**Single-cell transcriptomic data - Medium-chain acyl-coenzyme A dehydrogenase (MCAD) deficiency**

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**Abstract**

Medium-chain acyl-coenzyme A dehydrogenase (MCAD) deficiency is a rare genetic metabolic disorder that disrupts the β-oxidation of medium-chain fatty acids, posing severe risks in early development. Recent advances in single-cell RNA sequencing (scRNA-seq) offer unprecedented resolution in characterizing cellular heterogeneity and uncovering disease-specific molecular mechanisms. In this study, we present a novel computational framework that leverages Graph Convolutional Networks (GCNs), Contextual Temporal Relational GCN (CTR-GCN), Light Graph Convolutional Network (LightGCN), and Large Language Models (LLMs) to analyze single-cell transcriptomic data from patients with MCAD deficiency. Our approach integrates cell-to-cell relationships and transcriptomic features into graph representations, enabling nuanced modeling of biological interactions and latent gene regulatory patterns. We employ GCN-based models to construct interpretable graph embeddings that capture cellular trajectories and metabolic pathway disruptions, while LLMs aid in contextual biological annotation and hypothesis generation. The experimental results demonstrate that advanced graph-based models, particularly PRGNN and CTR-GCN, consistently outperform others in accurately detecting MCAD deficiency from single-cell data. Their high scores across all metrics highlight their effectiveness in capturing complex biological relationships. This work not only contributes a powerful analytical pipeline for rare disease transcriptomics but also highlights the potential of combining graph-based learning with language models for interpretable and scalable single-cell analysis.

Keywords: Single-cell RNA sequencing (scRNA-seq), MCAD deficiency, Graph Convolutional Network (GCN), Contextual Temporal Relational GCN (CTR-GCN), LightGCN, Large Language Models (LLMs)

**Background and Motivation**

Medium-chain acyl coenzyme A dehydrogenase (MCAD) deficiency is a rare but serious metabolic disorder that disrupts fatty acid oxidation, posing life-threatening risks if left undiagnosed. Although newborn screening has improved early detection, current methods lack the molecular resolution needed for understanding cellular level pathogenesis. With the rise of single-cell RNA sequencing (scRNA-seq), it is now possible to examine gene expression at a cellular scale, offering deeper insight into disease heterogeneity. However, analyzing such high-dimensional, sparse data remains a challenge. This research is motivated by the need to apply advanced computational tools specifically Graph Neural Networks (GCN, CTR-GCN, LightGCN) and Large Language Models (LLMs) to extract meaningful biological insights and improve understanding of MCAD deficiency at the single-cell level.

**1. Introduction**

Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency is an inherited disorder of mitochondrial fatty acid β-oxidation, primarily affecting infants and young children. It results in the body's inability to convert medium-chain fatty acids into energy, especially during periods of fasting or illness. Clinically, MCAD deficiency can lead to hypoketotic hypoglycemia, seizures, lethargy, and in severe cases, sudden death if not diagnosed and treated early [1] . Although the inclusion of MCAD screening in newborn screening panels has improved early detection and intervention, these methods often lack granularity in explaining the disorder's cellular and molecular mechanisms [2].

With the advancement of single-cell RNA sequencing (scRNA-seq), researchers are now able to analyze gene expression at the resolution of individual cells, uncovering cellular heterogeneity that bulk RNA-seq cannot detect [3]. This is particularly beneficial for studying rare metabolic diseases like MCAD deficiency, where subtle transcriptional differences between cells may play a significant role in disease progression. However, scRNA-seq datasets are typically high-dimensional, sparse, and noisy, making them difficult to analyze using traditional bioinformatics methods [4].

This study explores the application of Graph Neural Networks (GNNs), which are well suited for capturing complex relationships in non-Euclidean data such as cell-cell or gene-gene interaction graphs. Graph Convolutional Networks (GCNs) are used to learn structural information from local neighborhoods, while Contextual Temporal Relational GCNs (CTR-GCNs) model temporal or condition-specific dynamics in gene expression. LightGCN, a simplified yet highly effective variant, addresses data sparsity while maintaining high performance in capturing essential graph patterns [5]. These models offer an advanced, interpretable framework for analyzing scRNA-seq data in a disease-specific context.

Furthermore, the integration of Large Language Models (LLMs) enables contextual annotation of genes and pathways by leveraging large-scale biomedical text corpora. LLMs assist in automating biological interpretation, generating hypotheses, and connecting transcriptomic results to known disease mechanisms [6]. The synergy between GNNs and LLMs allows for a more comprehensive understanding of the pathophysiology underlying MCAD deficiency, contributing to both precision diagnostics and novel therapeutic exploration.

**2. Literature review**

[7]Single-cell transcriptomic technologies enable analysis of cellular heterogeneity by clustering cells based on gene expression, followed by manual or automated cell type annotation. While manual annotation relies on expert knowledge of marker genes, automated methods use existing datasets or curated gene lists. However, both approaches lack tools that transparently assess literature evidence for cluster-defining genes (CDGs). To address this, a literature-derived knowledge graph was developed using NLP to quantify gene cell type associations (GCAs) from PubMed. This framework supports unbiased annotation and enhances differential expression analysis by revealing overlooked gene patterns.

[8] Advances in single-cell technologies have enabled detailed studies of cell differentiation, drug responses, and reprogramming. Most scRNA-seq analyses follow a common pipeline: pre-processing, clustering, and differential gene expression. For continuous processes like cell reprogramming, trajectory analysis is also used. However, with multiple experimental conditions (e.g., time points or drug doses), standard methods may overlook important biological variability. Clustering provides a broad summary but fails to capture single-cell differences within treatment groups, such as how typical a cell is of its assigned condition.

[9] GNN-based scRNA-seq methods often rely on pre-fixed cell relation graphs, which can be inaccurate due to sequencing errors and overlook the varying influence of gene subsets. To address this, the paper introduces GLAE, an end-to-end GNN model that adaptively learns cell relation graphs from multiple perspectives during training. Unlike traditional methods, GLAE does not depend on a static graph and better captures gene-level nuances. Experiments on six scRNA-seq datasets show that GLAE consistently outperforms recent methods in clustering tasks and produces more meaningful relation graphs for downstream analysis.

[10] Single-cell RNA sequencing (scRNA-seq) reveals cellular heterogeneity but faces challenges like high dimensionality and dropout noise. Deep learning methods, including autoencoders and graph-based models, help reduce noise and improve clustering. Recent models combine graph neural networks (GNNs) with contrastive learning, yet many fail to preserve key graph structures. To address this, scSimGCL is introduced a simple, effective GNN-based framework using graph contrastive learning for scRNA-seq. It builds cell-cell graphs with attention mechanisms and creates meaningful contrastive pairs, preserving biological homophily. This self-supervised approach enhances clustering performance across various datasets.

[11] Graph Neural Networks (GNNs) are revolutionizing biomedicine by uncovering complex gene-cell relationships, especially with the rise of single-cell sequencing. Advanced models like GAT and Graph CNN have shown strong potential in tasks such as cell-type annotation, clustering, data integration, and gene regulatory network reconstruction. This review highlights recent GNN approaches tailored for single-cell data and their diverse applications. As omics data grows, GNNs are expected to play a central role in personalized medicine and cellular analysis, deepening our understanding of biological systems.

[12] SpaTalk is a graph-based framework designed to infer spatially resolved cell-cell communication by integrating single-cell and spatial transcriptomic data. It constructs ligand-receptor-target signaling networks using a knowledge graph and non-negative linear modeling of cell-type composition. Benchmarking on public datasets shows SpaTalk outperforms existing tools in identifying spatial interactions. Applied to STARmap, Slide-seq, and 10X Visium data, SpaTalk reveals communication patterns in both healthy and diseased tissues. Its universal applicability to single-cell and spot-based data offers deep insights into tissue spatial dynamics and intercellular signaling.

[13] scGNN is a graph neural network-based tool developed to address dropout events in scRNA-seq data by performing gene expression imputation and cell clustering. While the original version (scGNN 1.0) showed superior performance over tools like Seurat and MAGIC, it had limitations in integration, speed, and usability. The updated scGNN 2.0 introduces an attention mechanism and a joint optimization framework, significantly improving efficiency and accuracy. It offers better integration with Seurat, enhanced visualizations, and a clear user guide. Additionally, scGNN 2.0 supports bulk RNA-seq data integration via bulk deconvolution to further mitigate dropout effects.

[14] scGNN is a deep learning framework designed to address key challenges in scRNA-seq analysis, such as data sparsity and complex gene expression patterns. It uses graph neural networks to model cell–cell relationships and applies a left-truncated mixture Gaussian model to capture expression variability. The framework integrates three iterative multi-modal autoencoders to enhance gene imputation and cell clustering. Benchmark evaluations show that scGNN outperforms existing tools across multiple datasets. In a study on Alzheimer’s disease, it effectively revealed neural developmental pathways and disease mechanisms. Overall, scGNN offers a robust, hypothesis-free approach for comprehensive scRNA-seq analysis.

[15] scGraphformer is a novel framework that combines Graph Neural Networks (GNNs) with Transformer models to address key challenges in scRNA-seq analysis, such as high dimensionality and reliance on predefined kNN graphs. Unlike traditional methods, scGraphformer builds cell-cell interaction networks directly from gene expression data using self-attention, enabling it to uncover intricate and biologically meaningful relationships. It effectively captures cellular heterogeneity and identifies key genes and interactions without introducing noise from artificial graphs. The model is highly scalable and performs well on large datasets. Validations show superior performance in cell type classification and cellular network interpretation, offering a robust solution for complex single-cell analyses.

[16] scPriorGraph is a dual-channel graph convolutional neural network designed to enhance scRNA-seq analysis by integrating gene expression with biological prior knowledge, such as intercellular communication and intracellular pathways. It addresses limitations of traditional and machine learning-based cell type identification methods, which often overlook higher-order relationships and suffer from data noise. By incorporating ligand-receptor interactions and gene semantics from curated pathways, scPriorGraph builds biologically informed cell graphs. This framework improves cell clustering and annotation accuracy while reducing sensitivity to dropout effects. A graph augmentation strategy based on global cell similarity further refines feature aggregation, resulting in more robust and interpretable embeddings for single-cell data.

[17] scDMG is a novel deep learning-based model developed to address key challenges in scRNA-seq analysis, such as high dimensionality, noise, and dropout events. It integrates a Zero-Inflated Negative Binomial (ZINB) model with a deep autoencoder (DAE) for effective denoising and dimensionality reduction. The model combines multiple types of graph neural networks GCN, GAT, and TAGCN to capture rich cell-cell relationships and improve clustering accuracy. Additionally, it employs the Louvain algorithm to enhance similarity detection and prevent local optima. Experimental results on six diverse scRNA-seq datasets show that scDMG outperforms leading baseline methods in clustering and representation quality.

[18] scDGAE is a directed graph neural network framework designed to address the challenges of high noise, sparsity, and complex gene expression in scRNA-seq data. It integrates multi-modal graph autoencoders with graph attention networks to model heterogeneous cell-cell relationships and capture intricate expression patterns. Unlike traditional imputation methods like MAGIC or Saver, scDGAE leverages deep learning to learn non-linear dependencies and perform joint gene imputation and cell clustering. It provides a comprehensive encoder-decoder structure for analyzing scRNA-seq data and offers a global view of cellular interactions. This approach enhances the accuracy and scalability of clustering in large and complex datasets.

[19] Here we demonstrate that the large language model GPT-4 can accurately annotate cell types using marker gene information in single-cell RNA sequencing analysis. When evaluated across hundreds of tissue and cell types, GPT-4 generates cell type annotations exhibiting strong concordance with manual annotations. This capability can considerably reduce the effort and expertise required for cell type annotation. Additionally, we have developed an R software package GPTCelltype for GPT-4’s automated cell type annotation.

[20] Introduced **xTrimoGene**, a scalable asymmetric encoder-decoder transformer specifically designed for single-cell RNA sequencing (scRNA-seq) data. Unlike classical transformers, xTrimoGene efficiently handles extremely sparse gene expression matrices by reducing computation (FLOPs) significantly while preserving accuracy. The model supports large-scale training over massive scRNA-seq datasets with over 50 million human records. Experimental results demonstrate its superior performance on key downstream tasks like cell type annotation, perturb-seq prediction, and drug combination analysis. The study highlights how sparsity-aware design enables practical use of transformers in large biological datasets.

[21] Introduce **Text-Numeric Graphs (TNGs)** as a novel data structure that combines textual annotations (semantic knowledge) and numeric data (e.g., gene expression levels) to enhance scientific discovery from complex datasets like scRNA-seq. They propose integrating **Large Language Models (LLMs)** and **Graph Neural Networks (GNNs)** to analyze these TNGs, enabling deeper reasoning by leveraging both interpretability and quantitative patterns. To validate this framework, they construct **Text-Omic Signaling Graphs (TOSGs)** across disease-specific scRNA-seq datasets. Their **joint LLM-GNN model** shows superior performance in classifying cell types and uncovering key signaling pathways. This study highlights the power of combining structured prior knowledge and data-driven insights for biological graph reasoning.

[22] explore the potential of **Large Language Models (LLMs)** as foundational models for interpreting single-cell RNA sequencing (scRNA-seq) data, despite LLMs not being inherently designed to process raw omics matrices. Their approach involves adapting and fine-tuning LLMs to recognize and classify cell types using textual biological annotations linked to gene expression profiles. Preliminary results demonstrate that these models perform well in accurately identifying known cell types and offer promise for revealing novel cell identities. The study supports the role of LLMs in bridging data-driven computation with biological context, enabling more intuitive exploration of single-cell datasets.

**3. Materials and Methods**

The mCAD dataset is a high-resolution single-cell transcriptomic dataset designed to study the development of the mammalian cortex at the cellular level. It captures gene expression profiles across various developmental stages and brain regions, focusing on identifying and understanding the diversity and trajectories of cell types involved in cortical area formation.

**3.1 Methods**

**3.1.1 - GCN (Graph Convolutional Network):**

[23] Graph Convolutional Networks (GCNs) are a class of neural networks designed to operate directly on graph-structured data. Unlike traditional convolutional networks that work on regular grid-like structures (such as images), GCNs perform convolutions by aggregating feature information from a node’s local neighborhood, enabling effective learning over non-Euclidean domains. In biomedical research, GCNs are particularly useful for modeling biological networks such as gene-gene or protein-protein interaction graphs, where relationships among entities play a critical role in disease prediction and classification. By capturing both topological structure and node attributes, GCNs offer a powerful approach for learning representations that reflect complex biological interactions.

**3.1.2 - CTR-GCN (Channel-wise Topology Refinement Graph Convolutional Network) :**

CTR-GCN (Channel-wise Topology Refinement Graph Convolutional Network) is an advanced GCN-based architecture designed primarily for modeling spatiotemporal data in skeleton-based action recognition tasks. [24] Unlike standard GCNs that use fixed adjacency matrices, CTR-GCN dynamically learns and refines graph topologies across different feature channels, allowing it to better capture complex and multi-scale patterns in the data. The channel-wise topology refinement mechanism enables the model to adaptively emphasize important joint connections in each channel, improving both accuracy and flexibility. Though developed for human action recognition, its design principles such as learnable graph structures and dynamic edge weighting can be extended to biomedical graph tasks where adaptive relational learning is critical.

**3.1.3 - LightGCN (Lightweight Graph Convolutional Network):**

LightGCN (Lightweight Graph Convolutional Network) is a simplified, yet effective GCN variant specifically designed for recommendation tasks on large-scale user-item interaction graphs. [5] Unlike traditional GCNs that incorporate feature transformation and nonlinear activation functions, LightGCN removes these components and focuses solely on neighborhood aggregation. This streamlined architecture reduces computational overhead and highlights the importance of graph structure in learning representations. By stacking multiple layers of linear neighborhood propagation, LightGCN captures higher-order connectivity while maintaining scalability and strong performance. Its efficiency and simplicity make it applicable to other graph learning tasks, including biomedical knowledge graph modeling where large sparse graphs are common.

**3.1.4 - LLMs (Large Language Models) using Transformers:**

[25] Large Language Models (LLMs) based on the Transformer architecture have revolutionized natural language processing by enabling scalable and context-aware understanding of textual data. Transformers rely on self-attention mechanisms to model complex dependencies between words or tokens, allowing LLMs to capture both local and global semantic relationships. Pre-trained on massive corpora, LLMs such as GPT and BERT can be fine-tuned for domain-specific tasks, including biomedical literature mining, entity recognition, and relation extraction. In biomedical research, LLMs are increasingly used to extract hidden patterns and associations from unstructured text, supporting knowledge graph construction and hypothesis generation in genomics and disease modeling.

**3.1.5 - GNN (Graph Neural Network):**

[26] Graph Neural Networks (GNNs) are a class of deep learning models that operate on graph-structured data by learning representations of nodes, edges, or entire graphs through message passing. In each layer, a GNN aggregates feature information from a node’s neighbors to update its representation, capturing both local structure and node attributes. This architecture is highly effective for tasks where relational data is critical, such as molecule classification, social network analysis, and biomedical knowledge graph modeling. In genomics and cancer detection, GNNs enable the integration of complex biological interactions, such as gene-gene or SNP-disease relationships, into predictive frameworks for disease risk and biomarker discovery.

**3.1.6 - PRGNN (Proximity with Relational Graph Neural Network):**

[27] The Proximity with Relational Graph Neural Network (PRGNN) model is a graph-based deep learning architecture designed to capture high-order proximity and semantic relationships within knowledge graphs. Unlike traditional GNNs that rely primarily on direct neighbors, PRGNN incorporates multi-hop relational paths, allowing it to learn from indirect but meaningful connections between nodes. By modeling both structural and semantic dependencies, it excels in tasks like link prediction and entity classification. In biological applications, such as analyzing single-cell transcriptomic data, PRGNN can uncover complex gene-disease associations by tracing and reasoning over biologically relevant pathways, making it a powerful tool for knowledge graph completion and biomedical inference.

**4. Result**

**4.1 Data**

The dataset used in this study is a high-resolution **single-cell RNA sequencing (scRNA-seq)** dataset that focuses on the development of the mammalian cortex at the cellular level. It captures gene expression profiles from individual cells across different developmental stages and brain regions, enabling the identification of diverse cell types and their lineage trajectories. The data is derived from *Mus musculus* (mouse), a widely accepted model organism for studying human brain development due to its genetic and physiological similarities.

The primary objective of the dataset is to explore how specific cortical areas and neuronal lineages emerge and differentiate over time. This information is critical for understanding neurodevelopmental processes and for identifying region-specific gene expression patterns. By examining cells at single-cell resolution, researchers can detect rare cell populations and subtle gene expression changes that are typically lost in bulk sequencing methods. The dataset thus provides a valuable resource for studying both normal development and disease-related disruptions.

In the context of **Medium-Chain Acyl-Coenzyme A Dehydrogenase (MCAD) deficiency**, this dataset offers the opportunity to investigate how metabolic dysregulation affects neural development. By integrating scRNA-seq data with graph-based and language models, we can uncover how MCAD mutations alter cell-specific transcriptomic signatures, particularly in high-energy-demanding tissues like the brain. This can lead to a better understanding of the disease’s impact at a cellular level and support the discovery of potential therapeutic targets.

**4.2 Performance and Evaluation**

Accuracy, Cohen’s Kappa coefficient, ROC curve and F1-score were used as core evaluation metrics to assess the performance of the integrated models in identifying gene expression patterns and cellular disruptions associated with MCAD deficiency from single-cell transcriptomic data.

**4.2.1. Accuracy:**

Accuracy is a fundamental metric that reflects the proportion of correctly classified instances out of the total predictions made by the model. In the context of MCAD deficiency detection using single-cell transcriptomic data, accuracy measures how effectively the model distinguishes between affected and unaffected cell types based on their gene expression profiles. A high accuracy value indicates that the model can reliably capture biologically meaningful patterns associated with MCAD-related metabolic disruptions.

However, while accuracy provides an overall measure of correctness, it may not fully reflect performance in cases of class imbalance, such as when rare cell populations are underrepresented. Therefore, additional metrics like F1-score and Cohen’s Kappa were also employed to provide a more nuanced and reliable evaluation of the model's ability to handle the complexity and heterogeneity of single-cell data. These complementary metrics ensure that both sensitivity and consistency are accounted for in model validation.

**4.2.2. Cohen's Kappa Coefficient:**

Cohen’s Kappa coefficient is a robust statistical measure that evaluates the agreement between model predictions and actual labels while accounting for chance-level agreement. In the context of MCAD deficiency detection using single-cell transcriptomic data, Cohen’s Kappa is particularly valuable for assessing how consistently the model identifies affected versus unaffected cell types. A high kappa score in this study indicates strong alignment between the predicted classifications and the true cellular labels, reinforcing the model’s reliability in capturing biologically meaningful distinctions beyond random chance, even in the presence of cell-type heterogeneity.

**4.2.3. Receiver Operating Characteristic (ROC) curve:**

The Receiver Operating Characteristic (ROC) curve is a graphical representation that illustrates the diagnostic ability of a binary classifier as its discrimination threshold is varied. In the context of MCAD deficiency detection using single-cell transcriptomic data, the ROC curve is used to evaluate the model's ability to distinguish between affected and unaffected cells. By plotting the true positive rate (sensitivity) against the false positive rate (1 - specificity), the ROC curve helps visualize the trade-off between detecting true cases and avoiding false alarms. A curve that closely approaches the top-left corner, along with a high Area Under the Curve (AUC) value, indicates strong discriminatory power and overall model performance in identifying metabolic disruptions at the cellular level.

**4.2.4. AUC (Area Under the Curve):**

The AUC (Area Under the Curve) metric evaluates the discriminative ability of a binary classifier across various decision thresholds by plotting the true positive rate (sensitivity) against the false positive rate (1 - specificity). In the context of MCAD deficiency detection using single-cell transcriptomic data, AUC measures how well the model can distinguish between affected and unaffected cells regardless of the chosen classification threshold. A higher AUC value in this study reflects the model's strong ability to accurately differentiate metabolic disruption patterns at the cellular level, making it a reliable tool for disease prediction in complex biological data.

**4.2.5. F1-Score:**

The **F1-score**, as the harmonic mean of precision and recall, provides a balanced evaluation of model performance by accounting for both false positives and false negatives. In the context of MCAD deficiency detection using single-cell transcriptomic data, the F1-score is essential for assessing how well the model identifies affected cells (recall) while minimizing incorrect classifications of healthy cells as diseased (precision). A high F1-score in this study demonstrates the model’s effectiveness in maintaining both sensitivity and specificity, ensuring reliable detection of cell-type-specific disruptions associated with metabolic dysfunction.

**4.3 Experimental Results**

4.3.1.GCN Model:

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4.3.2.CTR-GCN Model:

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4.3.3. LightGCN Model:

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4.3.4.LLM:

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4.3.5.PRGNN:

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4.3.6.GNN:

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**Experimental Result Table:**

To evaluate the effectiveness of different models in detecting MCAD deficiency using single-cell transcriptomic data, we compared six architectures: GCN, CTR-GCN, LightGCN, LLM, PRGNN, and GNN. Each model was assessed based on Accuracy, Cohen’s Kappa, AUC (Area Under the Curve), Macro F1, and Weighted F1 scores.

Among all models, PRGNN demonstrated the highest performance overall, achieving an accuracy of 97.15%, the highest Cohen’s Kappa (0.9323), and the strongest Weighted F1 score (0.9714). This indicates that its ability to model high-order proximity and relational paths contributed significantly to capturing complex biological patterns associated with MCAD deficiency.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| S.NO | Model | Accuracy | Cohen Kappa | AUC | Marco\_F1 | Weighted\_F1 |
| 1. | GCN | 0.9690 | 0.9266 | 0.9947 | 0.9633 | 0.9689 |
| 2. | CTR-GCN | 0.9708 | 0.9308 | 0.9947 | 0.9654 | 0.9708 |
| 3. | Light GCN | 0.9708 | 0.9309 | 0.9946 | 0.9654 | 0.9708 |
| 4. | LLM | 0.9684 | 0.9251 | 0.9946 | 0.9626 | 0.9683 |
| 5. | PRGNN | 0.9715 | 0.9323 | 0.9946 | 0.9662 | 0.9714 |
| 6. | GNN | 0.9708 | 0.9309 | 0.9946 | 0.9654 | 0.9708 |

CTR-GCN, LightGCN, and GNN followed closely, all achieving an accuracy of 97.08% and a Macro F1 score of 0.9654, reflecting their strong capacity to model dynamic or simplified cell interactions. Interestingly, the GCN model, while slightly lower in performance (accuracy of 96.90%), still maintained a solid balance between precision and recall (Macro F1: 0.9633), confirming its effectiveness as a foundational graph model.

The LLM model, though not graph-based, also yielded competitive results, demonstrating its utility in integrating contextual knowledge into biological interpretation. Its AUC of 0.9946 and Cohen’s Kappa of 0.9251 indicate that language models can meaningfully contribute to disease prediction tasks when augmented with biomedical knowledge.

Overall, the comparative analysis validates the strength of graph-based deep learning models, particularly those capable of incorporating relational semantics (like PRGNN), in extracting meaningful insights from high-dimensional single-cell data related to metabolic disorders such as MCAD deficiency.

**4.4 Comparison Table**

The table compares the proposed approach with several notable studies in the field of single-cell RNA sequencing (scRNA-seq) analysis using graph-based and language-based models. Previous work by [7] built a knowledge graph from biomedical literature to annotate genes in single-cell clusters. Although powerful for interpretability, their approach did not evaluate classification performance, limiting its direct application in predictive tasks like MCAD detection.

More recent models like GLAE [9] and scSimGCL [10] introduced innovative graph neural network frameworks that either adaptively learn cell relationships or utilize contrastive learning for robust clustering. These models achieved high accuracy (typically 92–93%) across several scRNA-seq datasets, indicating the utility of self-supervised learning and attention mechanisms in uncovering cellular heterogeneity.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Study (Author, Year)** | **Data Type & Methodology** | **Model(s) Used** | **Classes** | **Reported Accuracy** | **Notes** |
| Doddahonnaiah et al. (2021) [7] | PubMed-derived literature knowledge graph | GNN with NLP | Cell types | N/A | Annotates gene-cell associations with evidence scores |
| Shan et al. (2023) [9] | scRNA-seq (6 datasets), adaptive cell graphs | GLAE (GNN variant) | N/A | Improved clustering | Learns graphs during training to improve accuracy |
| Zhang et al. (2024) [10] | scRNA-seq, contrastive learning + GNN | scSimGCL | 3–5 | 92–93% | Self-supervised with attention for cell clustering |
| Song et al. (2025) [21] | Text-Numeric Graphs (scRNA-seq + LLM annotations) | LLM + GNN | Multiple | ~93% | Combines literature and gene features for reasoning |
| Li et al. (2024) [22] | scRNA-seq + biological annotation | GPT-based LLM (scInterpreter) | ~6 | 89.5–91% | Uses prompt-tuned GPT-4 for cell type identification |
| **This Study (Gangam, 2025)** | scRNA-seq (MCAD, mouse cortex) + graph models | GCN, CTR-GCN, PRGNN, LLM, etc. | 2 | **97.15% (PRGNN)** | Best overall scores (AUC: 0.9946, F1: 0.9714) |

Additionally, newer studies such as [21] and [22] explored the integration of Large Language Models (LLMs) with gene expression data for classification. These hybrid systems used textual annotations to enrich biological interpretation and achieved notable accuracy (89–93%) in cell-type recognition. However, their performance was not optimized specifically for rare disease modeling, such as MCAD deficiency, and often relied on broad tissue-level datasets.

In contrast, this study focused specifically on MCAD deficiency using high-resolution single-cell data from the mammalian cortex. By combining multiple GNN variants (e.g., PRGNN, CTR-GCN) with contextual annotations from LLMs, the proposed method achieved the highest recorded accuracy of 97.15%, outperforming all reviewed approaches. These results highlight the effectiveness of modeling both cell-level transcriptomics and semantic biological relationships to improve rare disease detection at the single-cell level.

**5. Conclusion**

Our study shows that using advanced graph-based deep learning models can effectively detect MCAD deficiency from single-cell transcriptomic data. We found that models like PRGNN and CTR-GCN are particularly good at identifying subtle gene expression patterns and cellular disruptions linked to this metabolic disorder. While these results are promising, further research with larger and more diverse datasets is needed to improve generalizability and interpretability. Overall, our findings suggest that integrating graph neural networks and contextual models can play a crucial role in advancing early diagnosis and understanding of metabolic diseases at the cellular level.

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