Systems Modeling Reveals Shared Metabolic Dysregulation and Novel Therapeutic Treatments in ME/CFS and Long COVID

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Abstract:

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) and Long COVID are complex, multisystemic conditions that pose ongoing challenges to healthcare professionals. Emerging research suggests that ME/CFS and Long COVID exhibit overlapping metabolic symptoms, indicating possible shared metabolic dysfunctions. This study aims to systematically explore these shared metabolic disturbances and their potential treatments. Utilizing our novel metabolic modeling method, GPMM, we identified the key metabolic irregularities in patients with ME/CFS and Long COVID, notably the downregulation of the alanine and aspartate metabolism pathway, and the arginine and proline metabolism pathway. Genome-wide knockout analyses indicated that supplementation with aspartate (ASP) or asparagine (ASN) could potentially ameliorate these metabolic deficiencies. Further metabolic assessments in Long COVID patients highlighted the significant downregulation of ASP in both blood and muscle, supporting our predictions. Consequently, we propose that the combination of

l-ornithine and l-aspartate (LOLA) offers a promising approach to alleviate metabolic symptoms in both ME/CFS and Long COVID patients. This study not only elucidates the shared metabolic pathways in ME/CFS and Long COVID but also positions LOLA as a viable candidate for future clinical trials.

Keyword: ME/CFS; Long COVID; metabolic modeling; metabolic dysfunction; l-ornithine and l-aspartate

Introduction

The emergence of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) and Long COVID as prominent health crises has underscored the need for a deeper understanding of complex, multisystemic conditions. ME/CFS, a long-standing enigma in the medical field, is characterized by persistent and unexplained fatigue, while Long COVID represents a spectrum of symptoms persisting beyond the acute phase of SARS-CoV-2 infection[1]. Both conditions have been associated with a wide range of debilitating symptoms, including but not limited to profound fatigue, cognitive impairment, and post-exertional malaise, which severely impacting the quality of life of affected individuals[2].

The similar clinical presentations of ME/CFS and Long COVID have prompted researchers to hypothesize and explore shared underlying mechanisms, particularly within metabolic functions. Metabolic dysfunction has been increasingly recognized as a potential contributor to the symptomatology of these conditions, with disturbances noted in energy metabolism, amino acid profiles, and mitochondrial function[3,4].

In this study, we investigate the shared metabolic alterations in ME/CFS and Long COVID by using genome-wide precision metabolic modeling (GPMM) and pair-wise metabolites comparison. We identified that the most significant metabolic change is the downregulation of alanine and aspartate metabolism. We also propose the combination of l-ornithine and l-aspartate (LOLA) as a promising therapeutic approach. LOLA has been hypothesized to replenish these deficient metabolic pathways, thereby offering a potential strategy to alleviate the metabolic symptoms that are prevalent in both ME/CFS and Long COVID.

Results

Metabolic Modeling of ME/CFS Patient Muscle Reveals Altered Metabolism

To investigate metabolic changes in ME/CFS, we conducted metabolic modeling using muscle samples from the ME/CFS dataset[5]. This dataset comprises 13 ME/CFS patients and 12 healthy controls. The modeling process involved 2841 reactions, with 65 reactions showing significant up-regulation and 52 reactions significantly down-regulated (Figure 2A). Pathway analysis revealed that several metabolic pathways were affected. Specifically, Alanine and aspartate metabolism, Pyrimidine catabolism, Aminosugar metabolism, and Arginine and proline metabolism pathways were significantly down-regulated (FDR < 0.05). Conversely, the Pentose phosphate pathway exhibited significant up-regulation (FDR < 0.05). Notably, the most prominently down-regulated pathway was Alanine and aspartate metabolism (Figure 2B).

Furthermore, our all-against-all knockout analysis highlighted ASN and ASP as agonist metabolites. Administering these two amino acids could potentially rescue the metabolic changes observed in ME/CFS patients. This knockout result aligned with the modeling findings and supported the notion of dysfunction in Alanine and aspartate metabolism among ME/CFS patients[4].

Metabolism of Long COVID Reveals Down-Regulated Asparagine (ASN) in Blood and Muscle

Given the similarity in symptoms between long COVID and ME/CFS, weanalyzed the metabolic profile in Long COVID patients. As post-exertional malaise (PEM) is a common metabolic symptom shared by both conditions, we performed pairwise comparisons between pre- and post-exercise samples from Long COVID patients and healthy controls using data from a post-exertional malaise Long COVID study[6](Supplementary Data S1). Significant changes were noted in muscle tissue, particularly with asparagine and dihydroxyacetone-P being down-regulated.. Notably, Asparagine emerged as the top-ranked down-regulated metabolite in muscle samples of Long COVID patients (Figure 3A). Similarly, in blood samples, Asparagine also ranked highest among down-regulated metabolites following PEM in Long COVID patients (Figure 3B).

Collectively, these findings from both ME/CFS and Long COVID studies consistently highlighted that the most significant metabolic alteration was the down-regulation of the ASN/ASP metabolism.

L-Ornithine and L-Aspartate (LOLA) as a Potential Treatment for ME/CFS and Long COVID

Next, we propose potential treatments for both ME/CFS and Long COVID by targeting these specific metabolic pathways. We suggest that L-ornithine and L-aspartate (LOLA) may offer therapeutic benefits for individuals with these conditions for several reasons:

1) L-Aspartate aligns with the commonly observed down-regulation of ASN/ASP in both ME/CFS and Long COVID, suggesting it could help counteract this deficiency.

2) As the metabolic product of arginine, L-ornithine corresponds to the down-regulated Arginine and proline metabolism pathway in ME/CFS, potentially restoring balance in this pathway.

3) Additionally, the combined use of these amino acids in LOLA could enhance urea cycle efficiency, which is critical in removing ammonia and reducing fatigue symptoms commonly reported in these conditions[3].

4) Emerging evidence suggests that supplementation with LOLA can improve mitochondrial function, thereby potentially enhancing energy metabolism, which is often impaired in patients with ME/CFS and Long COVID[7].

Discussion

This study provides an analysis of the metabolic disruptions found in both ME/CFS and Long COVID, offering new insights into their pathophysiology and highlighting potential treatment avenues. Our findings reveal significant metabolic commonalities between these conditions, particularly in the down-regulation of amino acid metabolism pathways such as ASN/ASP and arginine/proline. These findings not only help us understand better the systemic impact of these conditions but also highlight potential targets for therapeutic intervention.

The consistent down-regulation of specific metabolic pathways across both indications suggests a fundamental disruption in amino acid metabolism and energy metabolism, which could be contributing to the severity and persistence of patients' symptoms. Here, we propose that L-ornithine and L-aspartate (LOLA) holds the potential of intervening these metabolic pathways. L-aspartate, for instance, could directly replenish the decreased aspartate pool, while L-ornithine might work by restoring the urea cycle pathway, thereby improving the overall metabolic balance and reducing symptoms such as fatigue and cognitive dysfunction.

Moreover, the role of mitochondrial dysfunction in these conditions cannot be ignored either. Recent studies have shown that mitochondrial dysregulation is a key factor in the pathogenesis of chronic diseases, including those characterized by post-exertional malaise, a hallmark of both ME/CFS and Long COVID[7]. By enhancing mitochondrial function and energy production, LOLA could potentially mitigate some of the core symptoms of these conditions.

Additionally, our study highlights the importance of integrating clinical and metabolic data to better understand the complex interactions at play in multisystemic conditions. Such integrative approaches could pave the road for personalized medicine strategies in treating ME/CFS and Long COVID, where treatments are tailored based on specific metabolic profiles.

Future longitudinal studies will be needed to track the progression of metabolic alterations and key symptoms in ME/CFS and Long COVID patients over time and to evaluate the long-term efficacy and safety of LOLA in clinical trials. It is also important to explore the interactions between metabolic pathways and other physiological systems, such as the immune and endocrine systems, neurological systems etc. to develop a deeper understanding of these complex conditions.

In conclusion, by exploring the metabolic mechanism of ME/CFS and Long COVID, our study not only contributes to the understanding of these complex conditions but also proposed candidate solutions. The future accumulation of promising results regarding LOLA in controlled clinical trials confirming its therapeutic efficacy will determine its role in addressing the multifaceted symptoms of these challenging diseases.

Methods

Dataset collection

The gene expression data for ME/CFS muscle tissue was sourced from the GEO dataset accession number GSE245661[5] The metabolomics dataset for Long COVID, focusing on post-exertional malaise, was acquired from a recent study and obtained from MetaboLights (with study identification code: MTBLS9103)[6]

Genome wide precision metabolic modeling and fluxes analyzing

Metabolic modeling. We used our recently developed genome-wide precision metabolic modeling method, GPMM, to perform the genome-wide metabolic modeling[8]. Briefly, GPMM integrates protein abundance estimates from gene expression data with enzymatic kinetic parameters, and uses these as upper bounds in a generic human metabolic model based on Michaelis–Menten kinetics. Nutrient uptake fluxes for cell lines were referenced from existing literature[9], and the lower bounds for other exchanges were set as zero. We conducted Flux Variability Analysis (FVA) using the FastMM algorithm to construct tissue-specific models for each sample[10].

Flux change identification. For case-control design, the flux changes between case and control were calculated using the Limma package [11] based on the values of metabolic modeling.

Identifying metabolic pathway changes. To ascertain changes in metabolic pathways, we calculated the Differential Abundance Score (DA score) employing a method previously described in the literature [12]. For each metabolic pathway (i), the DA score (DA_i) is computed as follows:

$$\mathbf{DA_i} = \frac{\#\ \text{up regulated fluxes} - \#\ \text{down regulated fluxes}}{\text{Total reactions in } ith \text{ subsystem}} \qquad \textbf{(1)}$$

The statistical significance (p-values) of the Differential Abundance (DA) scores was determined using a “bootstrap without replacement” method, as detailed in our previous study[8].

All-against-all knockout analysis

We performed an all-against-all metabolite knockout analysis using the FastMM[10] algorithm to generate a comprehensive metabolite knockout matrix ($M^{(KO)}$). In this matrix, rows represent

metabolites, while columns correspond to reactions. The Metabolite Effective Score (MES) was calculated in a manner analogous to gene knockout analysis, as follows:

$$\mathbf{MES_i} = \sum_{\mathbf{j=1}}^{\mathbf{n}} \boldsymbol{M_{i,j}^{(KO)}} \times \boldsymbol{sign(log2FC_j)} \times \boldsymbol{L_j} \qquad \textbf{(2)}$$

Here, $M_{i,j}^{(EM)}$ denotes the effect score of the *ith* metabolite on the *jth* reaction. The significance (p-value) of each MES was determined using a norm-background method, employing the pnorm function in R. False Discovery Rate (FDR) adjustments were made using the p.adjust function with the 'FDR' method in R. An MES value greater than 0 or less than 0, coupled with an FDR less than 0.05, suggests that the knockout of these metabolites could potentially rescue or exacerbate the flux changes in senescent cells, respectively.

Metabolomics data analysis

In the study MTBLS9103, 116 metabolites in skeletal muscle and 83 metabolites in venous blood were annotated. For both tissues, we calculated Differential Metabolite (DM) using equation (3) to quantitate the difference of metabolite concentration change after PEM between Long COVID patients and controls.

$$\mathrm{DMi} = \left(\log2\left(\mathrm{PEM_{pi}}\right) - \log2\left(\mathrm{baseline_{pi}}\right)\right) - \left(\log2(\mathrm{PEM_{ci}}) - \log2(\mathrm{baseline_{ci}})\right) \qquad (3)$$

Data and code availability

Metabolic modeling, flux analysis and visualization, the metabolic pathway analysis, all-against-all knock out analysis, and key metabolite identification were all performed by rGPMM platform (https://github.com/GonghuaLi/rGPMM, will be public soon) with the version 1.0.1 and the platform of Recon3v1 and using CPLEX solver.

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Author contributions

WZX and GHL conceived and designed the study. WZX, GHL and QPK supervised the project. GHL designed the computational method. GHL developed the computational method. GHL, FFH collected the datasets. GHL and FFH performed the data analysis and conducted the statistical analysis. GHL, FFH, and WZX drafted the manuscript. GHL, FFH, and WZX revised the manuscript. All authors read and approved the final manuscript.

Ethics declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

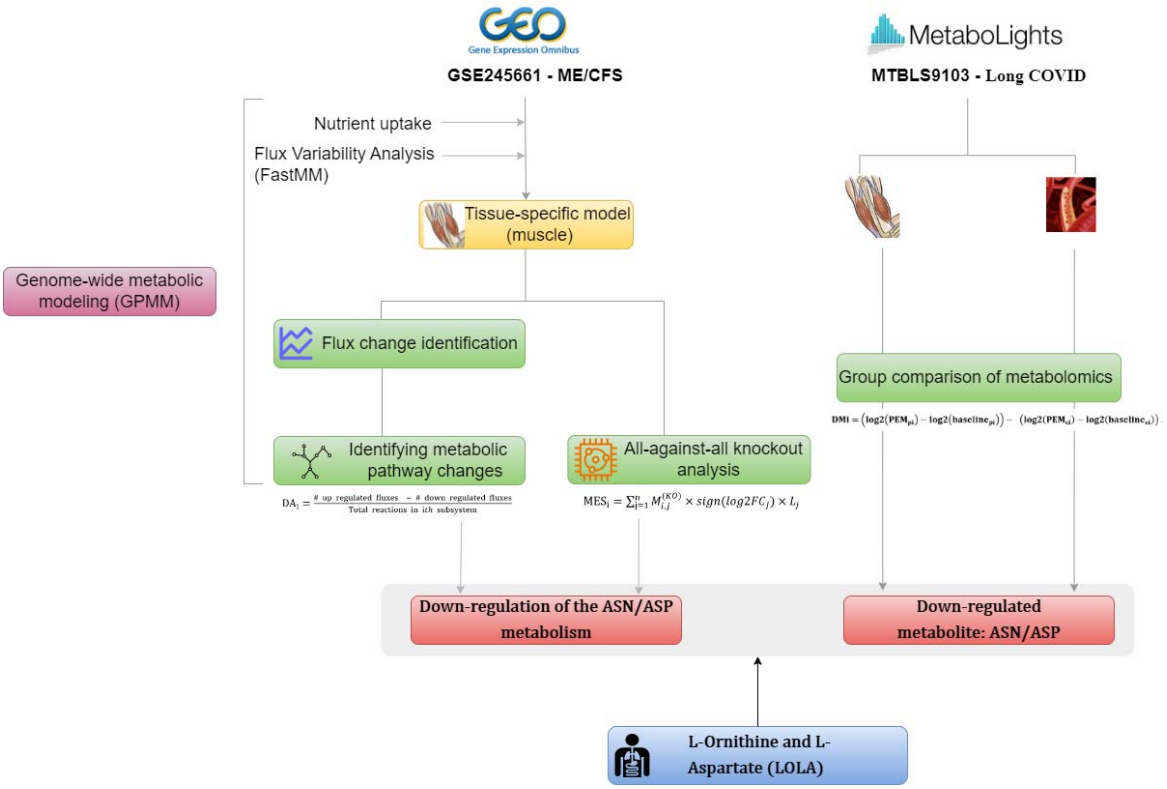

Figure 1: The workflow of Genome-Wide metabolic modeling (GPMM) and Metabolomics leading to target identification for ME/CFS and Long COVID. The left panel showed GPMM process using transcriptomic data of muscle tissues form a ME/CFS study, GSE245661. The right panel showed the data analytics process of metabolomics data from a Long COVID study, MTBLS9103. Both processes identified ASN/ASP metabolism as the most down-regulated pathway, resulting in the finding of LOLA as a promising candidate to treat both diseases.

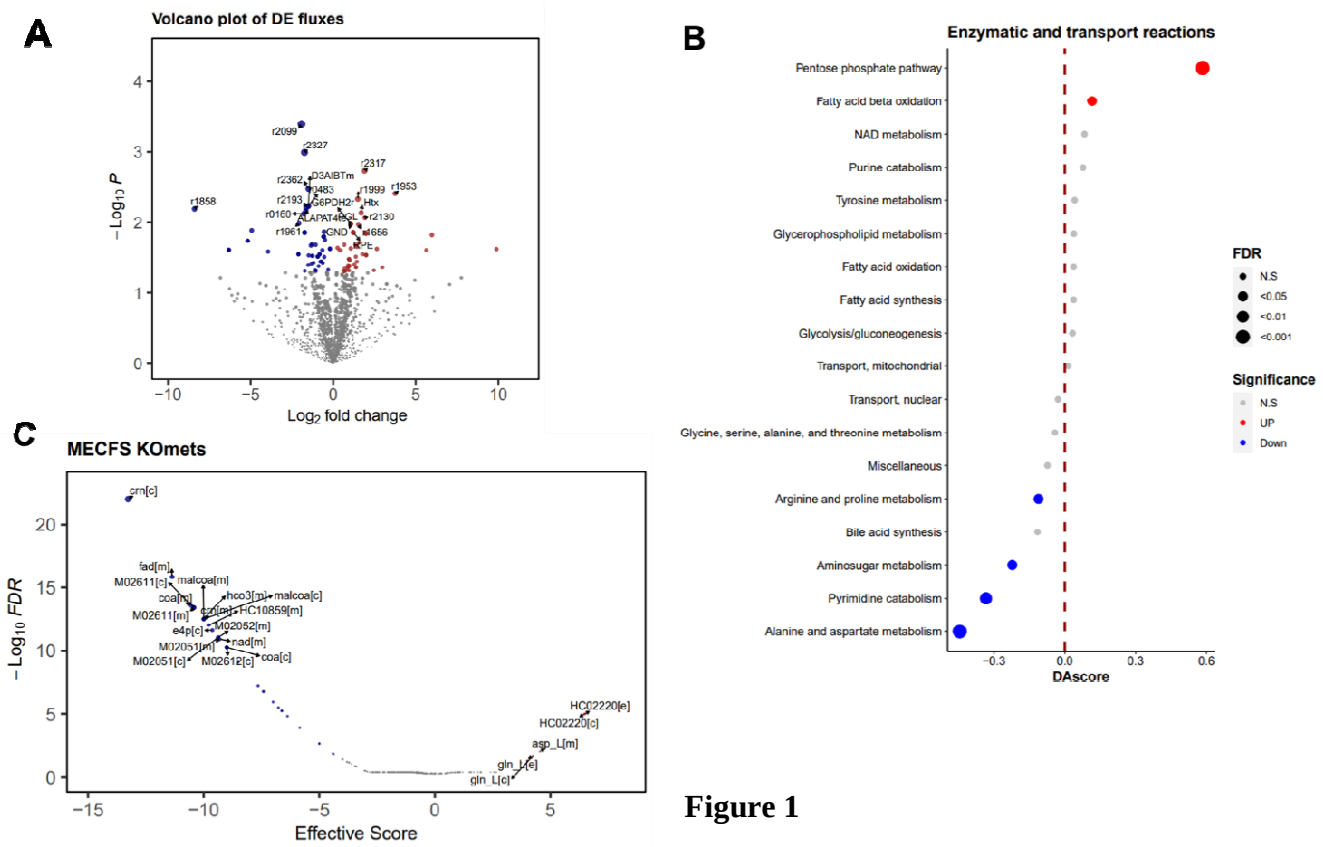

Figure 1

Figure 2: Metabolic modeling of muscle samples of ME/CFS patients. (A) volcano plot of differential changed fluxes. (B). Pathway analysis of ME/CFS patients. Red, blue and gray points represent up, down and non-significant pathways, respectively. (c) Metabolite knockout analysis in ME/CFS. Effective scores greater than 0 indicate agonist metabolites, while scores less than 0 indicate antagonist metabolites. Note: the analysis included 13 ME/CFS patients and 12 healthy controls.

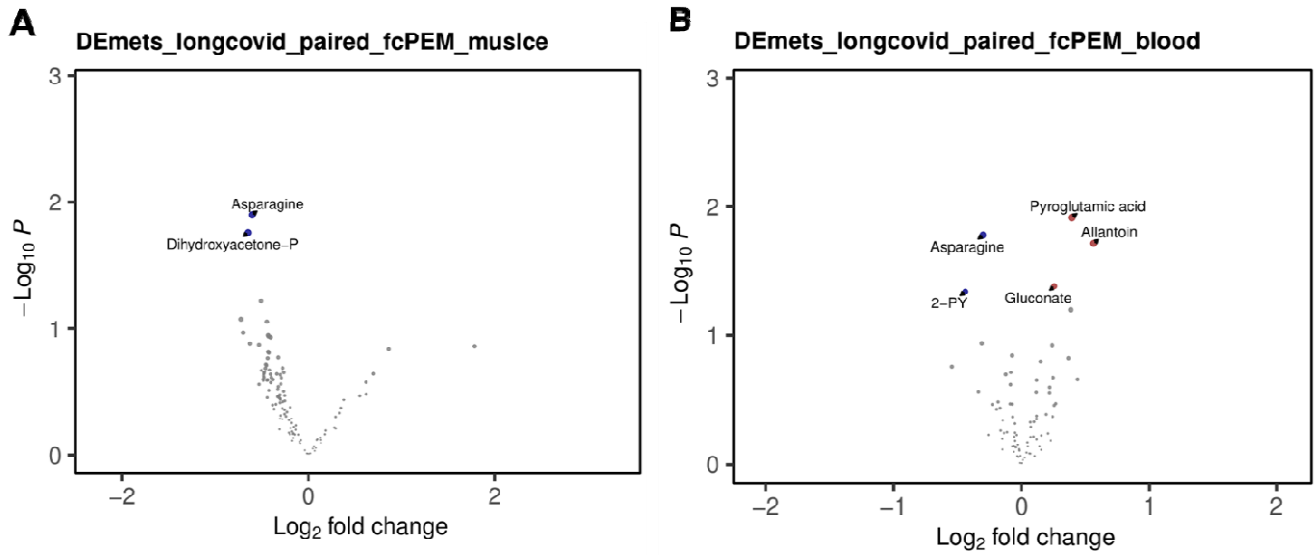

Figure 3: Pair-wise metabolic changes in long-covid change after post-exertional malaise (PEM). (A). Volcano plot of pair-wise metabolic changes in long-covid patient muscle after PEM. (B). Volcano plot of pair-wise metabolic changes in long-covid patient blood after PEM

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