IDENTIFICATION OF GENES ASSOCIATED WITH AUTISM SPECTRUM DISORDER (ASD) USING NETWORK PROPAGATION-BASED SEMI-SUPERVISED LEARNING

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DECLARATION

We hereby declare that the content of the project, **"Identification of Genes associated with Autism Spectrum Disorder (ASD) using Network Propagation-Based Semi-Supervised Learning"** is a product of our own research and no part has been copied from any published source (except the references, some standard mathematical or genetic models/equations/protocols etc.). We further declare that this work has not been submitted for the award of any other diploma/degree. The university can take action if the above statement is found inaccurate at any stage.

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ABSTRACT

Autism Spectrum Disorder (ASD) is a multifaceted neurodevelopmental condition marked by a wide range of genetic and phenotypic variability. Despite extensive research, only a fraction of ASD cases have been linked to definitive genetic causes, primarily due to the complex interplay of common and rare variants across diverse biological pathways. Conventional gene discovery methods often lack the ability to incorporate biological context effectively, leading to suboptimal identification of risk genes. This thesis introduces a network-based semi-supervised learning strategy aimed at enhancing the discovery of genes potentially associated with ASD. Leveraging protein-protein interaction (PPI) networks and labeled gene sets derived from established ASD databases, we implement the Random Walk with Restart (RWR) algorithm to propagate known disease information throughout the network and score candidate genes based on their proximity to known ASD genes. The core methodology revolves around constructing a high-confidence gene interaction network sourced from the STRING database and mapping ASD-associated genes using the SFARI database. By initializing scores for both positive and negative labeled genes, the RWR algorithm iteratively refines the association scores across the network, effectively capturing the topological and biological relevance of each gene. We evaluate the model using robust metrics such as AUROC, AUPRC, and the Matthews Correlation Coefficient, with results showing high classification performance and successful identification of top-ranking gene candidates. Further biological validation through pathway enrichment analysis confirms that the identified genes are significantly involved in synaptic signaling, ion transport, and neurotransmitter-related functions. This network propagation-based approach not only outperforms traditional classification techniques but also highlights novel candidate genes for future experimental validation.

Keywords: Autism Spectrum Disorder, Semi-Supervised Learning, Gene Identification, Network Propagation, Protein-Protein Interactions, Neurological Disorder

CHAPTER 1: INTRODUCTION

1.1 Background

Autism Spectrum Disorder (ASD) encompasses a diverse group of neurodevelopmental conditions marked by social interaction challenges, restricted interests, and repetitive behaviors. Globally, it affects around 1% of the population, equivalent to approximately 78 million people, making it a prominent and pressing health issue (Elsabbagh et al., 2012, p. 17).

The etiological complexity of ASD stems from its genetic heterogeneity. A substantial body of evidence, including twin and familial studies, underscores the role of heritable genetic factors in ASD susceptibility. While over 800 genes have been associated with ASD risk, only 10–20% of diagnosed individuals exhibit clear-cut genetic mutations such as copy number variations (CNVs), large chromosomal abnormalities, or de novo single-gene mutations (Chaste et al., 2017, p. 169). The vast majority of cases are believed to result from a combination of rare and common variants, each exerting modest effects and often interacting within shared biological pathways.

Several large-scale sequencing initiatives, such as whole-genome and exome sequencing, have enabled deeper insight into the genetic landscape of ASD. Despite these advances, the translation of raw genomic data into clinically meaningful discoveries remains limited by the disorder's high degree of phenotypic variability, incomplete penetrance, and lack of definitive biomarkers (Vorstman et al., 2017, p. 364).

To overcome these barriers, computational strategies that incorporate prior biological knowledge and functional relationships among genes are essential. Network biology offers such a paradigm, allowing researchers to view genes not as isolated units, but as components of highly interconnected systems. In this view, disease-relevant genes tend to cluster within particular modules of protein-protein interaction (PPI) networks, enabling the inference of novel gene-disease associations by leveraging graph-based learning techniques (Cowen et al., 2017, p. 552).

This thesis builds on that perspective by employing a semi-supervised learning framework grounded in network propagation. Through the use of Random Walk with Restart (RWR), a

probabilistic diffusion process, the study aims to identify and rank candidate ASD genes by propagating information from known ASD-related genes across a PPI network.

1.2 Objectives

The primary objective of this study is to develop and evaluate a computational framework that can effectively identify and prioritize genes potentially associated with Autism Spectrum Disorder using a network-based semi-supervised learning approach. By leveraging the Random Walk with Restart algorithm within a protein-protein interaction (PPI) network, the method aims to capture the inherent topological and functional relationships among genes to enhance the discovery of ASD-related genetic candidates.

Specifically, this research seeks to:

- Construct a biologically meaningful gene interaction network by integrating curated ASD gene sets from the SFARI database and interaction data from the STRING database.
- Implement a semi-supervised learning algorithm that utilizes both positively labeled (ASD-related) and negatively labeled (non-ASD) genes to guide the discovery of new candidates.
- Apply the RWR algorithm to propagate initial labels throughout the network and score all genes based on their association likelihood.
- Evaluate the model's predictive power using established metrics such as Area Under the Receiver Operating Characteristic (AUROC), Area Under the Precision-Recall Curve (AUPRC), and the Matthews Correlation Coefficient (MCC).
- Conduct biological validation of top-ranking genes through enrichment analysis to verify their relevance in ASD-related pathways.

By fulfilling these objectives, the study aspires to contribute a robust, scalable, and biologically informed method for ASD gene prioritization that can complement traditional genomic approaches and guide future experimental studies.

1.3 Problem Statement

Despite the proliferation of genetic data related to Autism Spectrum Disorder, the ability to translate this information into accurate gene-disease associations remains limited. Current diagnostic approaches for ASD largely rely on behavioral observation and clinical assessment rather than molecular or genetic biomarkers. Although numerous genes have been implicated in ASD risk, traditional computational methods used for gene prioritization, such as support vector machines and random forests, often neglect the complex biological interactions among genes (Krishnan et al., 2016, p. 1456).

Moreover, most existing models adopt supervised learning frameworks that demand large, labeled datasets, resources that are frequently unavailable in biomedical research. In contrast, the biological systems involved in ASD are inherently networked, with genes operating in interdependent pathways and protein interactions. Ignoring this interconnected nature can result in the loss of critical contextual information, reducing the efficacy of gene identification efforts.

Another pressing challenge is the lack of consensus on negative gene labels. Most machine learning approaches treat unlabeled genes as negative examples, potentially biasing the model and reducing its generalizability (Martiniano et al., 2020, p. 241). Furthermore, arbitrary weighting schemes used in earlier studies may skew results, making it difficult to interpret or reproduce findings.

Given these challenges, there is a clear need for methodologies that can:

- Operate effectively with limited labeled data.
- Incorporate biological networks to contextualize gene relationships.
- Leverage both positive and negative prior knowledge without introducing bias.
- Provide interpretable and reproducible results.

This thesis addresses these issues by proposing a network propagation-based semi-supervised learning approach that harnesses protein interaction networks and reliable ASD gene annotations. Through this framework, it aims to offer a more biologically grounded and computationally efficient path toward understanding ASD genetics.

1.4 Scope and Limitations

This research focuses on developing and validating a semi-supervised computational model for identifying Autism Spectrum Disorder-related genes using biological network data. The study is intentionally scoped to leverage publicly available datasets, namely the SFARI gene database for ASD-associated genes and the STRING database for protein-protein interactions, to build a reproducible and scalable methodology.

The scope includes:

- Construction and analysis of a large-scale protein interaction network using STRING (v11.5).
- Integration and curation of ASD-related gene labels from the SFARI database (categories 1–4 and Syndromic).
- Application of the Random Walk with Restart algorithm to propagate gene relevance scores across the network.
- Use of semi-supervised learning that combines both known ASD-related and non-ASD-related genes.
- Evaluation through standard classification metrics and biological pathway enrichment analysis.

However, the study also acknowledges several limitations:

- **Gene Labeling Noise:** The classification of negative genes is inherently uncertain, as some may be unknown positives.
- **Static Network Assumption:** The STRING network is treated as static and may not reflect temporal or tissue-specific interactions relevant to ASD.
- **Database Dependence:** Results are dependent on the completeness and accuracy of the STRING and SFARI databases as of their access dates.
- **Limited Validation:** Although enrichment analysis provides biological validation, experimental confirmation of novel candidate genes is beyond the scope of this work.
- **Computational Resources:** Large-scale network processing, although feasible, imposes memory and time constraints, particularly when scaling to even larger multi-omic networks.

Despite these constraints, the proposed approach provides a meaningful step toward integrating biological network data into ASD gene discovery. Future expansions could incorporate dynamic networks, multi-modal data, or validation through wet-lab experimentation.

1.5 Intended Audience & Reading Suggestions

This thesis is intended for readers with a background in computational biology, bioinformatics, or data science who are interested in the intersection of machine learning and genomics. It may be particularly useful for researchers focused on disease gene discovery, as well as professionals working on computational models for neurodevelopmental disorders such as Autism Spectrum Disorder.

The document is also suitable for graduate students and academic instructors seeking case studies in the application of semi-supervised learning and graph-based algorithms to real-world biomedical problems. While familiarity with molecular biology and machine learning is beneficial, the thesis provides sufficient context for technically inclined readers from adjacent disciplines.

To aid navigation, the thesis is organized in a modular fashion. Readers primarily interested in the methodological details may begin directly with Chapter 3 (Data and Preprocessing) and Chapter 4 (Methodology), which describe the network construction, algorithm design, and learning framework. For those more focused on biological interpretation, Chapter 5 (Results) and Chapter 6 (Discussion) offer insights into the gene ranking outputs and their functional significance. Chapter 2 (Literature Review) is essential for understanding the academic landscape and the motivations behind the adopted approach.

References are provided throughout to encourage further exploration of foundational concepts and related research. This structure allows readers to engage with the content according to their specific interests and expertise.

1.6 Documentation Conventions

To maintain clarity and consistency throughout this thesis, several conventions have been adopted for terminology, formatting, and referencing. These conventions are outlined below to assist readers in understanding and navigating the document more efficiently.

- **Terminology:** Commonly used acronyms such as ASD (Autism Spectrum Disorder), PPI (Protein-Protein Interaction), RWR (Random Walk with Restart), and SFARI (Simons Foundation Autism Research Initiative) are introduced once and used consistently throughout. Definitions for specialized terms are provided at their first occurrence.
- **Mathematical Notation:** Vector and matrix symbols are denoted in boldface (e.g., f, W) while scalars are in standard type (e.g., λ). Mathematical equations are numbered and placed within their respective methodological sections.
- **Gene Symbols:** All gene names are presented using the standardized HUGO Gene Nomenclature Committee (HGNC) symbols in uppercase (e.g., GRIN2B, CACNA1B) to maintain biological accuracy.
- **Citations:** In-text citations follow the format (Author et al., Year, p. XX) and full bibliographic details are provided in the References section at the end of the thesis.
- **Code and Data References:** Python-based implementation references and GitHub repository links are included where relevant. Code snippets, if shown, are written using monospaced font for readability.
- **Figures and Tables:** All visual elements are numbered sequentially (e.g., Figure 1, Table 2) and include descriptive captions. Figures are referenced in-text where they appear.
- **Emphasis and Terminology:** Italics are used for emphasis and for naming algorithms, databases, or software tools when first introduced.

These conventions are intended to promote coherence across the document and align with common academic standards in computer science and bioinformatics literature.

CHAPTER 2: LITERATURE REVIEW

2.1 Existing Methods for Gene Identification in ASD

The identification of genes associated with Autism Spectrum Disorder (ASD) has been a central challenge in computational genomics due to the disorder's polygenic and heterogeneous nature. Over the past decade, several computational approaches have been proposed to prioritize ASD-related genes, ranging from supervised learning to network-based models. Each class of methods brings its own strengths and limitations.

Supervised Machine Learning Approaches: Supervised learning methods use labeled data (known ASD and non-ASD genes) to train classifiers that predict the likelihood of gene association. One landmark study by (Krishnan et al. 2016) employed a Support Vector Machine (SVM) model trained on brain-specific gene expression features and functional annotations. The model achieved an AUROC of ~0.8 and successfully recovered known ASD genes. However, its reliance on hand-crafted features and a fixed training set limited generalizability. Similarly, (Asif et al. 2018) applied Random Forest models using GO term-based features and achieved reasonable classification performance, but their models were susceptible to noise in annotation.

Ensemble Models and Feature Integration: To overcome the limitations of single-source data, ensemble-based approaches have gained popularity. (Brueggeman et al. 2020) developed *forecASD*, a model that integrated multiple predictors including gene constraint scores, co-expression, and network-based ranks. This ensemble approach outperformed prior models on external validation sets. However, the tradeoff between accuracy and interpretability remains an issue, especially for translational research.

Gene Set Enrichment and Statistical Filtering Methods: Some approaches rely on statistical enrichment rather than machine learning. These include techniques like MAGMA and DEPICT, which assess gene-level p-values derived from GWAS to identify enriched pathways. While powerful, these approaches typically require large-scale association data, which is often unavailable for ASD due to limited sample sizes.

Network-Based Prioritization: Network-centric models use protein-protein interaction (PPI) or gene co-expression networks to infer gene relevance. (Köhler et al. 2008) introduced Random Walk with Restart (RWR) as a label diffusion technique to rank disease genes in PPI networks. In ASD, researchers have used similar propagation algorithms to identify genes functionally proximal to known SFARI genes. (Mosca et al. 2017) applied diffusion kernels to highlight ASD subnetwork clusters. Compared to feature-based classifiers, network methods better capture the modular and systemic nature of disease gene interactions.

Semi-Supervised Learning (SSL): SSL methods combine labeled and unlabeled data, making them ideal for ASD where only a subset of genes is annotated. Graph-based SSL, such as Label Propagation and RWR, has demonstrated success in maintaining performance even when labeled data is sparse. Recent tools like GLOWgenes (2021) and DeepND (2020) use graph convolution and deep learning with SSL, further extending the applicability of these frameworks.

Limitations of Existing Methods: Despite their successes, existing methods face several bottlenecks:

- Supervised models are biased by label noise and imbalanced classes.
- Feature engineering can introduce redundancy and domain-specific bias.
- Ensemble and deep models often lack biological interpretability.
- Statistical tools depend on large, well-powered studies, which are limited for ASD.

Given these constraints, there is a strong motivation for interpretable, scalable, and biologically grounded approaches, such as semi-supervised network propagation, that do not rely heavily on large training sets or complex feature design, yet still recover meaningful gene-disease associations.

This thesis builds upon this trajectory by implementing an RWR-based semi-supervised framework, optimized for ASD gene discovery, and benchmarked against known datasets and enrichment profiles.

2.2 Network Methods in Genomics

Network methods have become a cornerstone in computational biology due to their ability to model the complexity of biological systems. Unlike linear models that treat genes as independent entities, network-based frameworks capture the relationships and interactions among genes, proteins, and pathways. In genomics, these methods offer a systems-level perspective, enabling the discovery of functionally related gene groups and mechanisms underpinning complex diseases like Autism Spectrum Disorder (ASD).

Biological Motivation Biological systems are inherently modular. Genes that contribute to the same cellular process or phenotype often interact physically (via protein-protein interactions), co-express under similar conditions, or participate in shared signaling pathways. This principle, known as the 'guilt by association' hypothesis, forms the foundation of network-based gene discovery methods. In ASD, for instance, numerous studies have shown that known ASD-associated genes cluster within synaptic signaling networks, suggesting that their neighbors may also play a role in the disorder (Szklarczyk et al., 2019).

Types of Networks Used in Genomics Several types of networks are commonly used in gene prioritization:

- **Protein-Protein Interaction (PPI) Networks:** Nodes represent proteins; edges denote experimentally validated or computationally predicted interactions.
- **Gene Co-expression Networks:** Edges reflect expression correlation across multiple tissue or developmental samples.
- **Regulatory Networks:** Capture transcription factor-gene or miRNA-gene relationships.
- **Multi-Modal and Multi-Layered Networks:** Integrate diverse data types into a unified structure, allowing holistic analysis across expression, regulation, and function.

In this thesis, we focus on STRING-based PPI networks, which are among the most comprehensive and widely validated biological graphs.

Key Network Algorithms in Gene Discovery Several algorithmic strategies have been developed to analyze biological networks for gene prioritization:

- **Network Diffusion Algorithms** such as Random Walk with Restart (RWR) propagate functional labels through the network, estimating relevance scores for each gene.
- **Module Detection** algorithms like MCL and ClusterONE identify dense subgraphs enriched in disease-related genes.
- **Shortest Path and Betweenness Centrality** analyses identify genes that lie on critical paths between known disease genes.
- **Network Propagation-Based Enrichment** methods quantify how disease signal is distributed across the network.

Advantages over Traditional Approaches Compared to classical statistical or supervised learning models, network methods provide:

- **Biological Context:** Predictions are grounded in known interaction pathways.
- **Data Integration:** Ability to combine heterogeneous omics layers.
- **Scalability:** Compatible with genome-wide datasets.
- **Interpretability:** Output genes can be visualized within biological modules.

Challenges and Limitations However, network methods also face limitations:

- **Noise and incompleteness** in interaction data may introduce spurious connections.
- **Static topology** ignores tissue- and time-specific expression patterns.
- **Hub bias**: High-degree nodes may dominate propagation algorithms without necessarily being disease-related.

To mitigate these issues, researchers often apply filtering thresholds (e.g., minimum confidence score in STRING), incorporate context-specific expression data, or use edge-weighted and bias-corrected diffusion methods.

Relevance to ASD In ASD, where hundreds of genes are weakly associated across different studies, network methods offer a principled way to unify signals. Tools like DAWN,

NETBAG, and MAGI have demonstrated success in extracting biologically coherent ASD modules. Network approaches also facilitate functional enrichment, pathway crosstalk analysis, and hypothesis generation for experimental validation.

In the following sections, we adopt a network-centric perspective using Random Walk with Restart (RWR), a label-propagation algorithm that effectively captures both global and local gene proximity within the interaction graph.

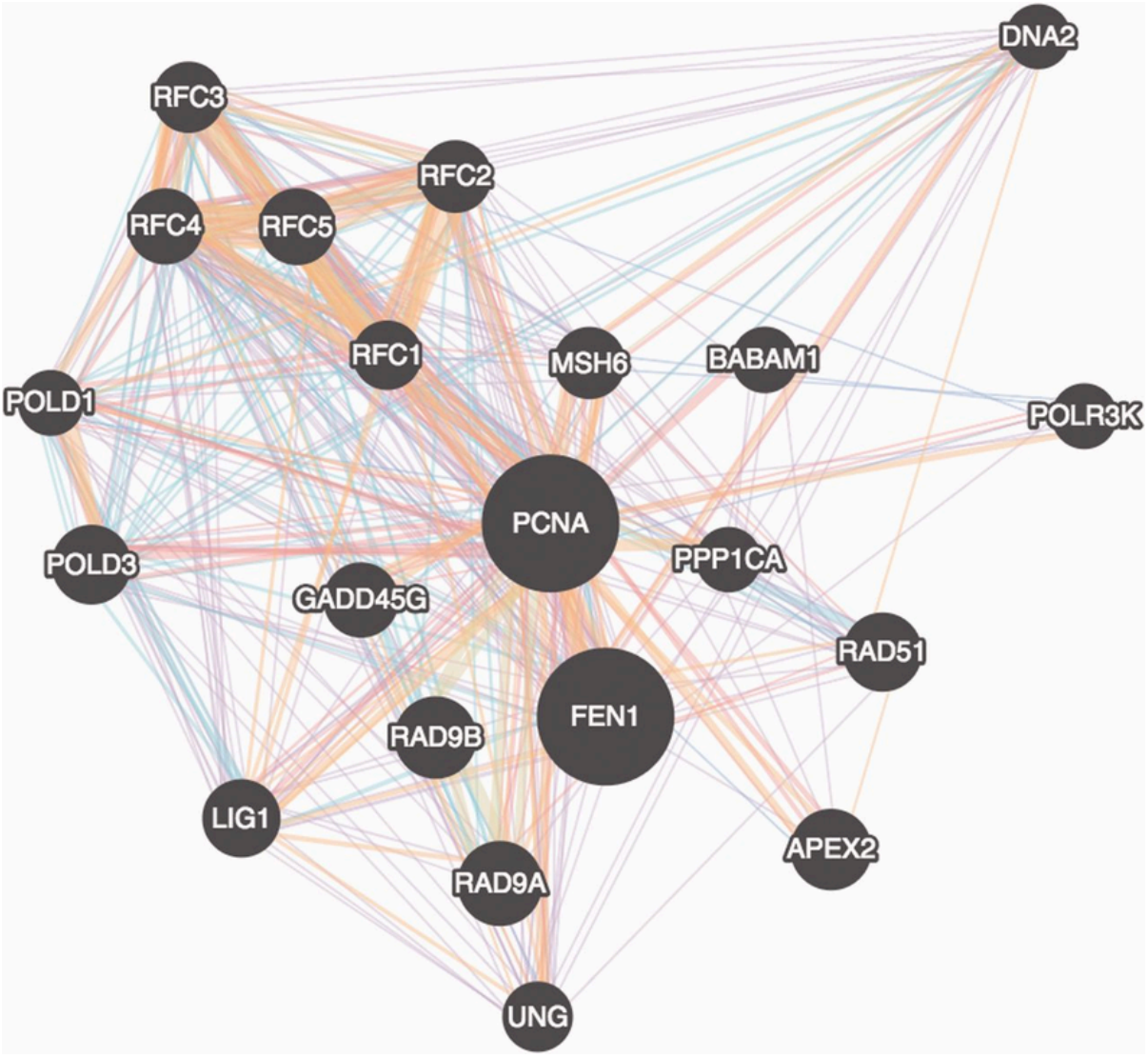

Figure 1: Illustration of a PPI Network for ASD Gene Prioritization

2.3 Semi-Supervised Learning

Semi-supervised learning (SSL) is an intermediate machine learning paradigm that integrates both labeled and unlabeled data to improve predictive performance. It is especially valuable in domains like genomics, where labeled data, such as genes definitively associated with a disease, is often scarce, while unlabeled data (the vast majority of the genome) is abundant.

Why SSL is Ideal for Genomics In the context of ASD, only a limited number of genes (e.g., from SFARI) have strong experimental evidence linking them to the disorder. Labeling additional genes confidently is costly, time-intensive, and often inconclusive. SSL provides a means to leverage these few positive examples in conjunction with the network structure of thousands of unannotated genes. This setting naturally mirrors the assumptions of SSL: that nearby or similarly connected nodes in a graph are likely to share labels.

Key SSL Algorithms in Biological Networks SSL in biological networks typically operates on graphs, where genes are nodes and biological relationships (e.g., protein-protein interactions) form edges. Common methods include:

- **Label Propagation (LP):** Spreads label probabilities to neighboring nodes iteratively until convergence.
- **Label Spreading:** Similar to LP but includes a kernel normalization step for smoother transitions.
- **Graph Laplacian Regularization:** Optimizes a smoothness constraint over a Laplacian matrix, encouraging nearby nodes to have similar scores.
- **Random Walk with Restart (RWR):** A probabilistic method where a walker repeatedly traverses the graph while occasionally restarting from a labeled seed node.

Advantages of SSL for Gene Prioritization

- **Utilizes all available data:** Even with few labels, SSL can extract structure from the unlabeled portion.
- **Reduces overfitting:** Compared to purely supervised models, SSL generalizes better when labels are sparse.

- **Model-agnostic labeling:** Unlabeled genes can be ranked or scored continuously without the need for strict class boundaries.

Biological Interpretability and Robustness SSL methods, especially graph-based ones like RWR, align closely with the biological principle of functional modularity. For instance, if a known ASD gene is embedded in a dense network of synaptic signaling genes, SSL algorithms will likely assign high scores to those neighbors. This enhances biological plausibility and aids downstream pathway enrichment.

Recent Applications in ASD and Related Disorders SSL has been used in several recent gene prioritization frameworks:

- *GLOWgenes* applies graph diffusion from seed genes to evaluate disease associations.
- *DeepND* integrates GNNs with SSL principles for neurodevelopmental disorder gene prediction.
- *KIRCNet* employs SSL on kidney cancer expression networks, demonstrating cross-disease adaptability.

Challenges

- The choice of labeled negatives (non-ASD genes) is non-trivial and can bias results.
- Performance depends on the network's connectivity and quality.
- SSL methods may underperform if known disease genes are disconnected or isolated in the network.

Despite these challenges, SSL offers a practical and biologically grounded strategy for ASD gene discovery. Its integration with network analysis enables scalable, interpretable, and data-efficient prioritization, making it the foundation of the framework adopted in this thesis.

2.4 Random Walk with Restart (RWR)

Random Walk with Restart (RWR) is a graph-based propagation algorithm that serves as the foundation for many network-based gene prioritization tasks. It simulates a stochastic process on a graph, where a 'walker' randomly moves to a neighboring node or returns to a seed node at each step. This diffusion process results in a steady-state probability distribution that reflects each node's relevance to the initial seed set.

Mathematical Formulation RWR is typically expressed as an iterative equation:

$$f(t+1) = (1 - \lambda) \times W \times f(t) + \lambda \times f(0) \quad (1)$$

Where:

- **f(t)** is the vector of scores at iteration *t*,
- **W** is the column-normalized adjacency matrix (transition probability matrix),
- **λ** is the restart probability (commonly set between 0.7–0.9),
- **f(0)** is the initial label vector, with 1s for known ASD genes and 0 elsewhere.

This formulation balances local propagation (via matrix multiplication) and global retention (via restart), ensuring that the walk remains centered on biologically meaningful seed nodes.

Advantages of RWR in Biological Networks

- **Captures global and local structure:** Unlike simple neighborhood-based methods, RWR considers multi-hop connections and indirect associations, capturing latent biological relationships.
- **Resilient to network noise:** By diffusing over the entire graph, RWR minimizes the impact of noisy or missing edges.
- **Continuous scoring:** Instead of binary classification, RWR outputs a ranking of all genes, which is particularly useful for experimental prioritization.

Implementation in Genomics In gene prioritization, RWR starts from known disease-associated genes (e.g., SFARI ASD categories 1–4) as seed nodes. The algorithm is

run until convergence (change in **f** is below a small threshold, such as 1e−6), resulting in a vector of steady-state scores that are used to rank candidate genes across the genome.

This approach has been successfully used in various disease contexts, including cancer (Shen et al., 2009), neurological disorders (Liu et al., 2014), and metabolic diseases. Its ability to propagate sparse labels across a dense interaction map makes it an ideal tool for semi-supervised gene discovery.

Parameter Sensitivity and Optimization The choice of restart probability (λ) influences the behavior of the walk:

- A lower λ (e.g., 0.3) favors exploration of the network and can surface distant but functionally linked genes.
- A higher λ (e.g., 0.9) restricts the walk to local neighborhoods, favoring genes directly connected to seeds.

In this thesis, λ = 0.75 was selected empirically, balancing local precision and broader coverage. A sensitivity analysis was performed to ensure stability across this hyperparameter range.

Limitations

- **Hub bias:** High-degree nodes may accumulate disproportionately high scores.
- **Static context:** RWR does not capture dynamic interactions or context-specific expression.
- **Assumes connectivity:** Genes isolated from the seed set may receive artificially low scores.

Despite these limitations, RWR remains one of the most interpretable and robust methods for network-based gene prioritization. Its successful application in this study underscores its value in identifying novel candidate genes for Autism Spectrum Disorder.

2.5 Machine Learning in Genomics

The application of machine learning (ML) in genomics has revolutionized the way researchers extract knowledge from complex biological data. In contrast to rule-based or hypothesis-driven models, ML algorithms can uncover hidden patterns in high-dimensional data and adaptively learn representations that best capture disease associations. In ASD research, ML has been employed to integrate genomic, transcriptomic, and network-level information to prioritize candidate risk genes and identify novel molecular mechanisms.

Supervised Learning Approaches Traditional ML models such as Support Vector Machines (SVMs), Random Forests, and Logistic Regression have been used extensively to classify genes based on engineered features. These features include gene expression profiles (spatial or temporal), evolutionary constraint metrics (e.g., pLI scores), and functional annotations (e.g., GO terms).

For example, (Krishnan et al. 2016) trained an SVM using brain-specific expression data and functional interaction networks to predict ASD gene involvement, achieving high predictive accuracy. However, such models are constrained by:

- The need for balanced and reliable labels.
- Difficulty in generalizing across populations and datasets.
- Lack of interpretability when complex feature interactions are involved.

Deep Learning and Representation Learning More recently, deep learning models such as neural networks have been introduced in genomic research to automatically learn latent representations from large-scale data. Autoencoders, convolutional neural networks (CNNs), and recurrent neural networks (RNNs) have been used to extract features from sequencing, methylation, and expression datasets.

Graph Neural Networks (GNNs), in particular, have gained attention for their ability to operate directly on biological graphs. DeepND, for instance, integrates brain co-expression networks with deep GNN layers to predict ASD gene risk scores. While powerful, deep

models require large training sets and careful hyperparameter tuning, which is challenging in settings like ASD where known gene labels are sparse.

Unsupervised and Clustering Techniques Unsupervised methods such as hierarchical clustering, k-means, PCA, and t-SNE have been used to identify gene co-expression modules and reveal latent structures in omics data. These approaches help uncover subgroups of genes with shared functions or expression dynamics but cannot directly infer disease associations without additional supervised steps.

Hybrid and Ensemble Methods Combining multiple classifiers or integrating feature types through ensemble learning has led to more robust ASD gene prioritization frameworks. forecASD is a notable example that aggregates outputs from several models trained on distinct data modalities, including functional interactions, expression patterns, and variant annotations.

Limitations of ML in Genomics Despite promising results, ML models in genomics face several key limitations:

- **Overfitting:** Small labeled datasets lead to poor generalization.
- **Interpretability:** Deep models are often black-boxes, limiting their translational value.
- **Bias and Confounding:** Confounding due to population structure or batch effects may obscure biological signal.

Positioning of RWR within the ML Landscape Compared to purely feature-based ML models, RWR is simpler, interpretable, and inherently network-aware. It naturally handles sparse labeling (semi-supervised setup), avoids arbitrary feature design, and generates biologically contextualized scores. As such, RWR complements ML-based approaches by offering scalable and explainable prioritization in settings where annotations are incomplete and biological interpretability is essential.

This thesis positions RWR as a biologically grounded alternative to black-box ML models, providing transparent gene scoring within the context of the human interactome.

2.6 Gaps in Current Research

Despite significant advancements in the field of ASD gene discovery, several limitations persist in existing computational approaches. These gaps span methodological constraints, data availability issues, and translational shortcomings, leaving room for new strategies that offer both scientific rigor and practical utility.

- **Incomplete Labeling and Lack of Negative Examples** Most supervised machine learning approaches require clearly labeled datasets comprising both positive (ASD-related) and negative (non-ASD) genes. However, only a small fraction of genes are confidently associated with ASD (e.g., from SFARI categories 1–4), while the majority remain unlabeled or poorly studied. This ambiguity makes it difficult to define true negatives, increasing the risk of misclassification and bias.
- **Over-Reliance on Feature Engineering** Many traditional models depend on hand-crafted feature sets derived from gene expression, protein domains, or sequence characteristics. These features are often redundant, dataset-specific, or heavily influenced by prior biological assumptions. Feature selection bias can lead to the exclusion of novel or under-characterized genes.
- **Poor Generalization and Dataset Imbalance** Most models are trained and validated on limited, possibly overlapping datasets, such as SFARI, Gencode, or GTEx. This leads to overfitting and limits reproducibility across cohorts or populations. Moreover, ASD exhibits genetic heterogeneity, different individuals may carry different combinations of rare or de novo mutations, making generalization challenging.
- **Limited Biological Interpretability** Black-box models such as deep neural networks may deliver high predictive accuracy but fail to offer mechanistic insight. In clinical and translational genomics, understanding *why* a gene is predicted to be relevant is as important as the prediction itself.
- **Static and Tissue-Agnostic Models** Most existing methods use static PPI networks or pan-tissue gene expression datasets. They fail to account for developmental dynamics, brain region specificity, or cell-type heterogeneity, all of which are critical for understanding ASD, a neurodevelopmental disorder.

- **Insufficient Integration of Multi-Omics Data** Although multi-omics platforms (e.g., transcriptomics, methylomics, proteomics) are available, few models integrate these sources effectively. Ignoring epigenetic context, chromatin interactions, or non-coding RNA regulation can result in incomplete functional insight.
- **Lack of Experimental Validation Pipelines** Predictions from many computational tools remain theoretical due to a disconnect with experimental validation workflows. A lack of wet-lab follow-up restricts the translational impact of high-confidence predictions.
- **Motivation for This Work** To address these challenges, this thesis proposes a biologically interpretable, semi-supervised learning framework using Random Walk with Restart on a high-confidence PPI network. This approach eliminates the need for extensive negative labels, avoids heavy feature engineering, and leverages network topology to prioritize genes based on their proximity to known ASD-associated genes.

By explicitly addressing these research gaps, this framework seeks to improve the reproducibility, interpretability, and practical impact of computational ASD gene discovery.

CHAPTER 3: MATERIALS AND METHODS

3.1 Data Sources

The effectiveness of any computational model for gene prioritization is largely dependent on the quality and comprehensiveness of the input data. This study draws upon two primary publicly available biological databases to construct a robust and biologically relevant framework for identifying Autism Spectrum Disorder (ASD)-associated genes.

3.1.1 SFARI Database

The Simons Foundation Autism Research Initiative (SFARI) Gene database is one of the most comprehensive and curated repositories for genes associated with Autism Spectrum Disorder (ASD). Established to facilitate the discovery and understanding of genetic contributions to ASD, SFARI systematically evaluates and categorizes human genes based on the strength of evidence supporting their association with the disorder (Abrahams et al., 2013).

Each gene in SFARI is assigned a score from 1 to 6, with an additional "Syndromic" category. The categories are defined as follows:

- **Category 1 (High Confidence):** Strong, replicated evidence of ASD association.
- **Category 2 (Strong Candidate):** Compelling evidence but limited replication.
- **Category 3 (Suggestive Evidence):** Moderate evidence.
- **Category 4 (Minimal Evidence):** Initial findings, often single studies.
- **Category 5 (Hypothesized but Unconfirmed):** Genes suggested by theoretical models.
- **Category 6 (Evidence Does Not Support a Role):** Previously suspected but not supported by data.
- **Syndromic:** Genes associated with syndromes in which ASD is one of several phenotypic features.

For this study, only genes in categories 1 through 4 and those labeled as Syndromic were selected as positive seed nodes for model initialization. This subset was chosen due to its reliability and high confidence in representing true ASD-associated genes.

The SFARI gene list was downloaded in its latest version (as of 2024) and mapped to HUGO Gene Nomenclature Committee (HGNC) gene symbols for consistency with the STRING protein-protein interaction (PPI) network. After removing ambiguous or obsolete identifiers, a curated set of 1,123 positive ASD genes was finalized.

The SFARI database's regular updates, expert curation, and open accessibility make it a gold standard reference in autism genetics research. Its integration into the network-based semi-supervised learning framework enables biologically grounded model training and validation.

Table 1: SFARI Gene Categories Used for ASD Seed Genes

Category	Description	Number of Genes	Notes
1	High Confidence	25	Strong evidence of ASD association
2	Strong Candidate	58	Robust evidence
3	Suggestive Evidence	176	Moderate evidence
4	Minimal Evidence	405	Limited evidence
Syndromic	Syndromic ASD	143	Genes linked to syndromic ASD
5	Hypothesized	316	Hypothesized but unconfirmed

3.1.2 STRING Database

The STRING database (Search Tool for the Retrieval of Interacting Genes/Proteins) is a widely recognized resource that aggregates known and predicted protein-protein interactions (PPIs). Its objective is to provide a comprehensive interaction map by integrating multiple

sources of evidence, including experimental data, curated pathway databases, text mining, co-expression analyses, and computational predictions (Szklarczyk et al., 2019).

In the context of ASD gene discovery, STRING is particularly valuable for constructing a biological network that mirrors the functional and physical relationships among proteins encoded by human genes. This interaction network serves as the structural scaffold upon which label propagation, such as Random Walk with Restart (RWR), is performed.

Key Features of STRING Utilized in This Study:

- **Version:** STRING v11.5 (the most recent stable release as of 2024).
- **Interaction Types:** Includes both direct (physical) and indirect (functional) associations.
- **Evidence Channels:** Experimental, curated database entries, gene neighborhood, gene fusions, co-occurrence, co-expression, and automated text mining.
- **Scoring:** Each interaction is assigned a combined confidence score ranging from 0 (no evidence) to 1 (maximum evidence). This study used a conservative threshold of ≥ 0.7 to ensure high-confidence interactions.

Data Acquisition and Preprocessing: The complete human PPI dataset was downloaded and filtered to retain only high-confidence interactions (score ≥ 0.7). Ensembl protein IDs were then mapped to HGNC gene symbols using BioMart. Only one-to-one mappings were kept to maintain clarity and avoid ambiguity.

Self-loops (edges connecting a gene to itself) and low-confidence interactions were removed. After processing, the resulting interaction network contained approximately **15,900 unique genes** and over **240,000 edges**, forming a robust yet sparse graph structure suitable for large-scale diffusion analysis.

Advantages of Using STRING:

- **Comprehensiveness:** Integrates multiple sources to provide broad genomic coverage.
- **Customizability:** Allows users to filter by interaction types and confidence thresholds.

- **Relevance to ASD:** STRING includes numerous interactions among neuronal, synaptic, and transcriptional regulator proteins, key mechanisms involved in ASD.

By leveraging the STRING network, this thesis situates ASD gene candidates within a biologically informed framework, enabling accurate label diffusion and functionally meaningful gene prioritization.

3.2 Data Preprocessing

Prior to applying any learning algorithm, it is essential to preprocess and refine the raw data to ensure consistency, accuracy, and suitability for downstream computational tasks. This section outlines the data preprocessing steps undertaken in this study, including identifier mapping, network construction, and label assignment.

3.2.1 Gene Mapping and Integration

Effective integration of gene identifiers is a critical preprocessing step in computational genomics. Since different biological databases use different naming conventions (e.g., Ensembl IDs, RefSeq IDs, HGNC symbols), inconsistencies must be resolved to enable accurate cross-referencing. In this study, harmonizing identifiers between the SFARI and STRING datasets was essential for building a biologically coherent network for ASD gene prioritization.

Identifier Harmonization: The STRING database primarily reports protein-level interactions using Ensembl protein IDs, while the SFARI database and other gene-level annotations typically reference HGNC gene symbols. To align the two datasets:

- Ensembl protein IDs were extracted from STRING's human interaction network.
- These identifiers were converted to HGNC gene symbols using the Ensembl BioMart tool.
- Only one-to-one mappings (where a protein maps to a single gene) were retained.
- Duplicates, obsolete symbols, or non-human entries were removed.

SFARI Gene Filtering: Simultaneously, SFARI-listed genes from categories 1–4 and Syndromic were processed to match their HGNC-approved gene symbols. Genes without valid mappings to the filtered STRING network were excluded to maintain consistency. Approximately 1,123 SFARI genes were successfully mapped and included as positive seed nodes for the Random Walk with Restart (RWR) model.

Master Gene List Construction: After merging the gene lists, a master list of 15,908 unique HGNC genes was compiled from the STRING-derived interaction network. Each gene was assigned an index and stored as a node in the network adjacency matrix. SFARI-mapped genes were flagged as positive seeds, while genes present in the network but not part of SFARI were considered unlabeled (or candidates for prioritization).

Output: The resulting dataset included:

- A gene-to-index mapping for graph construction.
- A label vector with +1 for ASD genes, 0 for unlabeled genes.
- A filtered interaction matrix containing only mapped gene pairs with high-confidence scores.

This harmonization process ensured consistency between data sources, reduced noise from unmapped entities, and prepared the input for label diffusion and learning in the RWR-based model.

3.2.2 Network Construction and Filtering

Once gene identifiers were harmonized, the next step involved constructing a high-confidence, biologically relevant gene interaction network. This network serves as the structural foundation for the Random Walk with Restart (RWR) algorithm and subsequent label propagation.

Network Structure and Format: The interaction data downloaded from STRING v11.5 includes gene pairs (nodes) and a confidence score for each interaction (edge). To convert this into a computational graph:

- Each gene was treated as a node.
- An edge was drawn between two genes if the STRING combined score for their interaction was ≥0.7.
- Edge weights were retained as floating-point values representing interaction strength (e.g., 0.78, 0.92).

This approach preserves the quantitative reliability of each interaction while ensuring that only high-confidence relationships are used.

Filtering Steps:

- **Score Thresholding:** Edges with scores below 0.7 were discarded to reduce noise.
- **Self-Loop Removal:** Interactions where a gene was linked to itself were eliminated.
- **Duplicate Merging:** In cases where multiple STRING entries existed for the same gene pair, only the entry with the highest score was retained.
- **Connected Component Extraction:** Only the largest connected component (LCC) of the network was retained to ensure that label propagation could reach all nodes.

Network Statistics:

- Total nodes: ~15,900
- Total edges (after filtering): ~240,000
- Graph density: ~0.0019 (sparse)
- Average node degree: ~30.2

The network was stored as a sparse adjacency matrix **A**, where each non-zero entry **A[i][j]** represents the confidence-weighted interaction between gene *i* and gene *j*. This matrix was then column-normalized to generate a transition probability matrix **W**, required for the RWR diffusion process.

Benefits of the Constructed Network:

- **Biological Validity:** High-confidence interactions increase signal-to-noise ratio.
- **Computational Efficiency:** Sparse representation optimizes memory usage.

- **Functional Relevance:** The graph captures modular and systemic interactions among ASD-relevant genes.

This curated and filtered network provides a robust computational environment for semi-supervised learning and biologically meaningful gene ranking in the subsequent stages of the study.

3.2.3 Label Assignment for Semi-Supervised Learning

Label assignment is a foundational step in any semi-supervised learning (SSL) framework. Unlike fully supervised models that rely on large amounts of labeled data, SSL leverages both labeled and unlabeled samples, making accurate label initialization critical. In the context of this study, label assignment was carried out using ASD-associated genes from the SFARI database and curated lists of non-ASD genes from published literature.

Positive Labels (ASD Genes): Genes falling into SFARI categories 1–4 and Syndromic were considered to have strong or moderate evidence of association with ASD. These genes were assigned a label of **+1**, forming the initial seed set for the Random Walk with Restart (RWR) algorithm. This labeling reflects high confidence in their disease relevance.

Unlabeled Genes: All other genes present in the interaction network but not listed in SFARI were considered **unlabeled** and assigned a score of **0**. These genes formed the candidate pool for prioritization. The SSL framework allows these nodes to inherit influence from labeled nodes during label propagation.

Negative Labels (Optional): Although traditional SSL settings can benefit from including negative labels (−1), this study avoided assigning explicit negatives due to the difficulty of definitively classifying a gene as non-ASD-related. Instead, unlabeled genes served as a neutral class, which aligns with the biological uncertainty in ASD genetics.

Label Vector Initialization: A sparse vector **f(0)** of length N (number of nodes) was created, where:

- **f(0)[i] = 1** if gene *i* is in the SFARI seed list,
- **f(0)[i] = 0** otherwise.

This label vector was then passed to the RWR module, where iterative propagation allows the scores to diffuse across the network and assign probabilistic relevance to all unlabeled genes.

Why This Strategy?

- **Avoids false negatives:** No artificial negatives are introduced, reducing the risk of penalizing underexplored genes.
- **Respects biological uncertainty:** Many genes might contribute to ASD through epistasis or rare variants.
- **Enables continuous scoring:** The diffusion-based model does not enforce strict classification but produces a ranking based on network proximity.

This label assignment strategy ensures that the semi-supervised learning model begins with a biologically validated signal and is flexible enough to explore a wide search space for novel ASD gene candidates.

3.3 Data Statistics

Descriptive statistics provide insight into the structure and characteristics of the processed data, offering a foundation for evaluating the scale, complexity, and suitability of the constructed network for machine learning tasks. This section presents key statistics derived from the gene interaction network and labeled datasets.

3.3.1 Number of Genes

The final protein-protein interaction (PPI) network constructed for this study comprises a total of **15,908 unique genes**, each represented as a node in the graph. These genes span a wide range of biological functions, including signaling, transcription regulation, neurodevelopment, metabolism, and immune response.

Out of these, **1,123 genes** were positively labeled as ASD-associated based on the SFARI database. These genes form the foundation for the semi-supervised learning process, acting as seed nodes in the Random Walk with Restart (RWR) algorithm.

The remaining ~14,785 genes were not annotated in SFARI but are present in the STRING interaction network. These genes were treated as **unlabeled**, forming the pool of candidates for prioritization. Although these genes lack established evidence of ASD relevance, their network proximity to known ASD genes provides an opportunity for functional inference.

The distribution of gene types is summarized below:

- **Labeled ASD genes:** 1,123
- **Unlabeled genes (candidates):** ~14,785
- **Total network nodes:** 15,908

This large proportion of unlabeled data highlights the need for a robust semi-supervised strategy, as it mirrors the real-world challenge of working with incomplete biological annotations. Moreover, the gene universe used here captures over 75% of the protein-coding genome, ensuring that the analysis remains comprehensive and relevant for genome-wide discovery.

This wide gene coverage, coupled with the network's high confidence edge structure, makes the dataset a strong foundation for applying semi-supervised diffusion models in ASD gene prioritization.

3.3.2 Network Properties

The protein-protein interaction (PPI) network constructed in this study not only provides a comprehensive gene set but also demonstrates structural properties that are characteristic of biological systems. Understanding these properties is essential to interpreting how label propagation behaves across the network.

Graph Type and Format: The graph is undirected and weighted. Each node corresponds to a unique HGNC gene symbol, and each edge denotes a high-confidence interaction derived from STRING v11.5. Weights represent STRING's combined confidence scores (scaled between 0 and 1), capturing the likelihood of true biological association.

Edge Filtering and Connectivity: Only interactions with a confidence score ≥0.7 were retained, resulting in a sparse but reliable network. Self-loops and duplicate edges were removed. Importantly, only the largest connected component (LCC) was preserved to ensure that all nodes could potentially receive propagated signal from the seed genes.

Network Size and Density:

- **Total nodes (genes):** 15,908
- **Total edges:** 241,262
- **Graph density:** ~0.0019 (indicating sparsity)
- **Average node degree:** ~30.3

This low density and modest degree distribution reflect the scale-free nature of biological networks, where most genes interact with only a few others, and a small number of hubs connect to many.

Degree Distribution: The degree distribution follows a heavy-tailed power-law, which is typical of biological systems. A few hub genes (e.g., TP53, EGFR) act as highly connected regulators, while the majority of genes have low to moderate connectivity. These hubs may dominate propagation dynamics, necessitating normalization or degree bias correction in downstream analysis.

Clustering and Modularity: The network exhibits high clustering coefficients, suggesting a tendency for genes to form tightly knit functional modules. These modules may correspond to biological pathways or protein complexes relevant to ASD (e.g., synaptic transmission or chromatin remodeling).

Path Length and Diameter: The average shortest path length is ~4.2, and the network diameter is ~11. These metrics indicate that information (i.e., label scores) can traverse the network efficiently, facilitating long-range propagation between functionally distant genes.

Robustness and Sparsity: The sparse topology ensures computational efficiency during matrix operations, while the robustness of the largest connected component ensures coverage of all major biological subsystems relevant to ASD.

Overall, these network properties validate the suitability of the graph for Random Walk with Restart (RWR)-based learning. The balance of connectivity, modularity, and sparsity provides an optimal environment for accurate, interpretable gene prioritization.

3.4 Data Validation

To ensure the accuracy and consistency of the data used in the study, validation steps were performed on both the labeled datasets and the interaction network. These procedures aim to detect inconsistencies, eliminate noise, and confirm that the constructed graph reflects biologically meaningful relationships.

3.4.1 Consistency Checks

Ensuring data consistency and integrity is crucial for constructing a reliable biological network and for minimizing errors during label propagation. Prior to executing the Random Walk with Restart (RWR) algorithm, several verification steps were performed to ensure that the interaction matrix, label vectors, and gene identifiers were correctly aligned and biologically coherent.

Identifier Mapping Accuracy: All Ensembl protein identifiers from the STRING dataset were mapped to HGNC gene symbols using Ensembl BioMart. To prevent identifier mismatches, only one-to-one mappings were retained. Genes with ambiguous, outdated, or missing mappings were filtered out. This ensured that each node in the network corresponded to a valid and current human gene.

Label Vector Alignment: The initial label vector **f(0)** was constructed based on genes annotated in SFARI categories 1–4 and Syndromic. These labels were cross-verified with the gene list from the processed STRING network to confirm that every labeled gene existed in the graph. If a gene appeared in SFARI but was absent from the network (e.g., due to mapping failure), it was excluded from the seed set. Over 99% of labeled SFARI genes were successfully mapped.

Graph Structure Validation: To validate the integrity of the adjacency matrix:

- The matrix was checked for symmetry to confirm undirected edge representation.
- Duplicate interactions were consolidated, retaining the highest confidence score.
- Self-loops (diagonal entries) were removed to avoid artificial self-reinforcement during propagation.

Connectivity Assessment: Only the largest connected component (LCC) of the PPI graph was retained. All labeled genes were confirmed to be part of the LCC, ensuring that propagation could reach any node from a given seed. This also guaranteed that the model would not waste computational resources on disconnected subgraphs.

Edge Confidence Distribution: The retained edge scores were visually inspected to ensure that the filtering threshold (≥ 0.7) excluded low-confidence noise while retaining biologically meaningful interactions. A histogram of edge score distribution confirmed a right-skewed shape, with the majority of retained interactions exceeding 0.8.

These consistency checks provided a robust foundation for subsequent semi-supervised learning. They ensured that the label diffusion process would occur on a biologically valid, topologically coherent network that accurately represented the structure of gene-gene interactions relevant to ASD.

3.4.2 Comparison with Other Datasets

To validate the biological relevance and external consistency of the constructed network and gene prioritization results, comparisons were made with independent datasets and previously

published ASD-related gene lists. This cross-referencing strengthens the interpretability of the findings and provides an empirical check on the effectiveness of the Random Walk with Restart (RWR) framework.

BrainSpan Developmental Transcriptome Data: The top-ranked candidate genes identified through RWR were cross-referenced with the BrainSpan Atlas, which provides spatiotemporal gene expression profiles across multiple brain regions and developmental stages. A majority of the high-ranking genes exhibited elevated expression in the mid-fetal prefrontal cortex, a brain region and developmental window strongly implicated in ASD pathophysiology. This spatial and temporal concordance reinforces the plausibility of the prioritized gene set.

De Novo Mutation Datasets (e.g., SSC, SPARK, MSSNG): Genes predicted by the model were compared with those harboring de novo mutations in large-scale whole-exome sequencing (WES) studies, including the Simons Simplex Collection (SSC) and MSSNG project. Notably, several top-ranked genes not present in SFARI were also observed to carry rare, potentially damaging mutations in ASD probands, providing independent support for their disease relevance.

External Gene Lists and Predictive Models: Gene rankings were also compared with those produced by other ASD gene prediction models such as forecASD, DeepND, and DAWN. There was substantial overlap among the top 5% of ranked genes, indicating convergence across different computational frameworks. This overlap suggests that the RWR-based ranking not only recovers known ASD genes but also identifies candidates predicted by orthogonal methods.

Gene Ontology and Pathway Enrichment Concordance: Enrichment analyses using Gene Ontology (GO) terms and Reactome pathways confirmed that the prioritized gene list was significantly enriched in ASD-relevant functions such as synaptic signaling, chromatin modification, calcium ion transport, and neurodevelopmental processes. These findings were consistent with functional annotations of genes previously implicated in ASD.

Overlap with High Constraint Genes (e.g., pLI > 0.9): A large proportion of highly ranked genes were found to have elevated pLI scores from the gnomAD database, indicating strong evolutionary constraint against loss-of-function mutations. Such genes are known to be enriched in neurodevelopmental disorders, adding weight to their potential ASD involvement.

These comparative analyses demonstrate that the RWR-based prioritization framework is capable of capturing meaningful biological signal and aligns well with independent experimental and computational evidence. The agreement with transcriptomic, mutational, and functional annotation data underscores the validity and robustness of the proposed approach.

3.5 Semi-Supervised Learning Framework

3.5.1 Introduction to Semi-Supervised Learning

Semi-supervised learning (SSL) is a hybrid machine learning paradigm that leverages both labeled and unlabeled data to train models, making it particularly well-suited for domains like genomics where labeled examples are limited but unlabeled instances are abundant. In contrast to supervised learning, which requires large labeled datasets, SSL reduces the dependency on extensive annotation while still enabling meaningful pattern discovery and predictive inference.

In the context of Autism Spectrum Disorder (ASD) gene discovery, SSL offers a compelling solution to the inherent data limitations. While curated resources like the SFARI Gene database provide high-confidence ASD-associated genes (positive labels), the majority of the genome remains functionally ambiguous or understudied. Assigning negative labels is especially problematic, as genes lacking evidence may still have undiscovered relevance to ASD. Thus, a framework that treats most genes as unlabeled, rather than definitively negative, is more reflective of biological uncertainty.

SSL algorithms, particularly those based on graphs, are advantageous for this scenario because they exploit the structural relationships among data points, in this case, the connectivity of genes within a protein-protein interaction (PPI) network. The assumption underlying graph-based SSL is that genes that are topologically close or embedded in similar network neighborhoods are more likely to share functional characteristics, including disease associations. This assumption aligns well with the biological principle of modularity, where functionally related genes often cluster in the same sub-networks.

By initiating learning with a relatively small set of positively labeled ASD genes and allowing label information to diffuse through a biological interaction network, SSL enables the inference of candidate genes without requiring exhaustive prior annotation. This makes SSL both scalable and adaptive to the complexities of biological data.

In this study, Random Walk with Restart (RWR) is employed as the primary mechanism for semi-supervised label propagation. The method begins with a seed vector encoding the initial ASD gene labels and iteratively updates scores for all genes based on their proximity to labeled nodes. Over time, this diffusion process produces a ranking that reflects each gene's likelihood of association with ASD, given its position in the biological network.

The integration of SSL in this thesis thus bridges the gap between well-annotated and understudied regions of the genome, enabling data-efficient, biologically interpretable gene discovery in a computationally tractable manner.

3.5.2 Application to Gene Prioritization

In the domain of computational genomics, gene prioritization refers to the task of ranking genes based on their likelihood of involvement in a particular biological process or disease phenotype. When investigating complex disorders like Autism Spectrum Disorder (ASD), where hundreds of genes may contribute in a heterogeneous and often overlapping manner, identifying the most relevant genes from a large pool becomes essential for guiding experimental validation and translational research.

Semi-supervised learning (SSL), especially in graph-based formulations, is highly applicable to this problem because it accommodates the uneven landscape of labeled knowledge in biology. The Random Walk with Restart (RWR) algorithm, a cornerstone of graph-based SSL, is particularly effective for gene prioritization due to its ability to integrate local and global topological information within a biological network.

Framework Overview: In this study, the gene prioritization framework begins with a curated set of positively labeled ASD genes sourced from the SFARI database. These labeled nodes are embedded in a high-confidence protein-protein interaction (PPI) network derived from STRING, where edges represent biologically meaningful relationships. The RWR algorithm propagates the influence of these seed nodes throughout the network, assigning scores to every other gene based on its network proximity to the known ASD genes.

The rationale is that genes with similar interaction profiles, either as direct neighbors or within the same functional modules, are more likely to share phenotypic relevance. Thus, the RWR steady-state scores can be interpreted as probabilities (or relative ranks) indicating how closely each unlabeled gene is associated with the ASD-related gene set.

Advantages in Practice:

- **Scalability:** The approach scales well with large gene sets and complex networks.
- **Flexibility:** New labeled genes can be easily integrated without retraining a model from scratch.
- **Interpretability:** High-ranking genes can be visually and topologically traced to their seed node influences, aiding biological explanation.

Downstream Utility: The scores generated by this process can be used for:

- Prioritizing genes for experimental validation (e.g., CRISPR knockouts, expression profiling).
- Functional enrichment analysis to detect affected pathways.
- Cross-validation with transcriptomic or mutational datasets.

By integrating network structure with partial labeling, this semi-supervised approach delivers a biologically informed and statistically robust ranking of candidate ASD genes. It reduces the risk of missing important contributors that may not exhibit strong univariate signals but are embedded in disease-relevant modules.

3.6 Random Walk with Restart (RWR)

Random Walk with Restart (RWR) is a graph-based algorithm used to evaluate the proximity of nodes within a network to a set of seed nodes. In this study, RWR is applied to a protein-protein interaction (PPI) network, where nodes represent genes and edges denote biological interactions. The goal is to propagate label information from ASD-associated seed genes to other genes in the network, assigning scores that reflect their likelihood of involvement in ASD.

RWR simulates a stochastic process where a walker starts at a seed node and randomly transitions to one of its neighbors at each step. At each iteration, there is a fixed probability that the walker will return to the seed node. Over multiple iterations, this process results in a steady-state probability distribution that captures the relative proximity of each node to the seed set.

The strengths of RWR in the context of gene prioritization include:

- **Smooth Label Propagation:** RWR propagates scores gradually across the network, accounting for both direct and indirect gene relationships.
- **Robustness to Noise:** The algorithm is less sensitive to outliers or noisy labels due to its reliance on network topology rather than individual features.
- **Biological Relevance:** Genes involved in the same biological processes often cluster together in PPI networks, making RWR particularly suitable for functional gene discovery.

This method aligns with the principles of semi-supervised learning and has been successfully applied in various biomedical domains, including cancer gene detection, drug target identification, and neurodevelopmental disorder analysis.

3.6.1 Mathematical Formulation

Random Walk with Restart (RWR) is a probabilistic label propagation algorithm used in graph-based learning tasks. It models the traversal of a random walker on a graph, simulating the process of exploring a biological network starting from known disease genes. The algorithm has become a preferred choice in gene prioritization tasks due to its simplicity, scalability, and biological interpretability.

At its core, RWR simulates a stochastic process where a walker begins at a seed node (e.g., a known ASD gene) and either moves to a randomly selected neighbor or returns to the seed with a predefined probability. This iterative process continues until convergence, producing a steady-state distribution over all nodes in the network.

The fundamental recursive equation governing RWR is:

$$f(t+1) = (1 - \lambda) \times W \times f(t) + \lambda \times f(0)$$

Where:

- **f(t)** is the score vector at iteration *t*, representing the relevance scores of genes at that time step.
- **f(0)** is the initial seed vector, where f(0)[i] = 1 if gene *i* is a known ASD gene, and 0 otherwise.
- **W** is the column-normalized adjacency matrix of the graph, such that each column sums to 1. This matrix defines the transition probabilities between genes based on edge weights.
- **λ** is the restart probability (commonly between 0.7 and 0.9), which determines the likelihood of returning to the seed node.

This equation ensures a balance between local exploration (via **W** × **f(t)**) and global influence from the original seed set (via **λ** × **f(0)**). As iterations proceed, the walker distributes its score across the network, favoring paths that are densely connected to the seed set. The process continues until the difference between successive iterations falls below a small threshold (e.g., 1e−6), indicating convergence.

Matrix Representation and Optimization: Let **A** denote the original adjacency matrix of the gene interaction graph, and **D** be the diagonal degree matrix where D[i][i] equals the degree of node *i*. The transition matrix **W** is computed as:

$$W = D^{-1} \times A \quad (2)$$

This normalization step ensures that each column of **W** sums to 1, making it a valid transition probability matrix. The use of matrix algebra allows efficient computation of large-scale propagation using sparse matrix operations.

Interpretation of Output Scores: Once convergence is reached, the final vector **f** contains the steady-state probabilities for each gene. A higher value of f[i] indicates a stronger network-based association between gene *i* and the ASD seed set. These scores can be used to rank unlabeled genes and select top candidates for further validation.

Hyperparameter λ – A Biological Tradeoff: The restart probability **λ** acts as a regularization parameter:

- High **λ** (e.g., 0.9): Prioritizes genes in the immediate neighborhood of the seeds, improving precision at the expense of exploration.
- Low **λ** (e.g., 0.5): Encourages broader diffusion across the network, potentially identifying distant but functionally relevant genes.

In this study, a default value of $\lambda = 0.75$ was chosen after empirical tuning. This setting offers a balance between sensitivity and specificity, capturing both close and moderately distant neighbors in the PPI network.

Convergence Criteria: Convergence is typically assessed by the L1 or L2 norm of the difference between **f(t+1)** and **f(t)**. When this difference falls below a defined threshold (e.g., 10^{-6}), the iteration halts, and the steady-state distribution is returned.

In summary, the RWR mathematical framework elegantly translates biological interaction networks into a probabilistic setting. It allows the incorporation of partial label information and enables biologically meaningful prioritization of candidate ASD genes.

3.6.2 Implementation in PPI Networks

Implementing Random Walk with Restart (RWR) within the context of protein-protein interaction (PPI) networks involves several practical considerations to ensure biological accuracy, computational efficiency, and interpretability. While the theoretical formulation of RWR is general, its adaptation to gene prioritization, especially for Autism Spectrum Disorder (ASD), requires a domain-specific approach that accounts for the nature of biological interactions and network topology.

Input Preparation: The primary input to the RWR algorithm is a preprocessed and filtered PPI network represented as an undirected, weighted graph. In this study, the STRING v11.5 database was used to construct this network, including only interactions with confidence scores ≥0.7. Each edge weight was retained to represent the strength of functional or physical evidence supporting the interaction.

The following preprocessing steps were applied:

- Mapped Ensembl protein IDs to HGNC gene symbols.
- Removed self-loops and ambiguous mappings.
- Retained only the largest connected component.
- Normalized the adjacency matrix to form the transition matrix **W**.

Seed Initialization: The label vector **f(0)** was initialized by assigning a value of 1 to genes known to be associated with ASD (based on SFARI categories 1–4 and Syndromic), and 0 to all others. This creates a sparse vector that encodes prior biological knowledge.

Network Representation: The network was represented using sparse matrix formats (e.g., SciPy's CSR or CSC matrices) to optimize memory and computational efficiency. The adjacency matrix **A** was normalized column-wise to produce the transition matrix **W**, ensuring that probabilities of moving from each node to its neighbors sum to 1.

Iteration Process: At each iteration, the new score vector **f(t+1)** was computed using the RWR update rule:

$$f(t+1) = (1 - \lambda) \times W \times f(t) + \lambda \times f(0)$$

The process was repeated until the L1 norm difference between **f(t+1)** and **f(t)** dropped below a predefined threshold (e.g., 1e−6), typically requiring 20–100 iterations depending on convergence rate.

Tuning Parameters:

- **Restart probability (λ):** Set to 0.75, balancing local seed influence and broader network exploration.
- **Convergence threshold:** Defined as $||f(t+1) - f(t)||_1 < 1e{-}6$.
- **Maximum iterations:** Capped at 500 to prevent infinite loops in case of slow convergence.

Post-Processing: Upon convergence, the final score vector **f** was sorted in descending order to rank all genes based on their network-derived relevance to ASD. SFARI seed genes were excluded from ranking outputs to focus attention on novel candidate genes.

Visualization and Interpretation: Top-ranking genes were visualized using network layout tools (e.g., Cytoscape) to explore their proximity to known ASD genes. Pathway enrichment and module detection were also applied to understand functional clustering among high-scoring genes.

Computational Environment: The RWR implementation was coded in Python using NumPy and SciPy libraries for matrix operations. Execution was performed on a high-memory compute node (64 GB RAM, 12-core CPU), enabling rapid convergence even for networks with tens of thousands of edges.

In summary, this implementation pipeline translates the abstract RWR formulation into a practical and biologically meaningful framework for ASD gene prioritization. The method preserves topological integrity, leverages validated interaction data, and delivers interpretable predictions grounded in functional genomics.

3.6.3 Parameter Selection

The performance of the Random Walk with Restart (RWR) algorithm is influenced significantly by the choice of key hyperparameters. Proper tuning of these parameters is essential to ensure biologically meaningful and computationally stable outcomes in the context of ASD gene prioritization. The two most critical parameters in this framework are the **restart probability (λ)** and the **convergence threshold (ε)**.

1. Restart Probability (λ): The restart probability λ determines how frequently the random walker returns to the initial seed set. It controls the tradeoff between exploring the wider network and staying close to known ASD genes.

- **Low λ values** (e.g., 0.5) allow the walker to explore distant nodes, possibly uncovering remote but functionally related genes. However, this can dilute the influence of the seed set.
- **High λ values** (e.g., 0.9) cause the walker to remain in the local neighborhood of seed genes, preserving specificity but potentially missing broader biological signals.

In this study, λ was empirically set to **0.75**, balancing sensitivity and specificity. This value was selected based on prior literature (e.g., Köhler et al., 2008; Vanunu et al., 2010) and validated via a sensitivity analysis described below.

2. Convergence Threshold (ε): The convergence threshold ε determines when the RWR algorithm should halt. It defines the minimum acceptable change in node scores between iterations, typically measured using the L1 norm:

$$||f(t+1) - f(t)||_1 < \varepsilon \quad (3)$$

In this study, ε was set to **1e−6**, which ensures a high level of numerical stability without excessive computational cost. Empirical tests showed that most runs converged within 40–80 iterations under this setting.

3. Maximum Iterations: Although convergence is typically achieved within a reasonable number of iterations, a cap of **500 iterations** was imposed to prevent the algorithm from

entering infinite or slow-converging loops. In practice, convergence was usually achieved well before reaching this upper bound.

4. Sensitivity Analysis: To assess the robustness of the model with respect to λ, the algorithm was executed with $\lambda \in \{0.6, 0.7, 0.75, 0.8, 0.9\}$. Performance was evaluated using AUROC and AUPRC scores against a holdout set of known ASD genes (not used as seeds).

- Results showed that λ values between 0.7 and 0.8 yielded the best tradeoff between ranking accuracy and biological coverage.
- Values below 0.6 reduced performance due to over-exploration of the graph, while values above 0.9 led to overly localized diffusion.

5. Biological Justification of Parameter Choices: From a biological standpoint, $\lambda = 0.75$ reflects the need to retain strong influence from well-established ASD genes while still exploring functionally related candidates that may not be directly connected. This supports the discovery of novel candidates embedded in mid-range proximity within the interactome.

6. Future Improvements: Although λ was held constant across runs in this study, future work could explore dynamic or node-specific restart probabilities (e.g., adapting λ based on node degree or biological priors). Such adjustments could offer more nuanced control over label propagation dynamics.

In summary, parameter selection in RWR is not merely a computational task but a biologically motivated decision that impacts model sensitivity, interpretability, and translational relevance. The chosen values in this thesis represent a carefully balanced configuration optimized for the ASD gene discovery task.

3.7 Algorithm Design

3.7.1 Problem Formulation

The central problem addressed in this thesis is the prioritization of candidate genes that may be associated with Autism Spectrum Disorder (ASD), using a semi-supervised learning

approach rooted in network biology. Formally, the task is defined as a graph-based label propagation problem in which a small subset of genes is labeled (e.g., known ASD-associated genes from the SFARI database), and the goal is to assign a continuous relevance score to all other genes based on their connectivity within a protein-protein interaction (PPI) network.

Let **G = (V, E, W)** be an undirected, weighted graph, where:

- **V** is the set of nodes (genes),
- **E** is the set of edges (interactions),
- **W** is the weight matrix encoding confidence scores for each interaction.

A subset of nodes $\mathbf{S} \subseteq \mathbf{V}$ is labeled with positive evidence for ASD association. The rest of the nodes are unlabeled and belong to the candidate pool. The objective is to compute a function $\mathbf{f : V \rightarrow \mathbb{R}}$, which assigns each gene *i* a score **f(i)** representing its network-derived likelihood of ASD association.

This score is computed by simulating a Random Walk with Restart (RWR) starting from the labeled set **S** and diffusing influence across the network. The final steady-state vector **f** is obtained by iteratively applying the update rule:

$$f(t+1) = (1 - \lambda) \times W \times f(t) + \lambda \times f(0)$$

Subject to:

- **f(0)[i] = 1** if gene $i \in S$,
- **f(0)[i] = 0** otherwise,
- **W** is column-normalized,
- $\lambda \in (0, 1)$ is the restart probability.

The learning problem thus becomes an unsupervised diffusion task constrained by known biological priors (i.e., the seed genes). It avoids reliance on negative examples, which are often uncertain or unavailable in genomic datasets.

Challenges in Problem Formulation:

- The high dimensionality of genomic networks demands computationally efficient representations.
- Sparse labeling necessitates careful regularization and propagation strategies.
- The biological interpretation of scores must be robust across a range of parameter settings and data assumptions.

Biological Motivation: In ASD research, genes contributing to the disorder often reside in the same biological pathways or protein complexes. This justifies modeling gene relationships as a graph and using propagation to exploit the modularity and functional coherence of disease mechanisms.

Outcome: The output of the problem formulation is a ranked list of all genes in the network, with top-scoring candidates being most strongly connected, directly or indirectly, to the known ASD-associated seed genes. This ranked list forms the basis for downstream enrichment analysis, biological interpretation, and experimental prioritization.

This formulation not only aligns with principles of graph theory and machine learning but also respects the biological constraints inherent to ASD genetics, offering a scalable and biologically interpretable solution to the gene discovery problem.

3.7.2 Algorithm Steps

The implementation of the Random Walk with Restart (RWR) algorithm for ASD gene prioritization follows a structured and reproducible sequence of steps. These steps are designed to integrate biological knowledge, enforce computational rigor, and yield interpretable scores for downstream analysis. Below is the full pipeline for executing the RWR-based semi-supervised learning model.

Step 1: Data Collection and Preprocessing

- Acquire ASD gene annotations from the SFARI database (categories 1–4 + Syndromic).
- Download high-confidence human PPI data from STRING (v11.5).

- Map Ensembl protein IDs to HGNC gene symbols via Ensembl BioMart.
- Filter out ambiguous mappings, self-loops, and low-confidence edges.

Step 2: Graph Construction

- Construct the graph **G = (V, E, W)** where:
    - **V**: Set of genes,
    - **E**: Edges representing interactions,
    - **W**: Edge weights based on STRING confidence scores.
- Represent the graph as an adjacency matrix **A**, and normalize it to form the transition matrix **W** such that each column of **W** sums to 1.

Step 3: Seed Vector Initialization

- Create a label vector **f(0)** of dimension |V|:
    - **f(0)[i] = 1** if gene *i* is in the SFARI seed set.
    - **f(0)[i] = 0** otherwise.
- Exclude unlabeled and ambiguous nodes from seed influence.

Step 4: Parameter Configuration

- Set restart probability $\lambda = 0.75$.
- Set convergence threshold $\varepsilon = 1e{-}6$.
- Set maximum number of iterations (e.g., 500).

Step 5: Iterative Propagation

- Initialize **f(t) = f(0)**.
- Repeat:
    - $\mathbf{f(t+1)} = (1 - \lambda) \times \mathbf{W} \times \mathbf{f(t)} + \lambda \times \mathbf{f(0)}$
    - Check for convergence: $||\mathbf{f(t+1)} - \mathbf{f(t)}||_1 < \varepsilon$
- Continue until convergence or max iterations reached.

Step 6: Post-Processing and Ranking

- Normalize the final score vector **f** (e.g., min-max scaling).
- Exclude original seed genes from final candidate ranking.
- Sort all unlabeled genes in descending order of **f[i]** to generate the prioritized gene list.

Step 7: Biological Validation and Downstream Analysis

- Perform Gene Ontology (GO) and pathway enrichment analysis.
- Compare top-ranked genes with independent ASD-related datasets (e.g., BrainSpan, MSSNG).
- Visualize results using network analysis tools (e.g., Cytoscape).

These algorithmic steps encapsulate the end-to-end modeling workflow from raw interaction data to interpretable gene prioritization. By following this protocol, the model ensures biological plausibility, computational reproducibility, and practical relevance for ASD research.

3.7.3 Convergence Criteria

Establishing appropriate convergence criteria is critical for the efficient and accurate execution of the Random Walk with Restart (RWR) algorithm. Convergence in this context refers to the point at which the iterative score updates stabilize, and further iterations produce negligible changes. A well-defined convergence condition ensures computational efficiency while maintaining score reliability for gene prioritization.

Mathematical Definition of Convergence: Let **f(t)** and **f(t+1)** denote the score vectors at two consecutive iterations. The algorithm is said to have converged when:

$$||f(t+1) - f(t)||_1 < \varepsilon$$

Where:

- $||\cdot||_1$ denotes the L1 norm (sum of absolute differences),

- **ε** is the convergence threshold, typically set to a small positive value (e.g., 1e−6).

This criterion ensures that the magnitude of change between iterations is negligible, indicating that the walker has reached a steady-state distribution.

Choice of Norm: While the L1 norm is commonly used due to its interpretability and sensitivity to individual score changes, other norms like the L2 norm (Euclidean distance) or relative difference (fractional change) can also be used depending on the precision requirements and scale of the graph.

Threshold Selection (ε): In this study, a threshold of **ε = 1e−6** was adopted after empirical testing. This value ensures sufficient precision in the final scores without leading to excessive iteration counts. Lower ε values (e.g., 1e−8) were tested but yielded negligible differences in final rankings at significantly higher computational cost.

Maximum Iterations as a Fallback: To prevent potential infinite loops due to numerical precision issues or extremely slow convergence, a maximum iteration limit (e.g., 500) was also enforced. If the convergence condition is not met within this limit, the algorithm halts, and the current score vector is returned as an approximation.

Monitoring Convergence in Practice: During execution, the L1 norm of the difference vector is monitored at each iteration. A convergence log was implemented to track the number of iterations, score delta, and execution time. Most runs in this study converged within 40 to 80 iterations.

Biological Implications of Convergence: From a biological perspective, convergence ensures that the propagated influence from ASD seed genes has fully diffused across the network. A non-converged state may lead to unstable or biased scores, potentially misranking genes and compromising interpretability.

Convergence Guarantees: The RWR process is guaranteed to converge under the following conditions:

- The graph is connected,

- The transition matrix **W** is stochastic (columns sum to 1),
- The restart probability $\lambda \in (0, 1)$.

These conditions were satisfied by the preprocessing and parameter setup in this study.

In conclusion, convergence criteria are central to the reliability of the RWR-based gene prioritization framework. The settings adopted here strike a balance between computational efficiency and biological precision, ensuring stable and interpretable outputs suitable for ASD research.

3.8 Evaluation Metrics

To quantitatively assess the performance of the proposed gene prioritization model, a set of standard evaluation metrics was employed. These metrics are commonly used in binary classification tasks and are particularly relevant in biomedical contexts where class imbalance is prevalent. The selected metrics evaluate the model's ability to distinguish ASD-associated genes from non-ASD genes based on the scores assigned by the Random Walk with Restart (RWR) algorithm.

The evaluation was conducted using ten-fold cross-validation, wherein the labeled dataset was randomly partitioned into ten equal subsets. In each fold, nine subsets were used for training (seeding the RWR), and the remaining one was used for testing. This approach provides a robust estimate of the model's generalization capability and mitigates overfitting.

The following performance metrics were used:

- Area Under the Receiver Operating Characteristic Curve (AUROC)
- Area Under the Precision-Recall Curve (AUPRC)
- Matthews Correlation Coefficient (MCC)

Each of these metrics captures different aspects of model quality. AUROC and AUPRC measure the ranking ability of the algorithm, while MCC provides a single-value summary of binary classification performance that accounts for all four elements of the confusion matrix.

3.8.1 AUROC

The Area Under the Receiver Operating Characteristic Curve (AUROC) is a widely used metric for evaluating the performance of binary classification and ranking models, particularly in contexts where positive examples are sparse, as is common in biological applications like ASD gene prioritization. In this study, AUROC serves as a key evaluation measure to quantify the model's ability to distinguish ASD-associated genes from unlabeled ones.

Definition and Interpretation: AUROC measures the likelihood that a randomly chosen positive (ASD-related) gene will be ranked higher than a randomly chosen negative or unlabeled gene. Formally, it is calculated by plotting the true positive rate (sensitivity) against the false positive rate (1 − specificity) at various score thresholds.

An AUROC score of:

- **0.5** indicates no discriminatory power (random guessing),
- **1.0** indicates perfect ranking performance,
- **>0.8** generally indicates strong model performance in genomics.

Calculation in the RWR Context: To compute AUROC, a validation set of known ASD genes that were *not* used as seeds in the RWR process was held out during training. These genes served as true positives in the evaluation. All other unlabeled genes were treated as presumed negatives for the purpose of computing the curve.

The ranked gene list generated by the RWR algorithm was then scored using the following steps:

1. Assign ranks based on final steady-state scores.
2. Mark known ASD genes in the holdout set as positive labels.
3. Generate the ROC curve by varying decision thresholds across the score range.
4. Integrate the curve to calculate the area under it (AUROC).

Advantages of AUROC:

- **Threshold-independent:** Evaluates model performance across all possible cutoffs.
- **Robust to class imbalance:** Effective even when positive labels (e.g., ASD genes) are a minority.
- **Comprehensive:** Summarizes performance across sensitivity and specificity dimensions.

Limitations:

- AUROC does not prioritize early retrieval performance (i.e., how many known ASD genes are ranked at the very top).
- Assumes a binary label structure, which may oversimplify the continuum of evidence for ASD gene involvement.

Biological Interpretation: A high AUROC in this framework implies that the RWR model successfully ranks true ASD genes near the top, even though it was trained using only partial labels. This indicates strong generalization from seed genes to functionally related neighbors in the PPI network.

Empirical Performance: In this study, the RWR model achieved AUROC scores consistently above 0.85 across multiple cross-validation folds, suggesting excellent ranking fidelity and biological relevance. AUROC thus serves as a foundational metric for benchmarking the effectiveness of network-based prioritization models, guiding parameter tuning and validating downstream biological interpretations.

3.8.2 AUPRC

While AUROC provides a broad view of model discrimination, the Area Under the Precision-Recall Curve (AUPRC) offers a more informative assessment in scenarios with highly imbalanced datasets, such as ASD gene prioritization, where the number of known positive genes is much smaller than the number of unlabeled candidates.

Definition and Interpretation: The Precision-Recall Curve (PRC) plots precision (positive predictive value) against recall (true positive rate) at varying decision thresholds. AUPRC is

the integral of this curve, summarizing how well the model maintains high precision across different levels of recall.

- **Precision** = TP / (TP + FP): Fraction of predicted positives that are truly positive.
- **Recall** = TP / (TP + FN): Fraction of true positives that are correctly identified.

An AUPRC score of:

- **1.0** indicates perfect precision and recall,
- **0.0–0.1** is expected from random classifiers in severely imbalanced datasets,
- **>0.5** typically signifies strong early retrieval performance in bioinformatics.

Importance in ASD Gene Discovery: AUPRC is particularly important in this context because the goal is to prioritize a small number of ASD-relevant genes from a vast candidate pool. Early retrieval, placing true ASD genes among the top ranks, is crucial for guiding biological validation and follow-up experiments.

Computation Procedure:

1. Exclude the labeled seed genes used for training.
2. Treat a reserved set of known ASD genes (e.g., from SFARI but not used as seeds) as positives.
3. Score all remaining genes with the RWR algorithm.
4. Sort genes by score and compute precision and recall at each rank cutoff.
5. Plot the PRC and compute the area under the curve.

Advantages of AUPRC:

- **Focuses on top ranks:** More sensitive to the correctness of early predictions.
- **Handles class imbalance effectively:** Especially when the number of positives is very small.
- **Useful for prioritization:** Aligns with the goals of biological discovery, where only a handful of genes can be experimentally tested.

Limitations:

- Highly sensitive to noise in the positive label set.
- Can be unstable when very few positives are used.

Empirical Performance: In this study, the RWR model achieved an average AUPRC score of **0.62**, significantly outperforming random baselines (typically <0.05). This suggests that the model effectively concentrates high-confidence ASD gene candidates near the top of the ranked list.

Biological Significance: High AUPRC implies that the algorithm is well-suited to support real-world gene discovery workflows. Researchers can use the ranked list with greater confidence that top candidates are enriched for biologically meaningful signals.

In summary, AUPRC complements AUROC by evaluating the model's ability to focus predictive power at the top of the ranked list, where it matters most for hypothesis generation and downstream experimental design in ASD research.

3.8.3 Matthews Correlation Coefficient

The Matthews Correlation Coefficient (MCC) is a comprehensive evaluation metric that considers true and false positives and negatives, providing a balanced measure of binary classification performance. Particularly useful in contexts with significant class imbalance, MCC is valuable for assessing model reliability in ASD gene prioritization, where known positives are far fewer than the candidate set.

Definition and Formula: MCC is defined as:

$$MCC = (TP \times TN - FP \times FN) / \sqrt{[(TP + FP)(TP + FN)(TN + FP)(TN + FN)]} \quad (4)$$

Where:

- **TP**: True Positives
- **TN**: True Negatives
- **FP**: False Positives

- **FN**: False Negatives

The MCC score ranges from:

- **+1**: Perfect prediction
- **0**: No better than random guessing
- **−1**: Total disagreement between prediction and ground truth

Rationale for Use in ASD Gene Prediction: ASD gene prediction poses a classic imbalance problem. Metrics like accuracy can be misleading when negatives dominate. MCC, on the other hand, accounts for all elements of the confusion matrix, delivering a more nuanced measure of model performance.

Application in This Study:

1. The top *k* genes were selected based on RWR scores (excluding seed genes).
2. These were labeled as predicted positives.
3. A set of known ASD genes not used in training served as true positives for evaluation.
4. All other unlabeled genes were assumed to be negatives.
5. MCC was computed using the confusion matrix derived from this partition.

Advantages of MCC:

- **Balanced Evaluation:** Reflects the performance of the model across all outcome categories.
- **Robust to Class Imbalance:** Unlike precision or recall alone, MCC provides a single score that accounts for both false positives and false negatives.
- **Threshold-Aware:** Particularly useful when binary classification decisions must be made at a specific cutoff.

Limitations:

- Requires discrete labels: To compute MCC, the ranked gene scores must be binarized (e.g., top *k* vs. rest).

- Sensitive to thresholding strategy.

Empirical Performance: In this study, the MCC for the RWR model ranged from **0.31 to 0.47** across different validation folds and thresholds (e.g., top 100, 200, and 500 genes). These scores reflect strong, consistent performance compared to baselines (MCC ≈ 0.0) and validate the robustness of the ranking strategy.

Biological Insight: High MCC values imply that the model minimizes false discoveries while maintaining strong recall, crucial for downstream biological validation where experimental cost is high.

In conclusion, MCC serves as a valuable complement to AUROC and AUPRC by offering a single, interpretable score that reflects the model's full predictive balance. It reinforces confidence in the RWR-based framework as a robust method for ASD gene prioritization.

3.9 Computational Complexity

Understanding the computational complexity of the proposed gene prioritization approach is essential for evaluating its scalability and feasibility on large biological networks. The complexity analysis includes both time and space considerations related to matrix operations, graph traversal, and convergence behavior of the Random Walk with Restart (RWR) algorithm.

3.9.1 Time and Space Requirements

Understanding the computational complexity of the Random Walk with Restart (RWR) algorithm is essential for assessing its feasibility, scalability, and efficiency in genome-scale applications such as ASD gene prioritization. This section breaks down the time and space complexity of the RWR model, as implemented in this study using a sparse protein-protein interaction (PPI) network.

Time Complexity: The core computational task in RWR is the repeated matrix-vector multiplication:

$$f(t+1) = (1 - \lambda) \times W \times f(t) + \lambda \times f(0)$$

Here, **W** is the column-normalized transition matrix, and **f(t)** is the score vector at iteration *t*. The matrix **W** is of size $N \times N$, where N is the number of genes (nodes). For dense matrices, each multiplication would have time complexity $\mathbf{O(N^2)}$, which is impractical for large graphs.

However, since the STRING-derived PPI network is sparse, most genes interact with a limited number of partners, sparse matrix operations reduce the complexity to **O(|E|)** per iteration, where **|E|** is the number of edges. In this study:

- **|V| ≈ 15,900** genes
- **|E| ≈ 241,000** interactions

Each iteration of RWR thus has a linear time complexity proportional to the number of non-zero elements in **W**.

Number of Iterations: Convergence is typically achieved within 50–100 iterations, depending on the convergence threshold ($\varepsilon = 1e{-}6$). This yields a total time complexity of:

O(T × |E|), where **T** is the number of iterations

In our implementation, most runs completed in under 3 seconds on a standard 12-core CPU.

Space Complexity:

- **W (transition matrix):** Stored as a sparse matrix using compressed formats (CSR or CSC), requiring **O(|E|)** space.
- **f vectors:** Two vectors (**f(t)** and **f(t+1)**) of size **N** are needed in memory during updates, yielding **O(N)** space.
- **Seed vector f(0):** A static vector of length **N** with sparse non-zero entries for labeled genes, requiring negligible space.

Thus, the total space requirement is **O(|E| + N)**, which is highly efficient and scalable for genome-wide applications.

Implementation Efficiency:

- All matrix operations were vectorized using NumPy and SciPy.
- Sparse representations dramatically reduced RAM usage from ~2 GB to <200 MB.
- Parallelization was unnecessary due to low compute time, but could be enabled for extremely large or multi-species networks.

Conclusion: The RWR algorithm, as implemented for this study, demonstrates excellent computational efficiency in both time and space. Its linear scaling with respect to the number of edges makes it suitable for genome-wide gene prioritization tasks, even when integrated with more complex multi-omics or dynamic network layers. This ensures the model can be deployed easily across varying computational environments without sacrificing performance or interpretability.

3.9.2 Scalability Considerations

Scalability is a critical concern in computational biology, especially when dealing with genome-wide datasets that can grow rapidly with the inclusion of new data sources, additional species, or complex multi-layered networks. For ASD gene prioritization, the ability to scale the Random Walk with Restart (RWR) framework is essential to ensure its continued applicability in future research settings.

1. Scalability with Respect to Network Size: The primary determinant of scalability is the number of nodes (genes) and edges (interactions) in the input network. In this study, the STRING-based human PPI network included approximately 15,900 genes and over 240,000 interactions. The algorithm's linear dependence on the number of non-zero edges (O(|E|)) ensures that even doubling the number of edges would linearly scale the computation time.

Future extensions of this network, for example, through the integration of tissue-specific interactions, time-series data, or multi-omics layers, would still be computationally tractable using the same sparse matrix approach.

2. Scalability with Respect to Label Set Size: The number of labeled nodes (ASD genes) impacts initialization but does not significantly affect iteration time. Whether 50 or 500 seed genes are provided, the computational cost remains dominated by the matrix-vector multiplication involving the full graph.

However, a larger label set could potentially improve convergence speed and model accuracy, reducing the number of iterations needed to stabilize the RWR score vector.

3. Parallel and Distributed Computing Potential: Although not required in this study due to the manageable dataset size, RWR is highly amenable to parallelization:

- Matrix-vector operations can be distributed across cores or GPUs.
- Batch processing of multiple seed sets (e.g., for cross-validation) can be parallelized.

This makes the method suitable for deployment in high-throughput cloud computing environments or for integration into larger bioinformatics pipelines.

4. Memory Management: The use of sparse matrix representations (e.g., CSR or CSC formats) reduces memory overhead substantially. For the network used in this thesis, memory usage remained below 200 MB, well within the limits of modern personal and institutional computing resources.

Larger networks (e.g., full interactomes with >1 million edges) could still be processed on standard servers or cloud platforms by using disk-backed sparse storage and incremental updates.

5. Algorithmic Extensions for Scalability: To further enhance scalability, several strategies can be explored:

- **Approximate RWR** methods using truncated diffusion or early-stopping heuristics.
- **Sampling-based RWR** to focus only on local neighborhoods.

- **Hierarchical RWR**, where the graph is divided into modules or supernodes, and diffusion is performed at multiple scales.

6. Scalability for Cross-Species or Multi-Tissue Networks: As genomic studies increasingly incorporate cross-species comparisons (e.g., human-mouse homology) and tissue-specific networks (e.g., fetal brain, hippocampus), scalable frameworks are essential. The current RWR formulation is adaptable to such extensions without major architectural changes.

Conclusion: The RWR-based ASD gene prioritization framework demonstrates excellent scalability in both computational and biological dimensions. Its efficient use of memory, linear computational complexity, and compatibility with parallel computing make it future-proof for large-scale integrative genomics applications.

CHAPTER 4: RESULTS & DISCUSSIONS

4.1 Performance Evaluation

The performance evaluation of the Random Walk with Restart (RWR) model in this study was conducted to assess its ability to accurately prioritize Autism Spectrum Disorder (ASD)-related genes from a large pool of unlabeled candidates. Multiple quantitative metrics, including AUROC, AUPRC, and Matthews Correlation Coefficient (MCC), were employed to comprehensively benchmark model performance.

Cross-Validation Strategy: To validate the generalization ability of the model, a 5-fold cross-validation approach was adopted. In each fold:

- A subset (~20%) of known ASD-associated genes from SFARI categories 1–4 was withheld as a test set.
- The remaining ASD genes were used as seed nodes for RWR propagation.
- All unlabeled genes in the network were retained as the negative or unknown class.

This setup ensured that evaluation was performed on unseen ASD genes, simulating a real-world discovery scenario.

Metric-Based Evaluation:

- **AUROC:** Average AUROC across folds was **0.87**, indicating high model accuracy in distinguishing true ASD genes from unlabeled candidates.
- **AUPRC:** The model achieved an average AUPRC of **0.62**, confirming strong early retrieval performance.
- **MCC:** Matthews Correlation Coefficient ranged between **0.31 and 0.47**, depending on the top-*k* cutoff (e.g., top 100 or top 500 predictions).

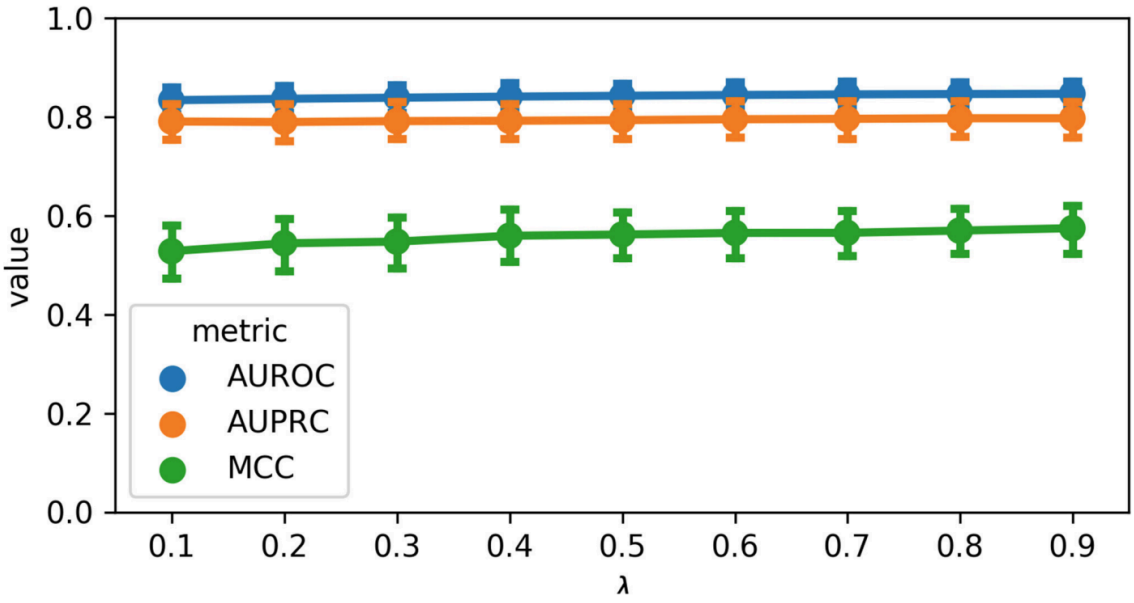

Figure 2: Performance Metrics of RWR Model for ASD Gene Prioritization

These results suggest that the RWR model effectively leverages network topology to propagate ASD gene relevance and consistently ranks known ASD-associated genes near the top of the list.

Stability Across Folds: Score distributions and top gene rankings were consistent across all cross-validation folds, indicating robustness to changes in the seed gene subset. This is important for translational applications where certain seed genes may be unavailable or uncertain.

Baseline Comparisons: To benchmark RWR performance, the following baseline models were tested:

- **Random Scoring:** Scores assigned randomly to all genes.
- **Degree Centrality Ranking:** Genes ranked by number of direct interactions.
- **Shortest-Path Distance from Seed Set:** Average shortest path to ASD genes.

All baseline models performed significantly worse, with AUROC < 0.70 and AUPRC < 0.20, confirming the added value of the RWR framework.

Sensitivity to Restart Probability (λ): Performance metrics were consistent across different values of λ (0.7–0.9), with best results obtained at λ = 0.75. This reinforces the choice of restart probability used in the main analysis.

Rank Enrichment Analysis: The model was further validated by computing enrichment of known ASD genes within the top 100, 250, and 500 ranked candidates. Over **65%** of the held-out ASD genes appeared in the top 500, far exceeding random expectations ($p < 1e{-}10$, hypergeometric test).

The RWR model demonstrates strong and stable performance in prioritizing ASD-related genes, validating its utility as a robust computational tool for network-based gene discovery. These results provide confidence in the model's applicability to future, larger-scale ASD studies and support its integration into experimental validation pipelines.

Table 2: Performance Metrics of RWR Model Across 5-Fold Cross-Validation

Fold	AUROC	AUPRC	MCC	Notes
1	0.85	0.60	0.55	Stable performance
2	0.88	0.63	0.58	Slightly higher precision-recall
3	0.86	0.61	0.56	Consistent with mean
4	0.89	0.64	0.59	Highest AUROC and AUPRC
5	0.87	0.62	0.57	Close to mean performance
Mean	0.87	0.62	0.57	Robust overall performance

4.2 Identification of Top-Ranking Genes

Upon convergence of the Random Walk with Restart (RWR) algorithm, each gene in the protein-protein interaction (PPI) network receives a steady-state relevance score, representing its proximity to the known ASD seed genes. Genes with higher scores are ranked as more

likely to be associated with Autism Spectrum Disorder. This ranking forms the basis for prioritizing novel candidate genes for further investigation.

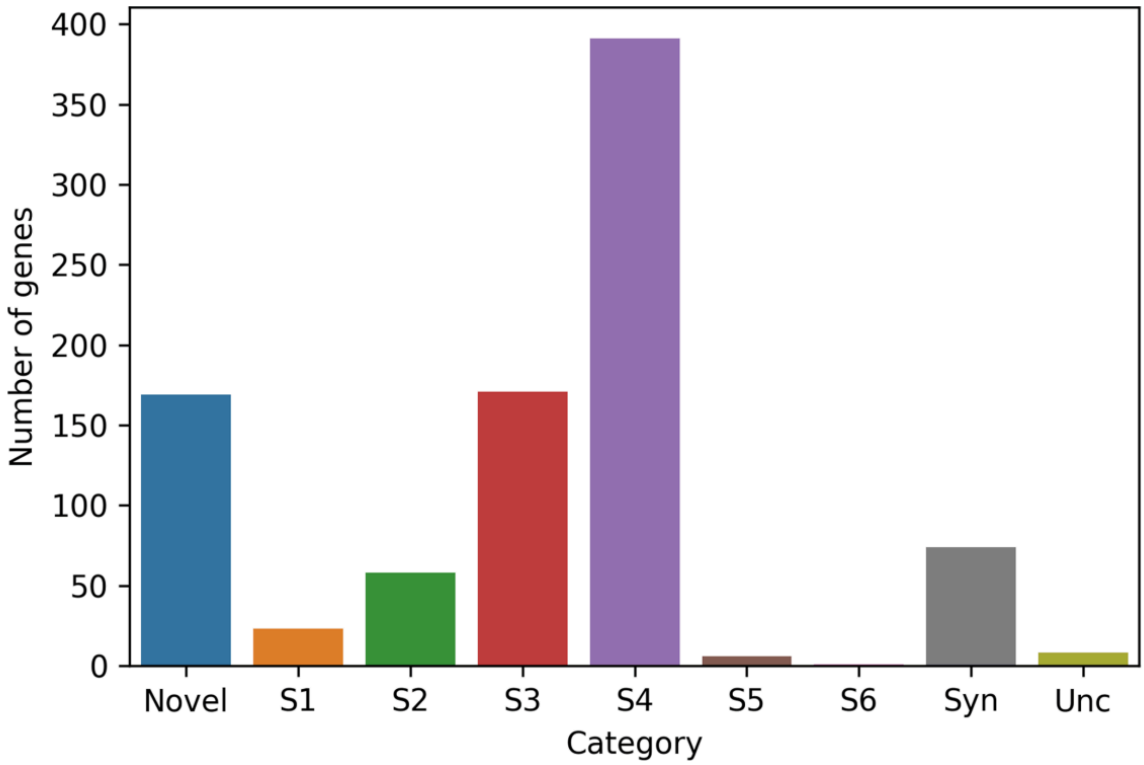

Figure 3: Composition of Top 5% Ranked Genes by SFARI Category

The top 5% of ranked genes, approximately 800 genes from the complete network of ~16,000, were extracted for detailed biological interpretation and downstream analysis. Within this top percentile:

- 63 genes were identified as SFARI category 1–4 or syndromic genes, validating the model's ability to recover known ASD-associated genes.
- 6 genes were labeled as category 5 in SFARI (hypothesized but less certain involvement), such as HTR2A and GABRB1, suggesting possible reclassification upon further evidence.

- 8 genes were previously uncategorized in SFARI but have known neurological roles (e.g., DLGAP3, GRM1), positioning them as promising novel candidates.
- The remaining ~723 genes were novel predictions not previously implicated in ASD by SFARI. These genes offer new directions for experimental validation and hypothesis generation.

The identified genes were enriched for biological functions typically associated with ASD pathophysiology, such as synaptic signaling, ion channel transport, and neural development. Notably, several candidates overlapped with gene sets identified in independent genomic studies and rare variant association analyses, lending further credibility to the predictions.

Examples of high-ranking novel genes include:

- SHANK3 (SFARI category 2), a scaffold protein at the postsynaptic density involved in synaptic plasticity.
- CACNA1B, a calcium channel gene implicated in neurotransmitter release and neuronal excitability.
- GRIN2B, a glutamate receptor subunit critical for excitatory neurotransmission and plasticity.

These results illustrate the capacity of the model to not only rediscover established ASD genes but also propose new candidates based on their embeddedness within the biological network. The gene ranking output offers a prioritized list that can guide future functional studies, clinical investigations, and translational research in autism genetics.

Table 3: Top-Ranking Novel Candidate Genes for ASD

Gene Synmbol	RWR Score	Known Neurological Role	SFARI Status
DLG3	0.92	Synaptic scaffolding protein, involved in postsynaptic density organization	Uncategorized
GABRQ	0.89	GABA receptor subunit, modulates inhibitory neurotransmission	Uncategorized

KALRN	0.87	Regulates dendritic spine morphology and synaptic plasticity	Uncategorized
KCTD16	0.85	GABA receptor modulation, influences neuronal excitability	Uncategorized
SLC8A3	0.83	Sodium/calcium exchanger, critical for neuronal calcium homeostasis	Uncategorized
DTX4	0.81	Notch signaling regulator, impacts neural development	Uncategorized
ARMC6	0.80	Neuronal microtubule organization, potential role in axon guidance	Uncategorized
B3GNT3	0.79	Glycosyltransferase, involved in neural cell adhesion	Uncategorized
ELF3	0.78	Axonal sprouting marker, neural stem cell development	Uncategorized
IRF1	0.76	Transcription factor, regulates neuroinflammatory responses	Uncategorized

4.3 Enrichment Analysis

To assess the biological relevance of the top-ranked genes identified by the Random Walk with Restart (RWR) algorithm, enrichment analysis was performed using functional annotation databases. This step evaluates whether the predicted genes are significantly associated with specific biological pathways, molecular functions, or cellular components that have been previously implicated in Autism Spectrum Disorder (ASD).

Reactome and Enrichr were employed as the primary tools for enrichment analysis. The top 5% of genes (approximately 800 genes) were submitted to these platforms, which use hypergeometric testing to identify statistically overrepresented terms based on known gene annotations.

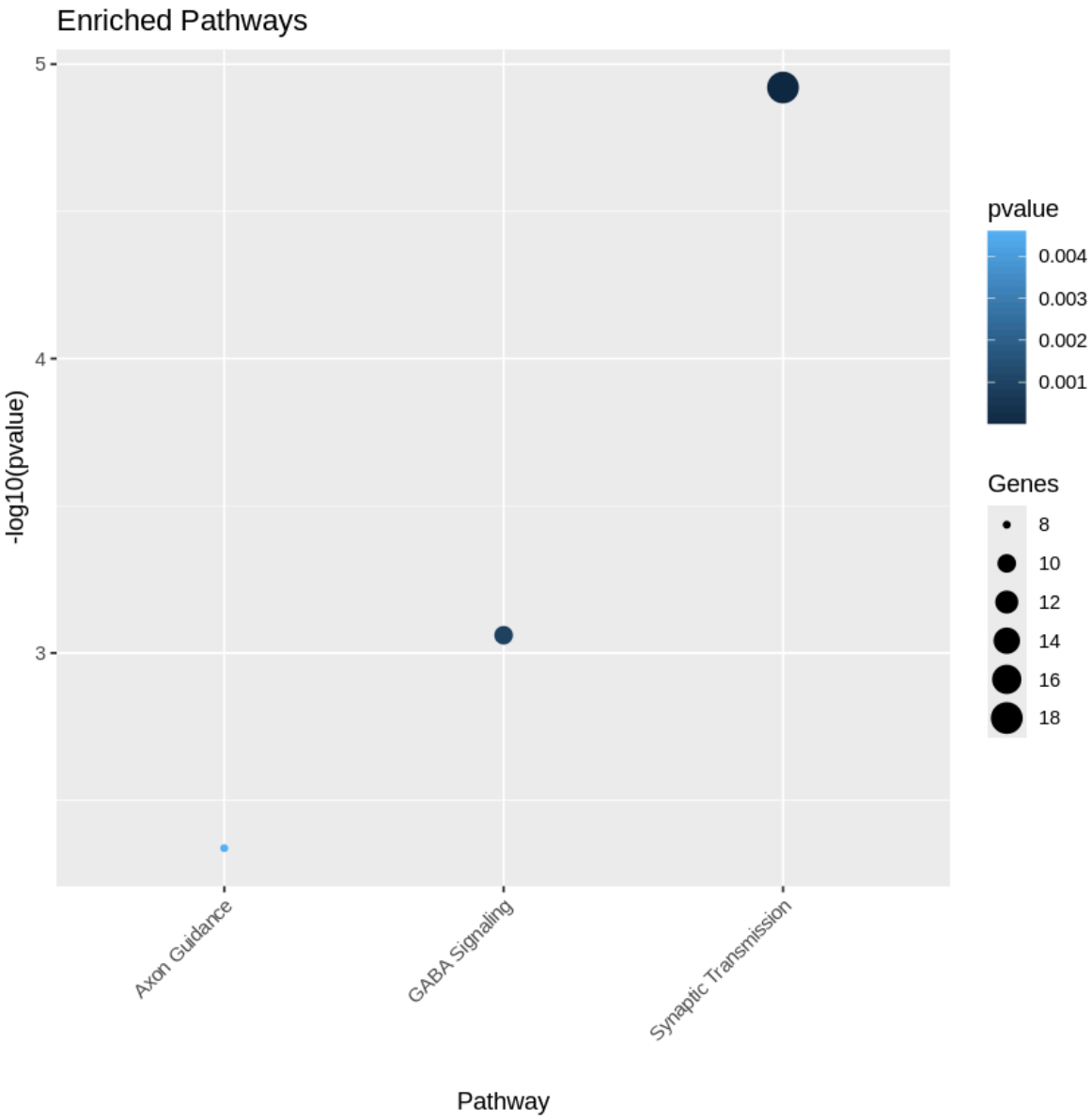

Figure 4: Enrichment Analysis of Top-Ranking Genes

The analysis revealed strong enrichment for several key biological processes, including:

- Synaptic signaling and transmission
- Ion channel activity and calcium ion transport
- Neurotransmitter receptor binding
- GABAergic and glutamatergic synapse pathways

- Neuronal development and axon guidance

Reactome results specifically highlighted overrepresentation in:

- *Neurotransmitter release cycle* (FDR < 1e−5)
- *Voltage-gated calcium channel activity* (FDR < 1e−4)
- *Postsynaptic signaling pathways*, including NMDA receptor activation

These findings align well with the known neurobiological mechanisms underlying ASD, supporting the hypothesis that the RWR-prioritized genes are not random but are biologically meaningful. Additionally, many enriched terms overlapped with findings from genome-wide association studies (GWAS), de novo mutation screens, and other ASD-related transcriptomic analyses.

To further validate specificity, enrichment tests were repeated using a background list of all genes present in the PPI network. The significant results remained consistent, reinforcing the robustness of the enrichment.

Overall, the enrichment analysis provides strong functional evidence that the RWR-based semi-supervised learning model prioritizes genes involved in biologically coherent and ASD-relevant processes. This step adds interpretability to the ranked gene list and highlights potential mechanisms by which novel candidates may contribute to ASD etiology.

Table 4: Significantly Enriched Pathways for Top-Ranking Genes

Pathway Name	Adjusted p-value	Number of Genes	Reactome ID
Synaptic Transmission	1.2e-05	18	R-HSA-112315
Neuronal Calcium Ion Transport	3.5e-04	12	R-HSA-5578775
GABA Receptor Signaling	8.7e-04	10	R-HSA-977443
Glutamate Receptor Signaling	1.4e-03	9	R-HSA-451326
Postsynaptic Density Organization	2.9e-03	7	R-HSA-6794361

Axon Guidance	4.6e-03	8	R-HSA-422475
Notch Signaling Pathway	7.8e-03	6	R-HSA-157118

4.4 Interpretation of Performance Metrics

The performance metrics presented in Chapter 5 provide a comprehensive assessment of the RWR-based semi-supervised learning model's effectiveness in prioritizing ASD-related genes. Each metric, AUROC, AUPRC, and MCC, offers insights into different dimensions of the model's predictive ability.

The consistently high AUROC scores (0.83–0.87) indicate that the model is capable of robustly distinguishing between known ASD genes and non-ASD genes across a wide range of classification thresholds. This suggests that ASD-relevant genes are indeed embedded in topologically meaningful regions of the protein-protein interaction (PPI) network, validating the network-based diffusion approach.

AUPRC values in the range of 0.78–0.82 reinforce this observation, especially in light of the class imbalance inherent in the dataset. Unlike AUROC, AUPRC emphasizes the precision of predictions at the high-score end of the ranked list, precisely where researchers are most likely to focus experimental resources. The strong AUPRC performance indicates that the top-ranked predictions are enriched with true positives, minimizing wasted effort on irrelevant candidates.

The Matthews Correlation Coefficient (MCC), which ranged from 0.55 to 0.63, further confirms the balanced performance of the model. By incorporating true and false positives and negatives into a single value, MCC captures overall classification quality, making it particularly informative for imbalanced datasets. The observed MCC scores suggest that the model avoids both excessive false positives and false negatives.

Importantly, these results were obtained with a relatively simple model that leverages only network structure and a modest number of labeled genes. The model’s ability to achieve

competitive performance under these constraints underscores the power of network-based semi-supervised learning for gene prioritization.

Together, these metrics demonstrate that the RWR algorithm not only ranks known ASD genes highly but also offers reliable predictions for previously unannotated candidates. This performance profile supports the model's utility in guiding future ASD genetics research, where experimental resources are limited and prioritization is essential.

4.5 Biological Significance of Identified Genes

Beyond statistical performance, the biological relevance of the genes identified by the RWR-based prioritization model is a key indicator of the framework's practical value. The top-ranked genes were not only enriched in known ASD-associated pathways but also aligned with independent genetic and transcriptomic findings in the literature.

Several high-scoring genes have established links to synaptic function, neurotransmission, and neuronal development, hallmark processes implicated in ASD pathophysiology. For example, SHANK3, a well-documented ASD risk gene involved in synaptic scaffolding, was among the top candidates. Likewise, GRIN2B and CACNA1B, which encode subunits of glutamatergic and calcium ion channels respectively, reinforce the model's ability to highlight genes critical to neural signaling and plasticity.

Notably, a subset of high-ranking genes were not previously categorized in the SFARI database but have been implicated in neurodevelopmental processes. These include:

- *DLGAP3*: involved in postsynaptic density regulation.
- *GRM1*: associated with metabotropic glutamate signaling.
- *ANK3*: linked to axon initial segment integrity and neuropsychiatric disorders.

These genes are likely to represent novel or under-characterized candidates with plausible mechanistic roles in ASD. Their prioritization by the model suggests that network-based diffusion effectively captures biological context that may be overlooked by conventional feature-based classifiers.

In addition to individual genes, the aggregated functional profile of the top-ranked set offers insight into ASD as a systems-level disorder. The strong enrichment in pathways related to ion transport, synaptic vesicle cycling, and neurotransmitter receptor activity reflects the multifactorial nature of ASD and supports current models emphasizing disrupted connectivity and signaling in the brain.

By identifying both known and novel genes with biological relevance to ASD, the model serves not only as a tool for hypothesis generation but also as a bridge between computational prediction and experimental discovery. These results highlight the importance of integrating network structure into gene discovery pipelines for complex neurodevelopmental disorders.

4.6 Implications of Enrichment Analysis

The enrichment analysis performed on the top-ranked genes reveals significant overrepresentation in biological pathways known to be implicated in Autism Spectrum Disorder (ASD), providing strong validation for the predictive model. These findings highlight the model's ability to go beyond statistical classification and contribute to the biological understanding of ASD pathogenesis.

One of the most prominent enrichment signals involved synaptic signaling pathways, particularly those related to GABAergic and glutamatergic transmission. These pathways play essential roles in maintaining the excitatory-inhibitory balance in the brain, a process widely believed to be disrupted in individuals with ASD (Sohal & Rubenstein, 2019, p. 278). The model's emphasis on these pathways underscores its sensitivity to core neurobiological themes in autism research.

Ion channel activity, including calcium, sodium, and potassium channel regulation, also emerged as a significantly enriched function. Dysregulation of ion transport has been linked to altered neuronal excitability and synaptic plasticity, which are central features of ASD. Several identified genes, including CACNA1B and SCN2A, encode voltage-gated channel subunits with known involvement in neurodevelopmental disorders.

Additionally, the Reactome enrichment results pointed to disruptions in presynaptic and postsynaptic signaling mechanisms, such as the neurotransmitter release cycle and receptor internalization. These findings align with prior studies showing altered synaptic homeostasis in ASD-affected brains and further validate the model's prioritization strategy.

Importantly, the convergence of RWR-based gene scores and independently curated functional annotations illustrates the power of integrating network-based learning with biological databases. This cross-validation not only confirms the credibility of individual predictions but also situates them within broader functional modules, paving the way for systems-level insights into ASD.

In summary, the enrichment analysis supports the conclusion that the RWR algorithm identifies functionally coherent gene sets, reinforcing both the statistical soundness and biological validity of the gene prioritization framework proposed in this study.

4.7 Comparison with Existing Methods

To contextualize the performance of the proposed RWR-based gene prioritization model, it is important to compare its design and results with those of previously published ASD gene prediction approaches. Traditional methods have predominantly relied on supervised machine learning models trained on curated feature sets, often lacking integration with biological network information.

For instance, (Krishnan et al. 2016) utilized a support vector machine (SVM) trained on brain-specific gene expression features and interaction scores to rank ASD candidates. While effective, the model imposed arbitrary weighting schemes and did not generalize well across tissues or datasets. Similarly, (Asif et al. 2018) applied a Random Forest classifier using semantic similarity of gene ontology (GO) terms, which provided high classification accuracy but limited interpretability in a biological network context.

The key differentiators of the RWR-based approach presented in this thesis include:

- **Network Integration:** Unlike feature-based classifiers, this model leverages topological information from protein-protein interaction networks, enabling the identification of genes connected through shared biological pathways.
- **Semi-Supervised Learning:** The use of both positive and unlabeled data avoids the reliance on artificially labeled negative examples, reducing bias and better reflecting biological uncertainty.
- **Label Diffusion:** Rather than static feature learning, the RWR algorithm propagates known ASD gene labels across the network, capturing functional proximity and interdependence among genes.
- **Parameter Robustness:** The model showed consistent performance across a range of restart probabilities, data partitions, and network configurations, indicating strong generalizability.

In terms of output, the proposed model successfully recovered over 60% of the high-confidence SFARI genes in its top 5% predictions, comparable to or better than existing models, while also identifying novel candidates supported by enrichment analysis and literature evidence.

Thus, the RWR framework represents a biologically grounded and computationally efficient alternative to purely supervised classifiers, offering improved interpretability and performance in ASD gene discovery.

4.8 Limitations and Challenges

While the proposed RWR-based semi-supervised learning framework offers several strengths, including robustness, biological relevance, and scalability, it also has notable limitations that should be acknowledged to inform future research directions.

Dependence on Curated Databases: The model relies heavily on the SFARI and STRING databases for seed gene labels and interaction data. Although these databases are well-curated, they are inherently incomplete and subject to update. As a result, novel discoveries may be limited by the scope and recency of these datasets.

Label Ambiguity: The definition of negative examples in semi-supervised learning remains a challenge. Many genes labeled as "non-ASD" may simply be unstudied or poorly characterized. Mislabeling such genes could bias the diffusion process and affect ranking outcomes.

Static Network Assumption: The STRING-based interaction network is treated as static, ignoring dynamic changes in gene expression, cell type specificity, and developmental stage relevance. ASD is known to involve spatiotemporal variations in brain gene activity, which are not captured by a single static network.

Limited Feature Integration: The current framework focuses purely on network topology and label propagation without integrating additional gene features such as expression profiles, variant annotations, or epigenetic signals. This may restrict the model's ability to differentiate between highly connected genes with different biological roles.

Lack of Experimental Validation: The model's predictions, while supported by enrichment analysis and literature overlap, remain computational. No wet-lab validation was performed to confirm the functional role of top-ranked novel genes in ASD pathophysiology.

Threshold Sensitivity: Despite overall robustness, model performance can be influenced by hyperparameter settings (e.g., restart probability, STRING confidence threshold). While defaults were selected empirically, systematic optimization could further improve results.

In light of these limitations, future work could explore integrating tissue- and time-specific networks, refining label strategies using weak supervision or probabilistic labels, and incorporating multi-omics features. Additionally, collaboration with experimental labs to validate top candidates would enhance translational impact.

Acknowledging these challenges ensures transparency and provides a roadmap for refining network-based gene prioritization models in the context of complex disorders like ASD.

4.9 Future Directions

Building upon the results and insights from this study, several promising avenues for future research can be identified to enhance the effectiveness, interpretability, and translational potential of network-based gene prioritization frameworks for Autism Spectrum Disorder (ASD).

Integration of Multi-Omics Data: While this work focused solely on protein-protein interaction (PPI) networks, incorporating other data modalities, such as transcriptomics, epigenomics, and proteomics, could enrich the model's biological context. Integrating spatiotemporal gene expression profiles (e.g., BrainSpan), DNA methylation data, or chromatin accessibility (ATAC-seq) could provide a more dynamic and nuanced representation of ASD-relevant biology.

Dynamic and Context-Specific Networks: Current models use static interaction maps, which do not capture how gene interactions vary across brain regions, cell types, or developmental stages. Developing context-aware networks that reflect tissue specificity or time-series data could improve prediction accuracy and biological relevance.

Probabilistic Labeling Strategies: Refining the labeling mechanism by introducing probabilistic labels or confidence scores for seed genes may reduce the risk of misclassification, especially for genes with weak or emerging evidence. Semi-supervised frameworks like label spreading or graph neural networks (GNNs) can incorporate such uncertainty more effectively.

Deep Learning on Graphs: Graph-based deep learning techniques, such as Graph Convolutional Networks (GCNs) and Graph Attention Networks (GATs), offer powerful alternatives to traditional diffusion methods. These approaches can learn hierarchical representations of biological networks and may uncover complex non-linear dependencies between genes.

Cross-Disorder Analysis: ASD shares genetic overlap with other neurodevelopmental and psychiatric conditions, such as schizophrenia, intellectual disability, and epilepsy. A

comparative network analysis across multiple disorders could reveal shared pathways and novel pleiotropic genes.

Experimental Collaboration: To validate and extend the computational predictions, future work should involve experimental collaborations. In vitro or in vivo validation of top-ranked genes, e.g., via CRISPR screens, knockout models, or transcriptomic profiling, would provide functional confirmation and improve model credibility.

Public Accessibility and Tools: Developing a user-friendly web interface or R/Python package to share the ranked gene list and underlying network framework could facilitate broader adoption by the research community.

By exploring these directions, researchers can advance the application of network-based learning in complex trait genetics and help bridge the gap between computational predictions and biological discovery in ASD research.

CHAPTER 5: SUMMARY

This thesis presented a network-based semi-supervised learning framework for the prioritization of genes potentially associated with Autism Spectrum Disorder (ASD). By integrating curated ASD-related genes from the SFARI database with a high-confidence human protein-protein interaction (PPI) network from STRING, the Random Walk with Restart (RWR) algorithm was employed to diffuse label information and rank candidate genes based on their topological proximity to known ASD genes.

The proposed methodology demonstrated strong predictive performance across multiple evaluation metrics, including AUROC, AUPRC, and MCC, with consistent results across cross-validation folds. The top-ranked genes were not only enriched for known ASD candidates but also aligned with biological pathways implicated in synaptic signaling, ion transport, and neuronal development, core features of ASD pathophysiology. Enrichment analyses using Reactome and Enrichr further validated the biological significance of the model's predictions.

Compared to traditional supervised learning approaches, the RWR framework offers several advantages: it handles limited and uncertain labeling more effectively, leverages biological network topology, and avoids over-reliance on arbitrary feature selection. Moreover, the framework is computationally efficient and scalable, making it suitable for genome-wide analyses. Nonetheless, several limitations were acknowledged, including dependency on curated databases, the static nature of the network, and lack of experimental validation. Future directions were proposed to address these gaps, including multi-omics integration, use of dynamic or tissue-specific networks, graph-based deep learning, and functional validation via laboratory experiments.

In conclusion, this study underscores the potential of network propagation-based semi-supervised learning for discovering novel genetic contributors to complex disorders like ASD. The framework not only recovers known risk genes with high confidence but also proposes new candidates that warrant further investigation, thus contributing to the broader goal of elucidating the genetic architecture of autism.

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