Synergistic Treatment of Multiple Sclerosis by Ketogenic Diet and Vitamin D

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Abstract. Multiple sclerosis (MS) is a chronic autoimmune disease in which the immune system mistakenly attacks the myelin sheath, resulting in the demyelination of neurons in the central nervous system (CNS). This damage leads to symptoms such as fatigue, blurred vision, muscle weakness, and cognitive impairment. Globally, about 2.8 million people live with MS, making it a significant neurological burden. Current treatments for MS are difficult to implement because they are expensive, associated with side effects, and vary in availability across countries, which impacts their effectiveness. Nutrition has emerged as an important aspect of MS treatment. Ketogenic diets (KDs), which are low in carbohydrates and high in fats, have been shown to protect the nervous system and reduce inflammation in MS. In addition, low vitamin D levels are recognized as a risk factor for MS. Using single-nucleus and single-cell RNA sequencing analyses, together with oligodendrocyte cell line experiments, this study provides evidence that vitamin D and the KD may act synergistically to prevent MS development. These findings suggest a potential dietary approach to lowering MS risk that is simple to implement and could benefit populations across diverse social and economic conditions.

Keywords: Multiple sclerosis (MS), Ketogenic diet, Vitamin D, Single cell RNA-sequencing, Single cell ATAC-sequencing, Oligodendrocytes.

1. Introduction

Multiple sclerosis (MS) is a chronic autoimmune and inflammatory disease, affecting about 2.8 million people worldwide (Walton et al., 2020). It is characterized by demyelination in the central nervous system (CNS), caused by abnormal immune responses in which T cells enter through blood vessels and damage nerve cells (Baecher-Allan et al., 2018). MS may impair cognition and affect the mobility of patients, decreasing their quality of life. Clinically, MS can advance in four disease courses: clinically isolated syndrome, relapsing-remitting, secondary progressive, and primary progressive. Although there are types of disease-modifying therapies (DMT) that can modify disease progression, currently, there is still no effective cure for MS (Mayo Clinic, 2018). Also, there are significant global inequalities in access to treatment and comprehensive care because of disparities in the availability of specialists and funding of therapies by governments (Alonso et al., 2019). For example, in China, only 10% of patients receive standard DMTs. Because MS has a long disease course and receives limited public attention in China, only a small proportion of patients adhere to long-term DMT treatment. In addition, high costs, limited effectiveness, inconvenience, and side effects further contribute to this remarkably low treatment rate (*Chinese Multiple Sclerosis Patients Survival Report*, 2019). Here, the unmet medical question is the need for a long-term stable form of treatment to alter the disease progression of MS. Given this context, dietary regulation emerges as a potential solution. Accordingly, this study asks: How can dietary regulation alter the disease progression of MS?

Ketogenic diets (KDs) are low in carbohydrates and high in fat and protein. Conventionally, KD has been used to treat epilepsy and seizures. KDs can increase the production of ketone bodies, including acetone, acetoacetic acid, and beta-hydroxybutyrate (Buscemi et al., 2021). Recent studies have also explored the use of KDs in Alzheimer's disease (AD). A randomized controlled trial demonstrated that a 12-week modified ketogenic diet improved daily functioning and quality of life in patients with mild to moderate AD (Kashi et al., 2021). Additionally, a narrative review concluded that KD could enhance cognitive abilities and quality of life across various stages of AD (Pereira et

al., 2021). These ketone bodies, generated during fat metabolism, can stimulate the production of neurotransmitters and reduce inflammation and oxidation (Prudencio et al., 2021). With the ability to cross the blood-brain barrier, ketone bodies also serve as an energy source for the brain (Detopoulou et al., 2022). Previous studies have reported a positive correlation between the use of KD and serum Vitamin D levels in certain groups of people, like obese patients and patients with type 2 diabetes mellitus (Buscemi et al., 2021; Almsaid & Khalfa, 2020). A possible causation is that the Vitamin D stored in the adipose tissue of the patients can be released upon taking KD (Buscemi et al., 2021). Also, in multiple animal studies, including murine models and rat models, KD reduced the levels of many inflammatory-related cytokines, like TNF-α, IL-1β, and IFN-γ (Kim et al., 2012; Lu et al., 2018), suggesting its anti-inflammatory effects on MS patients.

KD have been shown to influence serum Vitamin D levels, suggesting a potential link between dietary interventions and MS-related risk factors. Vitamin D, a fat-soluble vitamin, plays crucial roles in the nervous, immune, and cardiovascular systems, and most of the daily Vitamin D need is fulfilled by sunlight exposure (Buscemi et al., 2021; Almsaid & Khalfa, 2020). Serum vitamin D levels are related to 25-hydroxy vitamin D [25(OH)D] levels (Buscemi et al, 2021), which have been proven to be having a causal effect on preventing the developing MS (Rhead et al., 2016). Low Vitamin D levels have been identified as a risk factor for MS, which is a major factor in the remarkable MS prevalence at high latitudes (Lucas et al., 2011). The important effect Vitamin D has on MS onset may be explained by its ability to reduce demyelination and lower the expression of inflammation-causing enzymes (Miclea et al., 2020). While the association between KD and vitamin D levels is established, a critical gap remains in understanding the precise mechanistic pathway through which this interaction influences the pathogenesis of MS.

The central hypothesis of this research is that the synergistic treatment of KD and Vitamin D can slow MS progression and reduce risks of MS. Both Vitamin D and KD can alleviate inflammation and the promoting effect of KD on Vitamin D levels. A possible mechanism for the anti-inflammatory effects of KD is its stimulation of proliferator-activated receptor-gamma, lowering inflammation levels (Zhang et al., 2018). The fatty acid metabolism increased by KD produces β-hydroxybutyrate (BHB), which can reduce inflammation through pathways like regulating the activation of inflammatory macrophages and microglia cells and promoting the production of anti-inflammatory enzymes (Deng et al., 2021; Goldberg et al., 2017). As mentioned previously, low serum 25(OH)D levels have a causal effect on developing MS (Rhead et al., 2016). In experimental autoimmune encephalomyelitis (EAE) models, which are commonly used for simulating MS progression, the activated metabolized form of Vitamin D, $1,25(OH)_2D_3$, has been shown by multiple studies to be able to decrease the autoimmune effect by preventing the entering of T cells into the brain, impeding the activation of Helper T cells, and promoting the formation of Interleukin-10, a critical anti-inflammatory chemical (Fernandes de Abreu et al., 2009; Grishkan et al., 2013; Molina-Holgado et al., 2001). Apart from its effects in reducing inflammation, $1,25(OH)_2D_3$ can attenuate demyelination in the brain, because it can promote the removal of myelin debris by microglia, accelerate the remyelination via speeding the growth of oligodendrocytes, and stimulate the clearance of harmful proteins like β-amyloid polypeptides (Garcion et al., 1997; Masoumi et al., 2009; Nystad et al., 2014).

To investigate the molecular and cellular mechanisms underlying these effects, we employed a multi-level experimental approach. Single-cell RNA-sequencing (scRNA-seq) and single-cell ATAC-sequencing (scATAC-seq) were used to capture gene expression and chromatin accessibility at the single-cell level, which allowed us to identify cell type-specific transcriptional and regulatory changes in response to KD and Vitamin D. Complementing the single-cell analyses, protein expression assays, including Western blotting and fluorescent labeling of myelin-related proteins, and cell viability assays were performed to validate molecular changes and assess the functional impact of KD and Vitamin D supplementation. Together, this multi-level approach provides a comprehensive framework to elucidate how dietary regulation and Vitamin D modulate MS progression. These mechanisms may explain the effect of Vitamin D on MS incidence and progression. Together, this

strategy provides a comprehensive framework for elucidating how dietary regulation and Vitamin D modulate MS progression.

2. Methods

2.1. Bioinformatics analysis

2.1.1 Data Source

Data from multiple datasets were analyzed to ensure the objectivity and accuracy of the results. First, a study done by Zheng et al. (2023) was utilized, which is an open-access single-cell multiome ATAC and RNA sequencing dataset. In the research, Zheng et al. created a mouse experimental autoimmune encephalomyelitis (EAE) model, a commonly used standard for studying the immunology of MS, using Sox10: Cre-RCE:LoxP mice in order to specifically target and isolate oligodendrocyte cells for analysis (Zheng et al., 2023). They did single-cell multiome ATAC and RNA sequencing to oligodendrocytes of the EAE model at different stages of disease progression. The dataset is available in Gene Expression Omnibus (GEO) under the access code GSE250589. Next, a study by Jäkel et al. (2019) were utilized, which is an open-access single-nuclei RNA-sequencing dataset. In the original research, the researchers investigated altered oligodendrocyte heterogeneity in human MS. They divided participants into different groups, including 5 control samples, 4 progressive MS samples, further divided into non-lesioned and lesioned matter groups (Jäkel et al., 2019). They then used single-nuclei RNA-sequencing to analyze gene expression in individual nuclei of the white matter.

2.1.2 Preprocessing and Quality Control

Single-cell RNA sequencing data were processed and analyzed using the Seurat package in R, a widely used toolkit for single-cell data analysis. Standard quality control procedures were applied to remove low-quality or damaged cells, such as cells with unusually high mitochondrial gene content, low total gene counts, or extreme library sizes. After filtering, expression values were normalized and scaled to account for differences in sequencing depth and technical variation, ensuring that downstream analyses reflect true biological differences. Principal component analysis (PCA) and other dimensionality reduction methods were applied to assess data structure and detect potential outliers.

2.1.3 Gene Expression and Differential Analysis

Gene expression profiles were compared across different disease stages to identify transcriptional changes associated with progression. Expression levels of selected genes of interest were quantified within each group and across disease phases. Gene expression patterns were visualized using violin plots and dot plots generated in Seurat, allowing for clear comparison of distribution and expression intensity across cells. Differential expression analyses were conducted to identify genes with significant changes between conditions, which were further examined for their potential biological relevance.

2.2. Cell Line and Maintenance

The human oligodendrocyte cell line **MO3.13** (Whelab, Cat. No. G0200) was used in this study. Cells were thawed rapidly in a 37 °C water bath for 1–2 minutes until just a small ice pellet remained, then immediately transferred into pre-warmed high-glucose DMEM containing 10% FBS and 1× penicillin-streptomycin (Pep/Strep). Cells were seeded into 60 mm culture dishes at an initial density of approximately 2-3 × 10^5 cells per dish and incubated at 37 °C in a humidified incubator with 5% CO_2. No additional substrate coating was required for attachment. The culture medium was replaced every 2–3 days. Once cultures reached 80–90% confluence, cells were detached with 0.25% trypsin-EDTA and subcultured at a ratio of 1:3–1:4. All experiments were performed using cells between passages 3 and 10 to minimize variability.

2.3. Treatment Conditions

To mimic ketogenic diet (KD)-like conditions, cells were cultured in glucose-free DMEM supplemented with high levels of fat, protein, and ketone bodies. Control groups were maintained in standard high-glucose DMEM supplemented with 10% FBS and 1× penicillin/streptomycin. For vitamin D supplementation, treatment groups received vitamin D at final concentrations of 10 nM, 100 nM, or 1 μM, while control groups were cultured under identical conditions without vitamin D. To model multiple sclerosis (MS)-like demyelination, cultures were treated with 50 μM lysophosphatidylcholine (LPC) for 24 h, as described previously (Tian et al., 2020). Corresponding control groups without LPC treatment were included for comparison. All treatments were performed under standard cell culture conditions, and experiments were repeated in triplicate to ensure reproducibility.

2.4. Protein Expression Analysis

The expression of myelin basic protein (MBP) and proteolipid protein (PLP) was assessed by Western blotting and fluorescent labeling. Briefly, proteins were separated by SDS-PAGE and transferred to PVDF membranes. After blocking to reduce non-specific binding, membranes were incubated with primary antibodies against MBP or PLP, followed by HRP-conjugated secondary antibodies. Chemiluminescent detection was performed using the Super ECL reagent (Yeasen, China) according to the manufacturer's protocol. Membranes were incubated with the substrate at room temperature, and signals were visualized on X-ray film with exposure times ranging from seconds to several minutes. Signal intensity was quantified by densitometry and normalized to a loading control to assess relative protein expression levels.

2.5. Cell Viability Assay

Cell viability was determined using the CCK-8 assay. Cells were seeded into 96-well plates and treated as described above. After treatment, 10 μL of CCK-8 reagent was added to each well and incubated for 2 h to allow for formazan development. Absorbance was measured at 450 nm using a Victor X4 2030 multilabel reader. Cell viability was calculated relative to untreated control wells, and all assays were performed in triplicate to ensure reproducibility.

3. Results

3.1. Quality Control of Sequencing Datasets

To ensure the reliability of downstream analyses, we performed rigorous quality control on the single-cell RNA and ATAC sequencing datasets (GSE118257 and GSE250589). As shown in Figure 1, low-quality cells with excessive mitochondrial gene expression or unusually low feature counts were removed. After filtering, the remaining cells showed stable distributions of detected features and sequencing depth, indicating that the datasets were free of major technical artifacts. Normalization further minimized batch effects and improved comparability between experimental groups. These results confirm that the processed datasets are of sufficient quality to support robust differential gene expression analysis in subsequent sections.

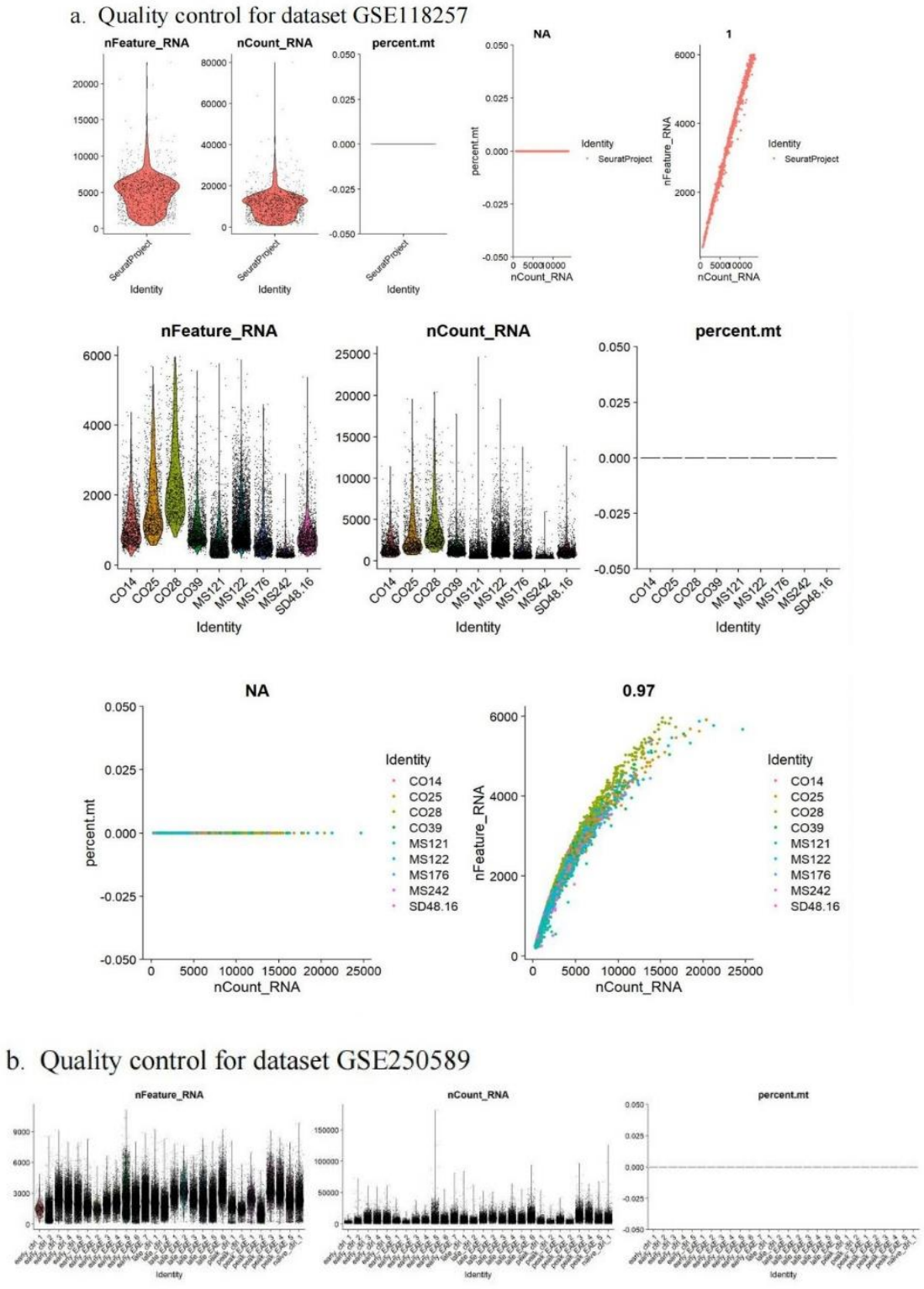

Fig. 1 Quality control for datasets GSE118257 and GSE 250589.

3.2. Differential Expression of Vitamin D-Related Genes

In the EAE model from Zheng et al. (2023), considerable variation was observed in the expression of several genes related to vitamin D (Figure 2). For the *Cyp2r1* gene, its expression in EAE mice is generally higher than in the control group, with the peak expression in the late phase of the EAE group. A similar pattern was observed for *Cyp27b1*, whose expression was elevated in EAE mice and highest in the early EAE phase. For the *Cyp27a1* gene, average expression was similar between the EAE and control groups, but the control group showed greater variance, with peak expression in the

early control phase. Finally, for the *Cbs* gene, the control group showed a higher expression of it. Its highest expression occurs in the late phase of the control group.

From past research on Vitamin D metabolism, the genes *Cyp2r1*, *Cyp27a1*, and *Cyp27b1* are shown to be involved in vitamin D metabolic pathways, such as the conversion of vitamin D to 25-hydroxyvitamin D (25-OHD) to the active VDR ligand (Bergadà et al., 2014; Cheng et al., 2004; Duan et al., 2018; Morán-Auth et al., 2013). Therefore, the variations of them suggest the correlation between MS and vitamin D metabolism. The increased expression of these genes in EAE mice suggests that the cells require more vitamin D metabolism and more vitamin D supplies. In contrast, the levels of *Cbs* genes, which have been found to be correlated with vitamin D metabolism (Kriebitzsch et al., 2011), were higher in control mice, potentially indicating greater basal vitamin D metabolism in this group.

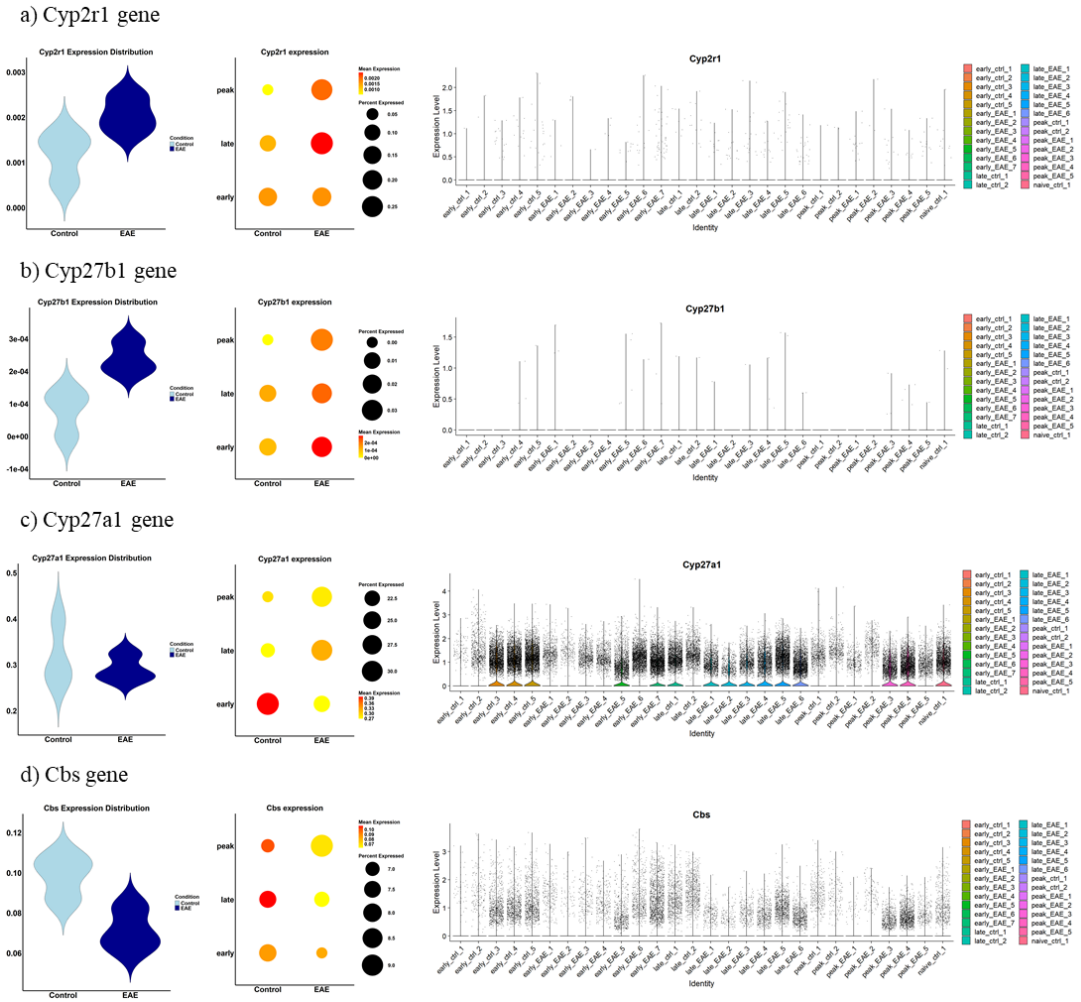

Fig. 2 Vitamin D-related genes on the dataset GSE250589.

3.3. Differential Expression of Ketogenic Diect-Related Genes

The single-cell RNA and ATAC sequencing also revealed significant differences in the expression of several KD-related genes (Figure 3). The expression of the *Otud7a* gene is remarkably higher in the EAE group than in the control group, with the peak phase of the EAE group having the highest expression. For the *Fgfrl1* gene, its expression is also notably higher in the EAE group than in the control group, with the highest expression in the peak phase of the EAE group. For the *Prdx4* gene, its expression is generally higher in the control group than in the EAE group. The group with the highest expression is the early control group. Finally, the expression of the *Ppargc1a* gene is higher in the control group than in the EAE group, with the highest expression in the late control group. These results imply a connection between MS and KD.

The *Otud7a* gene and *Fgfrl1* gene were found to be related to KD by their methylation status being altered (Ungaro et al., 2022). Their variance in expression by MS simulation suggests a relationship between KD and MS. The gene *Prdx4* is positively correlated with the usage of KD due to its action as part of a protective antioxidant response (Stafford et al., 2010). These results then suggest how KD may slow MS progression by upregulating antioxidative genes such as *Prdx4*. Finally, previous studies found a positive relationship between KD application and the *Ppargc1a* gene (Eudy, 2021), so these results can also suggest the possible treatment effects of KD on MS.

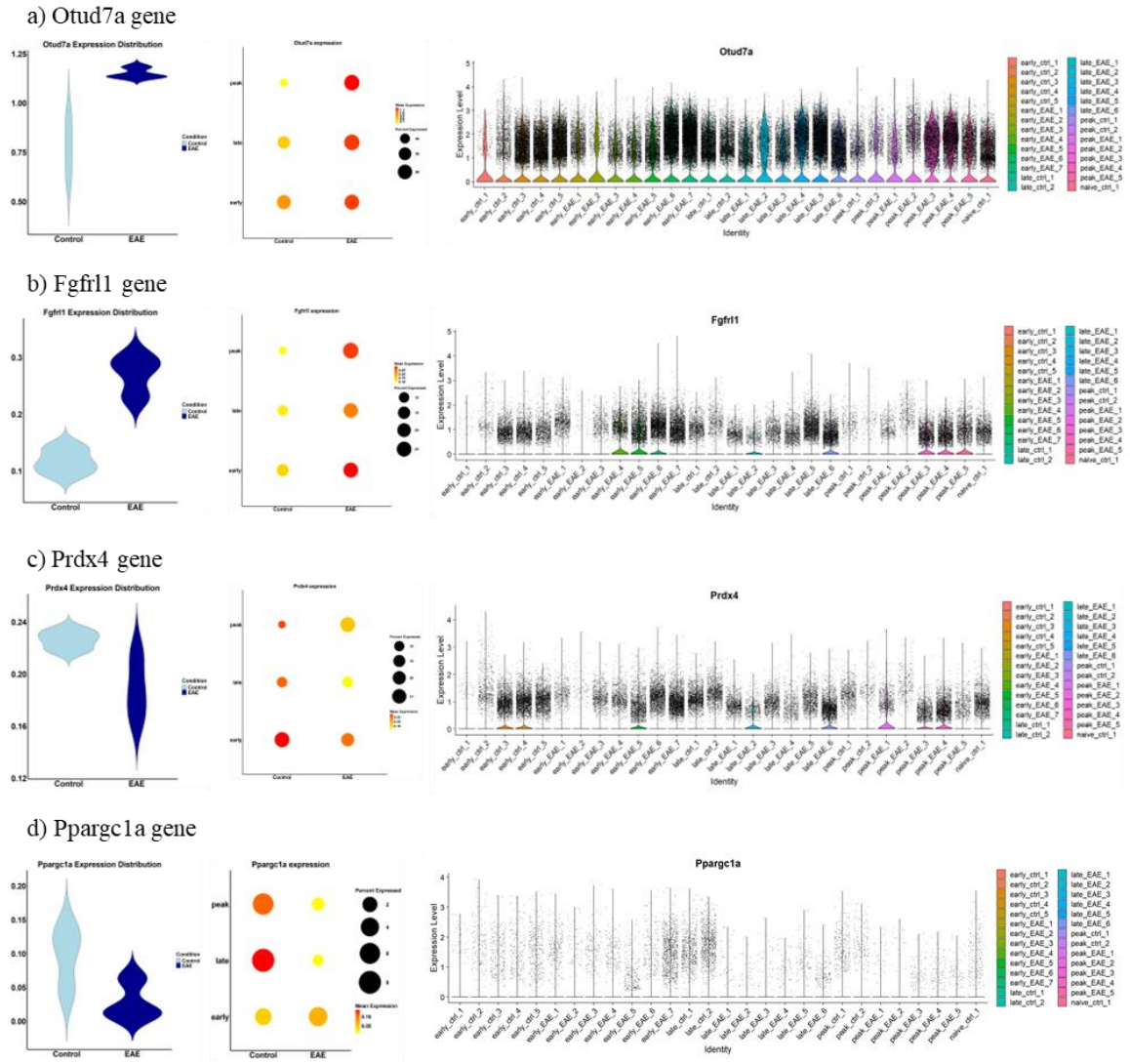

Fig. 3 KD-related genes on dataset GSE250589.

3.4. Cell microscopy results

For cell morphology, under Condition A (no LPC treatment), the oligodendrocyte cells generally have a healthier morphology in the sugar-free group (Figure 4a). Vertically comparing, the cells in the sugar-free and normal sugar group seem healthier by having a generally higher density, clearer cell membrane, a uniform cytoplasm, and a darker nucleus. The same trend occurs when looking horizontally. The cells with the most vitamin D added have a higher density and clearer cell membranes. A similar trend happens under Condition B (LPC-induced demyelination), with the cells that were cultured in sugar-free DMEM and the highest vitamin D added having the healthiest morphology, including higher cell density, clearer membranes, and darker nuclei (Figure 4b). The general trend of healthier cell morphology in high-vitamin and low-sugar groups suggests that KD conditions may improve the health conditions of oligodendrocyte cells and myelin sheaths, so KD may act as a potential treatment that decreases disease progression.

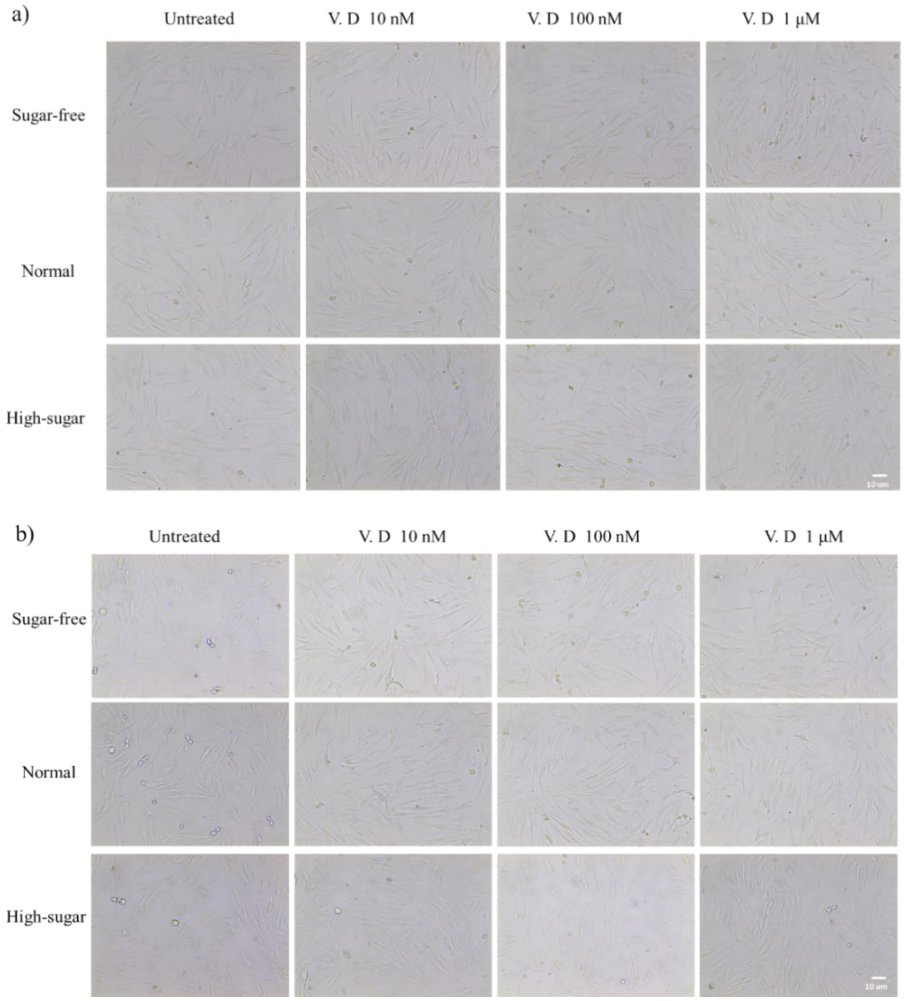

Fig. 4 Microscopic images of the MO 3.13 cell line. a) Different treatment groups under Condition A (no LPC treatment); b) Different treatment groups under Condition B (LPC-induced demyelination).

3.5. Western Blotting Results

In the Western blotting, the concentrations of β-actin, myelin basic protein (MBP), and proteolipid protein (PLP) were measured (Figure 5). Under Condition A (no LPC treatment), the Western blot bands are shown in Figure 5a, and the corresponding quantification is shown in Figure 5b. The intensity of the strands in darkness was measured and graphed, and the β-actin group was eliminated in normalizing. The intensity of the sugar-free groups of both MBP and PLP is significantly higher in both experiments than in the other groups, and their intensity follows a gradient negatively correlated with sugar concentrations. This represents that the expression of MBP and PLP in sugar-free groups is the highest. The stars in the graphs represent the p-value of certain groups, with one star meaning $p < 0.05$, two stars meaning $p < 0.01$, and so on. MBP and PLP are key proteins in the formation of myelin sheaths (Kister & Kister, 2023), so their improved formations show the possible effects of KD in the reformation of myelin sheaths and, therefore, treating MS. Also, the results are highly statistically significant.

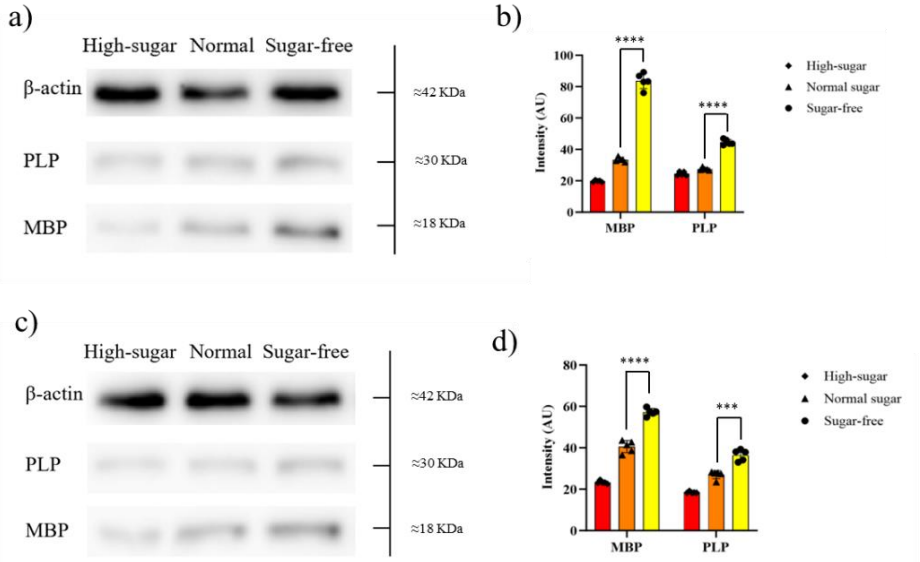

Fig. 5 Western Blotting Analysis. a) Western blotting strands under condition A (no LPC treatment); b) Western blotting strands' brightness under condition A (no LPC); c) Western blotting strands of condition B (LPC added); d) Western blotting strands' brightness under condition B (LPC added).

3.6. CCK-8 Cell Viability Results

The CCK-8 results show variations in cell viability for different treatment groups (Figure 6). For the 10 nM and 100 nM groups, the sugar-free group had the highest cell viability with significant results ($p < 0.05$). The results are not significant for the 1 μM group. Finally, for the CCK-8 test, the fact that the sugar-free groups have the highest viability suggests that KD conditions may improve the well-being of oligodendrocyte cells under MS conditions. Also, the 100 nM group has a higher viability than the 10 nM group, suggesting a positive relationship between vitamin D concentrations and cell vitality.

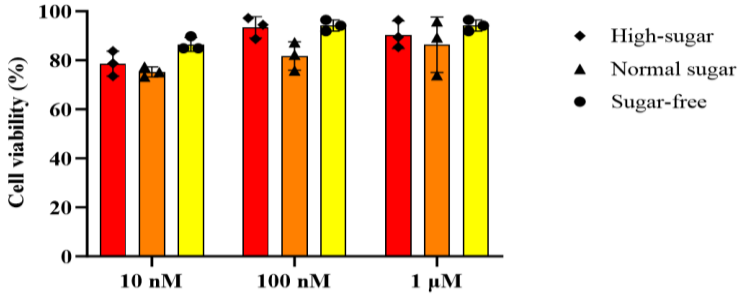

Fig. 6 CCK-8 cell viability data (50 μM LPC added).

4. Discussion

The results of the experiments suggest the effectiveness of the synergistic therapy of MS utilizing Vitamin D supplements and KD. The bioinformatics analysis proves the strong correlation between MS onset and progression and the change in expression levels of some crucial Vitamin D and KD-related genes. The altered expression of genes that are related to cell metabolism and Vitamin D metabolism reflects the effect cellular metabolism under MS conditions, suggesting oxidative stress and inflammation, and highlighting the importance of Vitamin D to the cells of MS patients. The change in the expression of KD-related genes implies the shared neurological and metabolic pathways affected by KD and MS, suggesting a potential alleviation of disease progress by KD. The wet lab experiments are complementary to the single-cell RNA and ATAC sequencing results. These in vitro

findings are complementary to the single-cell RNA and ATAC sequencing results. The observed improvement in oligodendrocyte morphology, alongside higher expression of myelin proteins and enhanced cell viability in high vitamin D and low-glucose DMEM media, indicates a promotive effect on oligodendrocyte health and myelin sheath integrity. This suggests the combined strategy of vitamin D and KD may slow MS progression and mitigate risk.

The anti-inflammatory effects of KD may be attributed to the regulation of productions of chemicals like proliferator-activated receptor-gamma, manipulated by altered gene expression (Zhang et al., 2018). In MS patients, fatty acid metabolism increased by KD produces β-hydroxybutyrate (BHB), a type of ketone body. When simulating KD condition, ketone bodies were added to different mediums. The results suggest the ability of ketone bodies to reduce inflammation through pathways like expressing anti-inflammation enzymes and decreasing inflammatory macrophages and microglia cells (Deng et al., 2021; Goldberg et al., 2017). Previous studies found a positive relationship of 25(OH)D concentrations in the cerebrospinal fluid and serum (Miclea et al., 2010). Knowing that low serum 25(OH)D levels have a causal effect on developing MS (Rhead et al., 2016), and how $1,25(OH)_2D_3$ can reduced inflammation after Vitamin D metabolism, this can explain the results of the experiment. Inflammatory conditions lead to the increase in need for Vitamin D for neural cells, leading to the increase in Vitamin D metabolism and the expression of genes related to Vitamin D metabolism. Consequently, the trafficking of T cells and activation of Helper T cells can be reduced, and key anti-inflammatory chemicals like Interleukin-10 are produced (Fernandes de Abreu et al., 2009; Grishkan et al., 2013; Molina-Holgado et al., 2001). The better cell morphology and viability can be explained by the effect of $1,25(OH)_2D_3$ on the removal of myelin debris and speeding the remyelination of oligodendrocytes (Garcion et al., 1997; Masoumi et al., 2009; Nystad et al., 2014).

There are several limitations of this research study. To begin with, in the single-cell RNA and ATAC sequencing, the study of Zheng et al. of dataset 250589 that utilized a mouse EAE model is selected. The EAE model is induced through activating autoreactive T cells against myelin antigens, simulating the autoimmune attacks of T cells on neural cells present in MS, therefore mimicking the immune responses that is believed to underlie MS (Mix et al., 2010). The EAE model can simulate many key aspects of MS, like inflammation caused by leukocyte infiltration, demyelination, and axonal damage (Mix et al., 2010). In the past years, EAE models have helped to identify many key insights of MS, including many critical genes and metabolic pathways, such as the HLA-DRB1*1501 risk allele (Chao et al., 2008). However, despite the historical contributions of EAE models and their resemblance of MS conditions, it has limitations that may induce diverged results. For one, EAE models are purely autoimmune-induced, while actual MS may be triggered by many factors, such as the Epstein-Barr virus. Also, the genetic diversity of the inbred mouse strain will still lack the variety of human genes (Mix et al., 2010). The Anti-TNF-α mAb infliximab was effective in slowing disease activity in mouse EAE models, but caused the opposite effect in MS patients (van Oosten et al., 1996); the Anti-CD3 and anti-CD4 antibodies were effective in control MS progression in EAE models, but showed no significant clinical effects in actual MS patients (Wiendl & Hohlfeld, 2002). These differences implies that the results of studies done on EAE models, including the single-cell RNA and ATAC sequencing done in this study on the dataset, may not be directly applicable to MS patients. Solution to mitigate this problem include including additional datasets based on humans and see if they produce similar results. Also, due to laboratory limitations, the wet lab experiments on human oligodendrocyte cell line only monitors the health of oligodendrocytes and the status of myelin sheaths. However, MS is a complex disease, and the current wet lab design cannot simulate the autoimmune and inflammatory responses in an actual patient. Next, in actual MS patients, Vitamin D is metabolized in the liver to form 25(OH)D, and then hydroxylated by CYP27B1 enzyme by immune cells or in kidneys, forming $1,25(OH)_2D$, which is the activated form of Vitamin D that can act on neural cells (Miclea et al., 2010). In this experiment, the lack of immune cells will prevent the happening of this pathway. Instead of $1,25(OH)_2D$, the relatively inactive form of Vitamin D is added into culture mediums, so in an actual case, the activated form of Vitamin D may yield more significant

results. Finally, although LPC can replicate many key aspects of MS, including demyelination and axonal damage, LPC fails to capture the immunological origin of MS. LPC-induced demyelination is caused by lipid disruption that does not involve T cells or antibodies, which is the core of MS (Plemel et al., 2017). LPC-induced injury is acute, so it cannot demonstrate the chronic progressive pathology, like oligodendrocyte precursor cell exhaustion and axonal transection (Irvine & Blakemore, 2008). The chronic and complex nature of MS cannot be fully simulated by LPC-induce demyelination, so these experiments might yield divergent results when carried out in actual MS patients.

Future research studies can expand on the current results. First, the effect of Vitamin D and KD on oligodendrocyte cell line can be done using $1,25(OH)_2D$, making the conditions to conform with actual Vitamin D's effect on neural cells. Also, the experiments on cell line can be replicated on a more complex model that better resembles actual MS cases, such as on EAE models. This addresses the limitations of cell line culturing experiments as mentioned previously. Actual long-term follow-up studies on MS patients utilizing KD with Vitamin D supplements are the best justification of the effectiveness of this synergistic therapy, which avoid the divergences of LPC-induced demyelination from actual MS conditions. Larger-scale clinical trials, ideally multicenter, randomized controlled trials with diverse patient cohorts, can validate the efficacy of this therapy statistically.

The synergistic effects of KD and Vitamin D supplementation demonstrated in this study may lead to substantial potential for translating into effective clinical interventions. This dietary strategy directly mitigates the barriers to MS care currently, including high costs, limited accessibility, and side effects of DMT. KD and Vitamin D are low-cost and requires no specialized medical infrastructure to implement, making the intervention broadly applicable. For relapsing-remitting MS, integrating KD with Vitamin D supplementation could serve as an alternative therapy to DMTs, potentially reducing relapse frequency by enhancing anti-inflammatory effects and supporting myelin health. For progressive MS patients, the intervention can offer a mean to slow disease progression. The crucial genes that are monitored during MS and with KD and Vitamin D may be further research of their effects in altering disease progression, and potential genes could be targeted for drug development. By suggesting the synergistic benefits of KD and Vitamin D, two interventions that are safe and accessible, on MS progression and myelin health, we can move closer to reducing the threat of MS in the world and improving quality of life for the numerous MS patients.

Acknowledgements

My interest in biology began in primary school when Jane Goodall, the renowned British ethologist, visited and delivered an inspiring speech about her experiences studying African chimpanzees. Beyond the science itself, her talk taught me the importance of challenging mainstream views and the necessity of persistence in scientific research. Then, from school biology courses to competitions like biology Olympiads and IGEM, as my path in biology progresses, my school always provides sufficient support. I would like to appreciate my school's hardcore biology courses and resources, including laboratory classes, that enabled me to be prepared for this research study. My interest in neuroscience began in middle school, where I learned about the Brainbee competition. The most direct motivation for me to conduct this research, however, is when my grandmother was affected by demyelination that was caused by MS. My focus on neuroscience became even more personal. This caused her to experience recurring headaches, impacted her mobility and vision, and, most distressingly, made her ineligible for vaccinations, leaving her vulnerable to COVID-19. Witnessing her struggles firsthand was a turning point. I realized that despite medical advancements, there was no cure and limited treatment options for many neurological disorders. From that moment, I was determined to explore ways to alleviate neurodegenerative diseases like hers through research. I would like to thank my grandmother for her care and for providing the chance to get to dive into neuroscience, and for being a motivation for me in pursuing my research. In a summer program at the University of California, Irvine, I wrote a short review about the socioeconomic burden of MS on Chinese patients. When writing the review, I learned that despite the many treatments available, their

effectiveness is limited due to their high costs and unavailability in many places. Therefore, I decided to focus on therapies that are easily attainable and cheap, and dietary interventions are the most apparent to me.

The bioinformatic analysis of single-cell RNA and ATAC sequencing was difficult for me to achieve. After learning about it in class, I have wanted to include it in my research study. In my process of single-cell RNA and ATAC sequencing analysis using Seurat in R, I would first like to thank the creators of the Seurat package. Also, when learning to use it for analysis, I encountered many challenges, like not knowing how to analyze a dataset of a format I was not familiar with, how to sort data and do quality control, and how to draw appropriate diagrams to demonstrate the results. I would like to thank the creators on the Bilibili online platform for providing guidance of using Seurat and solutions to many of my problems.

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