12), and adapter sequences together with low-quality bases were trimmed using Trimmomatic (v0.39)(13). High-quality reads were aligned to the human reference genome (GRCh38/hg38) using the Burrows–Wheeler Aligner (BWA-MEM v0.7.17)(14). PCR duplicates were identified and removed using Picard Tools (v3.1.1)(15), followed by base quality score recalibration according to the Genome Analysis Toolkit (GATK v4.5.0)(16) Best Practices workflow. Germline variants, including single nucleotide variants (SNVs) and small insertions/deletions (Indels), were identified using GATK HaplotypeCaller (v4.5.0). Variant annotation was performed using the Ensembl Variant Effect Predictor (VEP v99) based on Ensembl Release 99, together with publicly available databases including dbSNP(17), ClinVar(18), and gnomAD(19). Variants were filtered based on sequencing quality, read depth, mapping quality, and population allele frequency. Variants with a read depth $\geq 20\times$, mapping quality ≥ 30 , and minor allele frequency (MAF) $< 1\%$ in the gnomAD database were retained for further analysis. Rare variants were prioritized according to their predicted functional consequences, pathogenicity annotations in ClinVar, and biological relevance to genes previously implicated in dystonia and related movement disorders. Only high-confidence variants passing all quality-control criteria were included in downstream functional and pathway analyses(20).

Functional Enrichment Analysis

Functional enrichment analysis was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID, v6.8) to identify significantly enriched Gene Ontology (GO) categories, including Biological Process (BP), Molecular Function (MF), and Cellular Component (CC), as well as Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Enrichment significance was evaluated using a modified Fisher's exact test (EASE score), and Benjamini–Hochberg false discovery rate (FDR) correction was applied to account for multiple testing. Functional terms and pathways with an FDR-adjusted P value < 0.05 were considered statistically significant(19).

Protein–Protein Interaction Network Analysis

Protein–protein interaction (PPI) network analysis was performed using the STRING database (v12.0; <https://string-db.org/>) to investigate the functional interactions among the identified dystonia-associated genes. The analysis was conducted using Homo sapiens as the reference organism, and interactions with a minimum required confidence score of 0.70 (high confidence) were included in the network. The interaction network was subsequently imported into Cytoscape (v3.10.3) for visualization and topological analysis to identify key hub genes and functionally connected molecular networks(21).

Variant Annotation and Visualization

Identified variants were classified according to mutation type, genomic location, and clinical significance (pathogenic, likely pathogenic, or variants of uncertain significance) based on ClinVar annotation. Variant distribution and graphical visualization were generated using R software (version 4.5.1). Variant

pathogenicity was interpreted according to the American College of Medical Genetics and Genomics (ACMG) guidelines(22).

Statistical Analysis

Statistical analyses were performed using R software (version 4.5.1; R Foundation for Statistical Computing, Vienna, Austria). Descriptive statistics were used to summarize demographic and clinical characteristics of the study participants. Continuous variables are presented as mean $\pm$ standard deviation (SD) or median (interquartile range [IQR]), as appropriate, while categorical variables are expressed as frequencies and percentages. Statistical significance for functional enrichment analyses was determined using the modified Fisher's exact test (EASE score) implemented in DAVID (v6.8). For enrichment analyses, Benjamini–Hochberg false discovery rate (FDR) correction was applied to account for multiple comparisons, and an adjusted P value < 0.05 was considered statistically significant.

Results

Variant Distribution Across Genes

Genomic mapping of detected variants revealed their distribution across multiple dystonia-associated genes, including TOR1A, THAP1, SGCE, SPR, SDHA, SCN2A, and ANK2. Variants were located at different positions relative to gene start sites, indicating heterogeneous mutational localization, as shown in Fig. 1.

A pathogenic deletion in TOR1A (c.907_909delGAG) was observed at position 907. THAP1 harbored a likely pathogenic variant (c.305delC) at position 305. SGCE showed a pathogenic splice-site variant (c.933 + 2T > C) at position 933. SPR contained a variant of uncertain significance (c.262C > T) at position 262. SDHA exhibited a pathogenic deletion (c.1945_1946del) at position 1945, representing the most distal mutation among the analyzed genes. SCN2A demonstrated a variant of uncertain significance (c.1208T > C) at position 1208, while ANK2 showed a pathogenic duplication (c.8dup) near the gene start site at position 8. Overall, pathogenic variants were distributed across both proximal and distal gene regions.

Gene–Phenotype Association Patterns

Heatmap analysis demonstrated heterogeneous associations between genes and clinical features. Distinct patterns of presence (high values) and absence (low values) were observed across phenotypic domains including pain, fatigue, depression, anxiety, motor difficulties, and sleep disturbance, as shown in Fig. 2.

THAP1 and SCN2A showed widespread associations across multiple clinical parameters, whereas ANK2 displayed a more restricted pattern with strong signals in selected domains. SDHA and SGCE exhibited intermediate association patterns, while SPR showed selective enrichment in specific features.

These results indicate gene-specific phenotypic profiles and substantial clinical heterogeneity among dystonia patients.

Functional Enrichment Analysis (Gene Ontology)

Significant involvement of genes in metabolic and biosynthetic pathways was identified by biological process enrichment analysis, with prominent processes including regulation of bile acid metabolism, triglyceride metabolism, pantothenate metabolism, coenzyme A biosynthesis, and electron transport chain activity. Enrichment was also observed in the regulation of ketone body metabolism, neuronal action potential, coenzyme A metabolic processes, and nucleoside phosphate biosynthesis, indicating disturbances in energy metabolism and neuronal signaling. In cellular component analysis, significant enrichment was observed in membrane-associated and mitochondrial structures, including the sarcoglycan complex, dystrophin-associated complex, voltage-gated sodium channel complex, dendritic membranes, axonal and dendritic compartments, mitochondrial intermembrane space, mitochondrial matrix, and inner mitochondrial membrane, suggesting the involvement of synaptic structures, ion channel complexes, and mitochondrial compartments. In molecular function analysis, enrichment in ion channel activity and various enzymatic functions was also observed, notably voltage-gated sodium channel activity, flavin adenine dinucleotide binding, phosphotransferase activity, DNA-binding transcription repressor activity, kinase activity, zinc ion binding, anion binding, transition metal ion binding, and protein homodimerization activity, collectively indicating alterations in electrical signaling, transcriptional regulation, and catalytic processes, as shown in Fig. 3.

Protein–Protein Interaction Network

Protein–protein interaction (PPI) analysis revealed a highly interconnected network consisting of 7 nodes and 12 edges, indicating substantial functional connectivity among the analyzed genes. The network included key dystonia-related genes such as GCH1, SPR, THAP1, TOR1A, PANK2, PRRT2, and ANO3, as shown in Fig. 4.

Fig- 4 Protein–protein interaction network- PPI network illustrating functional interactions among dystonia-related proteins; nodes represent proteins and edges represent interactions

Central nodes exhibited multiple interactions, suggesting hub-like roles in the network. GCH1 and THAP1 showed extensive connectivity with other proteins, while TOR1A linked several components associated with neuronal function. The observed number of interactions was significantly greater than expected by chance, with a PPI enrichment p-value < 0.01, indicating that the proteins are biologically connected as a group rather than representing random associations. This network supports coordinated involvement of neurotransmitter synthesis, ion transport, and cellular signaling pathways in dystonia pathophysiology.

Variant Types by Clinical Significance

Analysis of variant types revealed a diverse mutational spectrum across genes. Identified alterations included frameshift mutations (PANK2, SGCE), an in-frame deletion (SDHA), a missense variant (THAP1),

a nonsense variant (SGCE), and a splice-site mutation (SPR).

Most variants were classified as pathogenic, while a smaller proportion were categorized as likely pathogenic or of uncertain significance. Frameshift and truncating mutations predominated among pathogenic variants, indicating potential loss-of-function mechanisms in several genes, as shown in Fig. 5.

Discussion

Dystonia is a complex and heterogeneous movement disorder characterized by sustained or intermittent muscle contractions that arise from dysfunction within distributed motor networks rather than a single localized lesion. Increasing evidence supports the concept that dystonia is a systems-level disorder involving the basal ganglia, cerebellum, thalamus, and cortical motor regions, which function in an integrated manner to regulate coordinated movement(23). The findings of the present study further reinforce this concept by demonstrating that multiple disease-associated genes converge on interconnected molecular pathways, ultimately leading to disruption of neuronal homeostasis and motor circuit integrity(24). The diversity of these molecular mechanisms provides a plausible explanation for the marked clinical heterogeneity observed across patients, including variability in age of onset, disease progression, and phenotypic expression(25). A central pathogenic mechanism involves impaired protein processing and endoplasmic reticulum (ER) homeostasis, particularly mediated by the TOR1A gene(26). TOR1A encodes torsinA, an ATP-dependent chaperone protein localized to the ER and nuclear envelope. TorsinA plays a critical role in protein folding, vesicular trafficking, and maintenance of nuclear envelope integrity(27). Mutations in TOR1A result in defective chaperone activity, leading to accumulation of misfolded proteins, impaired intracellular transport, and altered cellular stress responses(28). These abnormalities are especially detrimental in neurons due to their dependence on efficient protein quality control mechanisms. Disruption of nuclear envelope dynamics may further affect nucleocytoplasmic transport, thereby influencing gene expression and neuronal signaling pathways(29). Transcriptional dysregulation represents another important mechanism contributing to dystonia pathogenesis. The THAP1 gene encodes a DNA-binding transcription factor that regulates genes involved in neuronal differentiation, development, and synaptic function(30). Mutations in THAP1 can lead to altered transcriptional control, resulting in dysregulation of downstream gene expression necessary for proper neuronal connectivity and circuit formation. These alterations may impair synaptic plasticity and communication within motor pathways, thereby contributing to abnormal motor output(31). Membrane stability and synaptic signaling are also significantly affected in dystonia. The SGCE gene encodes epsilon-sarcoglycan, a component of the dystrophin-associated glycoprotein complex that contributes to membrane integrity and signal transduction. Disruption of SGCE function may weaken neuronal membranes, impair synaptic transmission, and alter cytoskeletal interactions(31). Such changes are consistent with abnormalities in sensorimotor integration, a hallmark feature of dystonia.

Neurotransmitter metabolism is another critical pathway involved in disease pathophysiology(32). The SPR gene encodes sepiapterin reductase, an enzyme required for the synthesis of tetrahydrobiopterin (BH4), which serves as an essential cofactor for dopamine production. Reduced availability of BH4 may

result in dopaminergic deficiency within basal ganglia circuits. Given the key role of dopamine in modulating motor activity, such imbalances can significantly disrupt motor control and contribute to dystonic symptoms(33).

Mitochondrial dysfunction represents an additional major contributor to dystonia. The SDHA gene encodes a catalytic subunit of succinate dehydrogenase (complex II), linking the tricarboxylic acid cycle to oxidative phosphorylation. Impaired SDHA function may lead to reduced ATP production and increased generation of reactive oxygen species, resulting in oxidative stress and cellular damage(34). Neurons within motor circuits are particularly vulnerable to such metabolic disturbances due to their high energy requirements and limited antioxidant capacity. Furthermore, the PANK2 gene, which regulates coenzyme A biosynthesis, plays a critical role in cellular metabolism and lipid homeostasis(34). Disruption of this pathway may further exacerbate mitochondrial dysfunction and compromise neuronal maintenance. Altered neuronal excitability is another key factor in dystonia pathogenesis. The SCN2A gene encodes a voltage-gated sodium channel essential for action potential initiation and propagation. Mutations in SCN2A may lead to abnormal channel function, resulting in either neuronal hyperexcitability or impaired signal conduction. Such disturbances disrupt the balance between excitatory and inhibitory signaling within motor circuits, contributing to sustained muscle contractions and abnormal postures(35, 36).

Importantly, these molecular pathways are highly interconnected and do not operate independently (Fig. 6). Dysfunction in one pathway can influence multiple others, leading to a cascade of cellular disturbances. For instance, mitochondrial dysfunction may impair synaptic transmission and alter gene expression, while membrane instability may increase susceptibility to metabolic stress(37). Similarly, transcriptional dysregulation can affect genes involved in protein folding, energy metabolism, and ion channel function. This extensive cross-talk underscores the systems-level nature of dystonia and explains why mutations in distinct genes often result in overlapping clinical phenotypes(38). The concept of dystonia as a network disorder is further supported by the observation that many disease-associated mutations lead to partial or complete loss of protein function, thereby reducing the ability of neurons to maintain homeostasis under physiological stress. This vulnerability may become more pronounced during development, aging, or environmental exposure, contributing to variability in disease onset and progression. Over time, the cumulative impact of these molecular disturbances may destabilize motor networks and give rise to the clinical manifestations of dystonia as shown in Fig. 7.

The present study is primarily based on genetic and pathway-level analyses and lacks extensive experimental validation. The number of genes analyzed is limited, and additional variants contributing to dystonia may not have been captured. Clinical heterogeneity among patients was not fully addressed, and potential gene–environment interactions were not explored. Moreover, the cross-sectional nature of the study limits the ability to assess disease progression and temporal relationships between molecular alterations and clinical outcomes. These factors may limit the generalizability of the findings.

Conclusion

In conclusion, dystonia is a multifactorial disorder resulting from the convergence of multiple molecular dysfunctions that collectively disrupt neuronal homeostasis and motor circuit function. Genes involved in protein processing, transcriptional regulation, membrane stability, neurotransmitter synthesis, mitochondrial metabolism, and ion channel activity contribute to disease pathogenesis through interconnected mechanisms. These findings emphasize that dystonia should be considered a systems-level disorder rather than a condition driven by isolated genetic defects. A comprehensive understanding of these shared molecular pathways provides a strong foundation for the development of targeted therapeutic strategies. Approaches aimed at improving mitochondrial function, restoring neurotransmitter balance, enhancing protein quality control, and stabilizing neuronal excitability may offer broader clinical benefits across diverse genetic forms of dystonia. Continued integration of genetic and bioinformatic analyses will be essential for advancing precision medicine approaches and improving clinical outcomes.

Abbreviations

ANK2

Ankyrin 2

bp

Base pairs

DAVID

Database for Annotation, Visualization and Integrated Discovery

ER

Endoplasmic reticulum

GO

Gene Ontology

LoF

Loss of function

PANK2

Pantothenate kinase 2

PPI

Protein–protein interaction

QC

Quality control

Declarations

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of the Institutional Ethical committee before starting the study No. Dean/2024/EC/7101

Consent to Participate

All research procedures were approved by and in accordance with relevant guidelines and regulations.

Funding

Not available.

Author Contribution

DJ, and RS conceived and designed the project. NRD, AA, And SM, performed all operations. SS analysed the data and SM drew the figures. NDR, SS and AA wrote the manuscript. AA, SM, AK and VKS revised the manuscript. All authors contributed to the article and approved the submitted version.

Acknowledgement

We want to extend our sincere gratitude to Multi-Disciplinary Research Units (MRUs) Laboratory, a grant by ICMR-Department of Health Research and Institute of Eminence, Banaras Hindu University.

Data Availability

The detailed data analyzed during the current study are available with the corresponding author. In the future, it will be made available on reasonable request.

References

1. Roze E, McClelland VM, Lange LM, Jinnah HA, Vidailhet M, Pisani A (2026) Diagnosis and pathogenesis of dystonia: clinical heterogeneity, shared mechanisms, and neurodevelopmental origins. *Lancet Neurol* 25(6):591–601. 10.1016/S1474-4422(26)00098-0 PubMed PMID: 42127934
2. Henschke JU, Pakan JMP (2023) Engaging distributed cortical and cerebellar networks through motor execution, observation, and imagery. *Front Syst Neurosci* 17:1165307. 10.3389/FNSYS.2023.1165307/FULL

3. Poston KL, Stephen CD, The Dystonias (2022) Continuum (Minneap Minn) 28(5):1435
doi:10.1212/CON.0000000000001159 PubMed PMID: 36222773
4. Rissardo JP, McGarry A, Shi Y, Caprara ALF, Walker IM (2025) Neurobiology of Dystonia: Review of Genetics, Animal Models, and Neuroimaging. Brain Sci 15(7):767. 10.3390/BRAINSCI15070767/S1
5. Domingo A, Yadav R, Shah S, Hendriks WT, Erdin S, Gao D et al (2021) Dystonia-specific mutations in THAP1 alter transcription of genes associated with neurodevelopment and myelin. Am J Hum Genet 108(11):2145–2158 .2021.09.017 PubMed PMID: 34672987
6. Thomsen M, Ott F, Loens S, Kilic-Berkmen G, Tan AH, Lim SY et al Genetic Diversity and Expanded Phenotypes in Dystonia: Insights from Large-Scale Exome Sequencing. medRxiv. 2024;2024.12.02.24316741. 10.1101/2024.12.02.24316741 PubMed PMID: 39677454.
7. Dzinovic I, Zech M, Dystonia (2026) Insights into Mechanisms and Novel Therapeutics. Curr Neurol Neurosci Rep 26(1):8. 10.1007/S11910-025-01479-7 PubMed PMID: 41518464
8. Yarıcı M, Cantürk F, Dursun S, Aydın HN, Karabekmez ME (2025) RSEA: A Web Server for Pathway Enrichment Analysis of Metabolic Reaction Sets. Biotechnol Bioeng 122(8):2251–2258 .29020 PubMed PMID: 40345143
9. Tong Z, Yan C, Dong YA, Yao M, Zhang H, Liu L et al (2020) Whole-exome sequencing reveals potential mechanisms of drug resistance to FGFR3-TACC3 targeted therapy and subsequent drug selection: Towards a personalized medicine. BMC Med Genomics 13(1). 10.1186/S12920-020-00794-X PubMed PMID: 32957974
10. Zech M, Dzinovic I, Skorvanek M, Harrer P, Necpal J, Kopajtich R et al (2025) Combined genomics and proteomics unveils elusive variants and vast aetiologic heterogeneity in dystonia. Brain 148(8):2827–2846. 10.1093 /BRAIN/AWAF059 PubMed PMID: 39937650
11. Diaz-Horta O, Bademci G, Tokgoz-Yilmaz S, Guo S, Zafeer F, Sineni CJ et al (2019) Novel variant p.E269K confirms causative role of PLS1 mutations in autosomal dominant hearing loss. Clin Genet 96(6):575–578. 101111/CGE.13626 PubMed PMID: 31432506
12. Babraham Bioinformatics - FastQC A Quality Control tool for High Throughput Sequence Data [Internet]. [cited 2026 Jul 21]. Available from: <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>
13. Bolger AM, Lohse M, Usadel B, Trimmomatic (2014) A flexible trimmer for Illumina sequence data. Bioinformatics 30(15):2114–2120. 10.1093/ BIOINFORMATICS/BTU170 PubMed PMID: 24695404
14. Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25(14):1754–1760. 10.1093/bioinformatics/btp324 PubMed PMID: 19451168
15. Picard Tools - By Broad Institute [Internet]. [cited 2026 Jul 21]. Available from: <https://broadinstitute.github.io/picard/>

16. Getting started with GATK4 – GATK [Internet] [cited 2026 Jul 21]. Available from:
<https://gatk.broadinstitute.org/hc/en-us/articles/360036194592-Getting-started-with-GATK4>
17. Home - SNP - NCBI [Internet]. [cited 2026 Jul 21]. Available from: <https://www.ncbi.nlm.nih.gov/snp/>
18. ClinVar - Home [Internet]. [cited 2026 Jul 21]. Available from: <https://www.ncbi.nlm.nih.gov/clinvar/>
19. Gudmundsson S, Singer-Berk M, Watts NA, Phu W, Goodrich JK, Solomonson M et al (2022) Variant interpretation using population databases: Lessons from gnomAD. *Hum Mutat* 43(8):1012–1030. 101002/HUMU.24309 PubMed PMID: 34859531
20. Collins RL, Glessner JT, Porcu E, Lepamets M, Brandon R, Lauricella C et al (2022) A cross-disorder dosage sensitivity map of the human genome. *Cell* 185(16):3041–3055e25 2022.06.036 PubMed PMID: 35917817
21. Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R et al (2023) The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Res* 51(1 D):D638–D646. 10.1093/NAR/GKAC1000 PubMed PMID: 36370105
22. Practice Guidelines [Internet] [cited 2026 Jul 21]. Available from:
<https://www.acmg.net/ACMG/Medical-Genetics-Practice-Resources/Practice-Guidelines.aspx>
23. Stephen CD, Dy-Hollins M, Gusmao CM, De, Qahtani X, Al SN (2023) Dystonias: Clinical Recognition and the Role of Additional Diagnostic Testing. *Semin Neurol* 43(1):17. 10.1055/S-0043-1764292 PubMed PMID: 36972613
24. Wilson DM, Cookson MR, Van Den Bosch L, Zetterberg H, Holtzman DM, Dewachter I (2023) Hallmarks of neurodegenerative diseases. *Cell* 186(4):693–714 .2022.12.032 PubMed PMID: 36803602
25. Sun YH, Wu YL, Liao BY (2023) Phenotypic heterogeneity in human genetic diseases: ultrasensitivity-mediated threshold effects as a unifying molecular mechanism. *J Biomedical Sci* 2023 30(1):1. 10.1186/S12929-023-00959-7 PubMed PMID: 37525275
26. Fan Y, Si Z, Wang L, Zhang L (2023) DYT-TOR1A dystonia: an update on pathogenesis and treatment. *Front Neurosci* 17:1216929. 10.3389/FNINS.2023.1216929 PubMed PMID: 37638318
27. Shroff K, Caffall ZF, Calakos N (2021) DYT-TOR1A subcellular proteomics reveals selective vulnerability of the nuclear proteome to cell stress. *Neurobiol Dis* 158:105464. 10.1016/J.NBD.2021.105464 PubMed PMID: 34358617
28. Jurek M, Obersztyn E, Milewski M (2018) The Mutation Responsible for Torsion Dystonia Type 1 Shows the Ability To Stimulate Intracellular Aggregation of Mutant Huntingtin. *Dev Period Med* 22(1):33. 10.34763/DEVPERIODMED.20182201.3338 PubMed PMID: 29641419

29. Riaz Z, Richardson GS, Jin H, Zenitsky G, Anantharam V, Kanthasamy A et al (2024) Nuclear pore and nucleocytoplasmic transport impairment in oxidative stress-induced neurodegeneration: relevance to molecular mechanisms in Pathogenesis of Parkinson's and other related neurodegenerative diseases. *Mol Neurodegener* 19(1):87. 10.1186/S13024-024-00774-0 PubMed PMID: 39578912
30. Diaw SH, Ott F, Münchau A, Lohmann K, Busch H (2022) Emerging role of a systems biology approach to elucidate factors of reduced penetrance: transcriptional changes in THAP1-linked dystonia as an example. *Med Gen* 34(2):131. 101515/MEDGEN-2022-2126 PubMed PMID: 38835919
31. Domingo A, Yadav R, Shah S, Hendriks WT, Erdin S, Gao D et al (2021) Dystonia-specific mutations in THAP1 alter transcription of genes associated with neurodevelopment and myelin. *Am J Hum Genet* 108(11):2145. 2021.09.017 PubMed PMID: 34672987
32. Peterson DA, Kim M, Chen R, Eidelberg D, Gallea C, Horn AG et al (2026) Brain network pathophysiology in dystonia. *Dystonia* 4:15446. 10.3389/DYST.2025.15446/FULL
33. Mohamed FE, Alzyoud L, Ghattas MA, Tabouni M, Fienemann A, Trinh J et al (2025) Clinical Features of Families with a Novel Pathogenic Mutation in Sepiapterin Reductase. *Int J Mol Sci* 2025 26(7):3056 doi:10.3390/IJMS26073056 PubMed PMID: 40243727
34. Esteban-Amo MJ, Jiménez-Cuadrado P, Serrano-Lorenzo P, de la Fuente M, Simarro M (2024) Succinate Dehydrogenase and Human Disease: Novel Insights into a Well-Known Enzyme. *Biomedicines* 12(9):2050 doi:10.3390/BIOMEDICINES12092050 PubMed PMID: 39335562
35. Hammer MF (2025) Transgenic mouse models of sodium and potassium channelopathies in epilepsy: insights into disease mechanisms and therapeutics. *Biosci Rep* 45(10):567–595 doi:10.1042/BSR20253356 PubMed PMID: 41151066
36. Indelicato E, Schlieben LD, Stenton SL, Boesch S, Skorvanek M, Necpal J et al (2024) Dystonia and mitochondrial disease: the movement disorder connection revisited in 900 genetically diagnosed patients. *J Neurol* 271(7):4685. 10.1007/S00415-024-12447-5 PubMed PMID: 38775934
37. Khan T, Waseem R, Zehra Z, Aiman A, Bhardwaj P, Ansari J et al (2022) Mitochondrial Dysfunction: Pathophysiology and Mitochondria-Targeted Drug Delivery Approaches. *Pharmaceutics* 14(12):2657. 103390/PHARMACEUTICS14122657 PubMed PMID: 36559149
38. Domingo A, Yadav R, Shah S, Hendriks WT, Erdin S, Gao D et al (2021) Dystonia-specific mutations in THAP1 alter transcription of genes associated with neurodevelopment and myelin. *Am J Hum Genet* 108(11):2145. 2021.09.017 PubMed PMID: 34672987

Figures

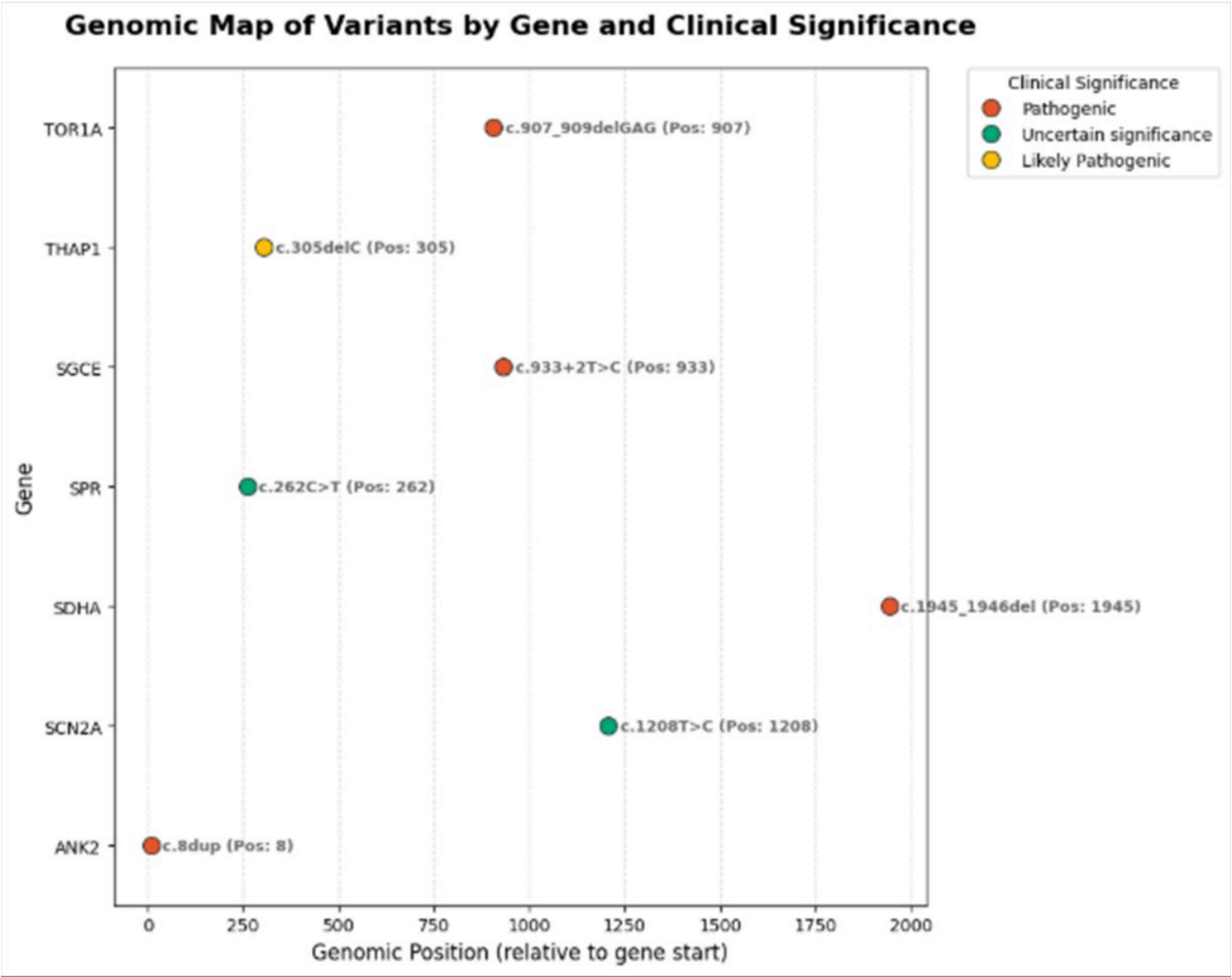


Figure 1

Variant distribution across genes- Distribution of identified variants across dystonia-associated genes according to their positions within the coding sequences.

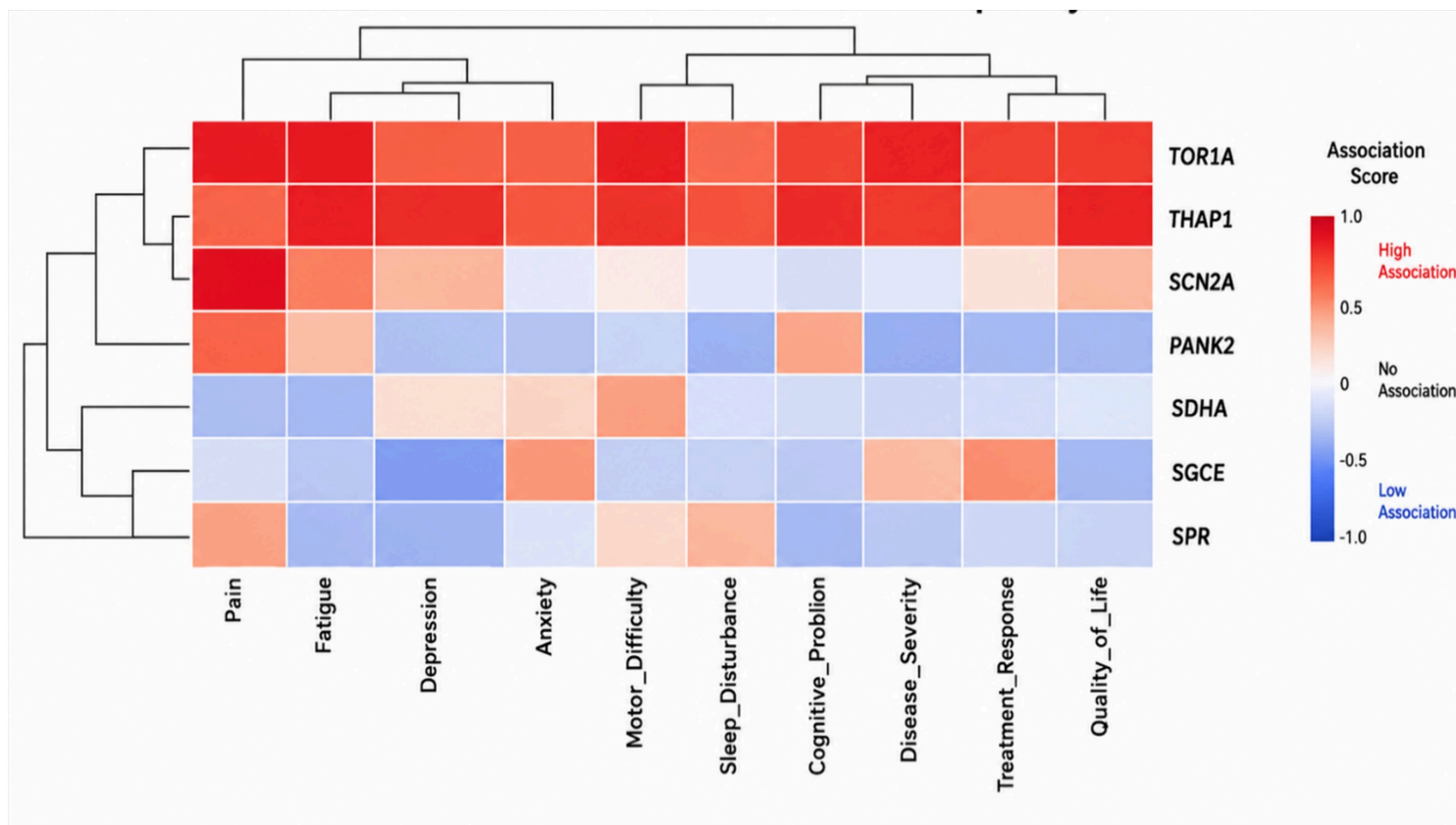


Figure 2

Gene-phenotype heatmap- Heatmap showing associations between dystonia genes and clinical features, with color intensity representing the strength of association.

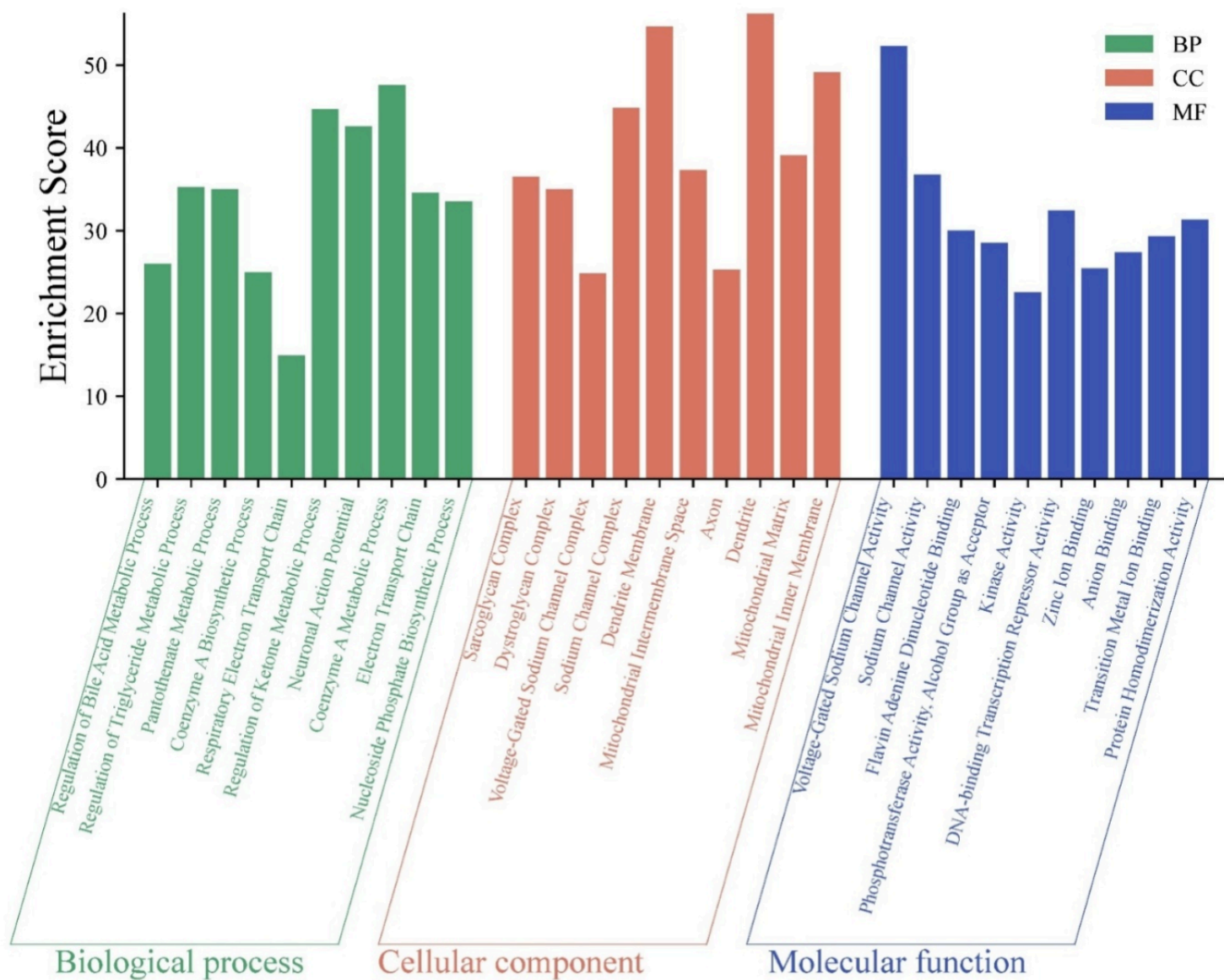


Figure 3

Gene Ontology enrichment analysis- Functional enrichment of dystonia-associated genes highlighting significant Biological Process, Cellular Component, and Molecular Function categories.

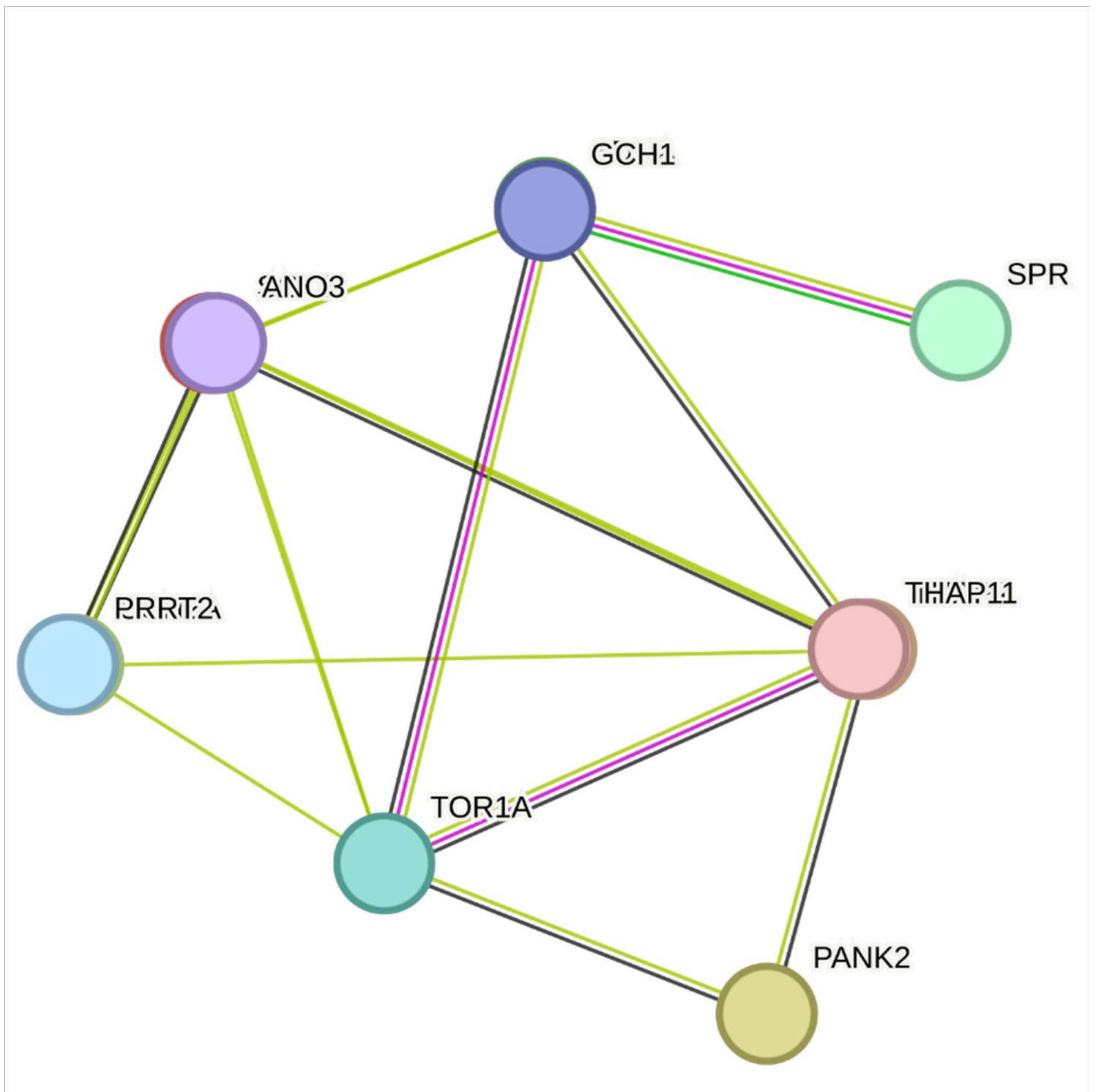


Figure 4

Protein–protein interaction network- PPI network illustrating functional interactions among dystonia-related proteins; nodes represent proteins and edges represent interactions

Central nodes exhibited multiple interactions, suggesting hub-like roles in the network. GCH1 and THAP11 showed extensive connectivity with other proteins, while TOR1A linked several components associated with neuronal function. The observed number of interactions was significantly greater than expected by

chance, with a PPI enrichment p-value < 0.01, indicating that the proteins are biologically connected as a group rather than representing random associations. This network supports coordinated involvement of neurotransmitter synthesis, ion transport, and cellular signaling pathways in dystonia pathophysiology.

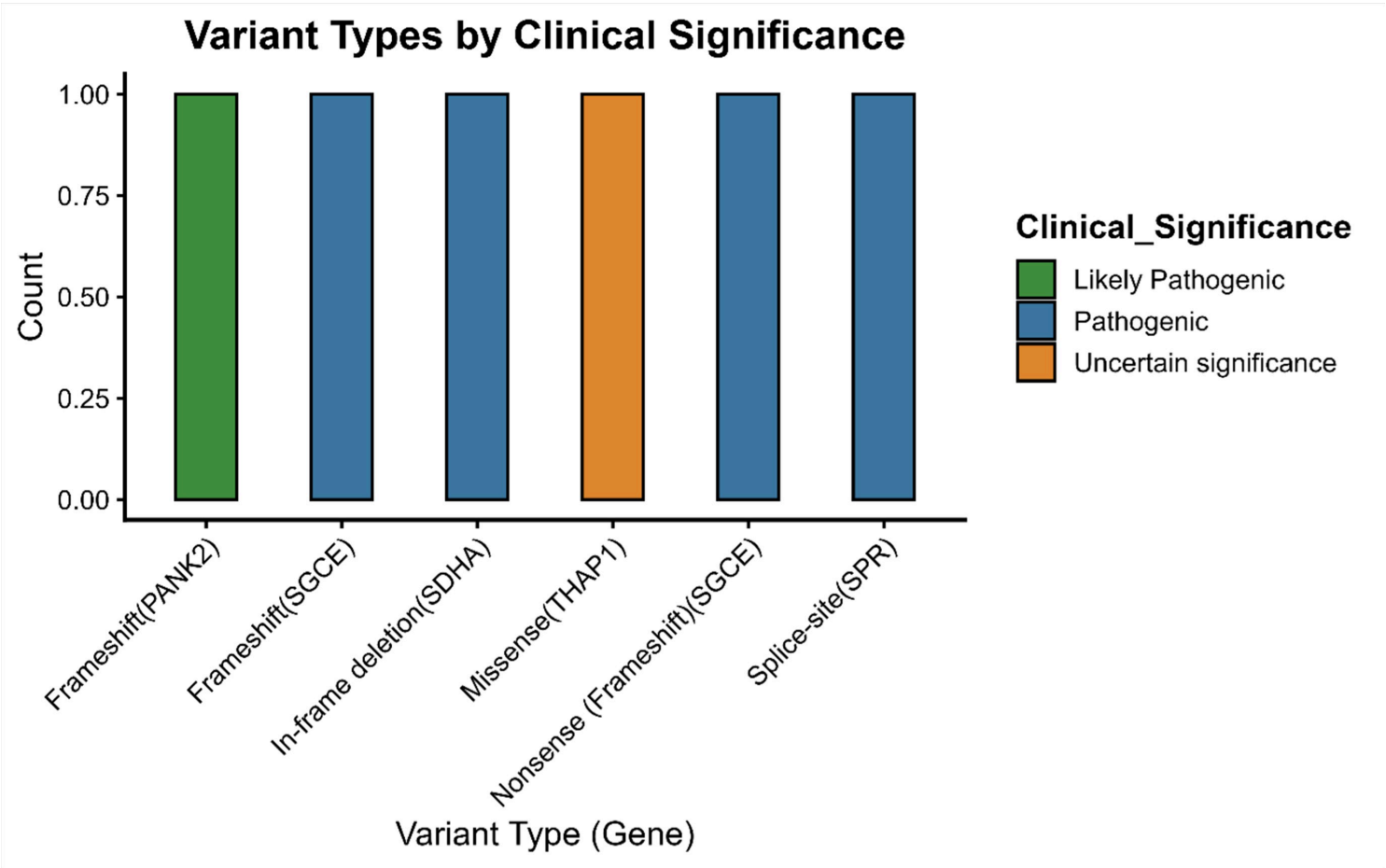


Figure 5

Distribution of variant types according to their clinical significance in dystonia-associated genes.

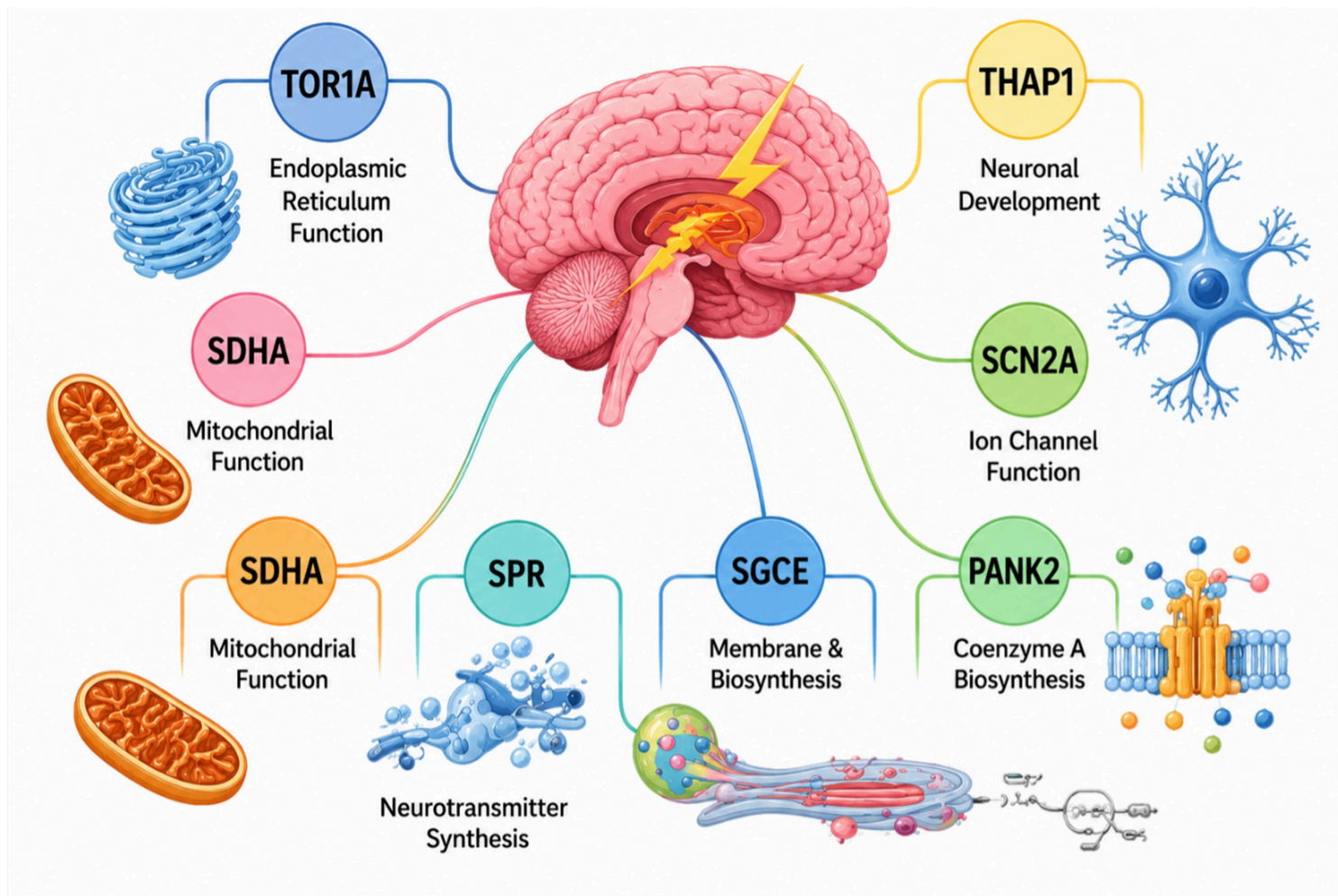


Figure 6

Schematic representation of dystonia-associated genes and their functional roles. Key genes including TOR1A, THAP1, SGCE, SPR, SDHA, SCN2A, and PANK2 are shown to be involved in endoplasmic reticulum function, transcriptional regulation, mitochondrial activity, neurotransmitter synthesis, ion channel function, and metabolic pathways, indicating their collective role in disease pathogenesis.

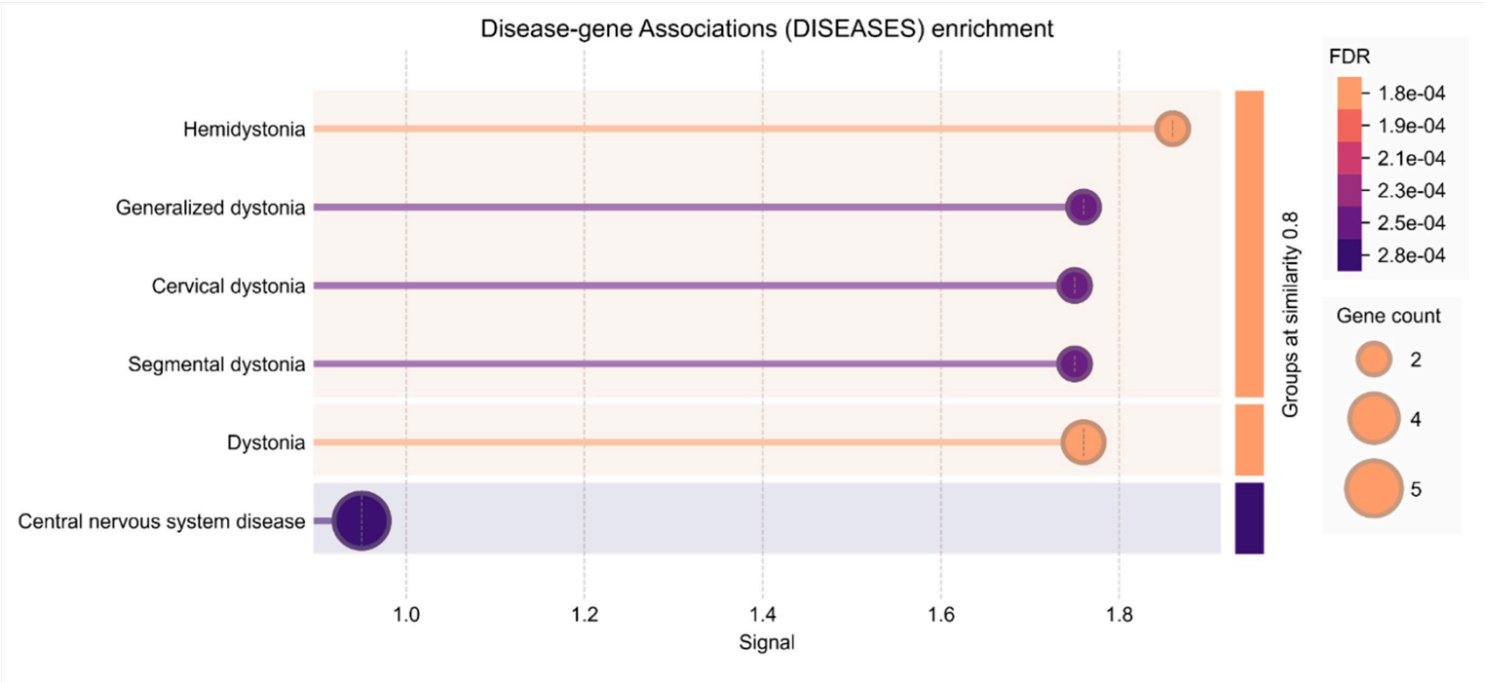


Figure 7

Disease–gene association enrichment showing the relationship between dystonia-associated genes and different clinical subtypes of dystonia.