Overexpressed pathways in patients with myalgic encephalomyelitis and chronic fatigue syndrome

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Introduction

What is ME/CFS?

ME/CFS is an untreatable chronic condition associated with extreme fatigue.

Symptoms

Chronic fatigue

Post-exertion malaise (PEM)

Flu-like symptoms

Incidence

836,000+

US adult population who have ME/CFS

1.7%

US adult women who have ME/CFS
(compared to 0.9% of adult men)

Why study ME/CFS?

Post-viral infections are associated with the onset of ME/CFS.

COVID-19

What We Know About Long Covid So Far

There is no universal definition of the complex condition, but clues about causes and potential treatments are beginning to emerge.

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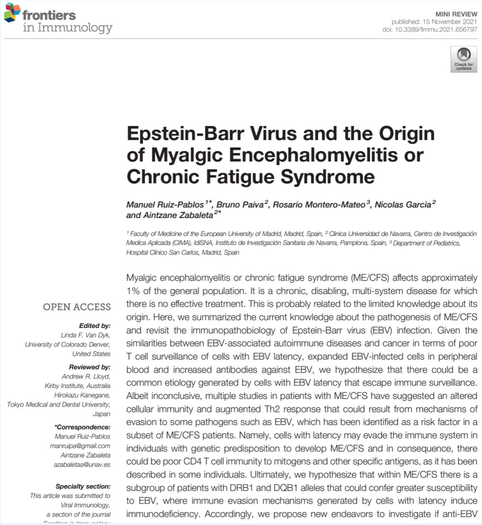
Epstein-Barr



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Epstein-Barr Virus and the Origin of Myalgic Encephalomyelitis or Chronic Fatigue Syndrome

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Myalgic encephalomyelitis or chronic fatigue syndrome (ME/CFS) affects approximately 1% of the general population. It is a chronic, disabling, multi-system disease for which there is no effective treatment. This is probably related to the limited knowledge about its origin. Here, we summarized the current knowledge about the pathogenesis of ME/CFS and revisit the immunopathobiology of Epstein-Barr virus (EBV) infection. Given the similarities between EBV-associated autoimmune diseases and cancer in terms of poor T cell surveillance of cells with EBV latency, expanded EBV-infected cells in peripheral blood and increased antibodies against EBV, we hypothesize that there could be a common etiology generated by cells with EBV latency that escape immune surveillance. Albeit inconclusive, multiple studies in patients with ME/CFS have suggested an altered cellular immunity and augmented Th2 response that could result from mechanisms of evasion to some pathogens such as EBV, which has been identified as a risk factor in a subset of ME/CFS patients. Namely, cells with latency may evade the immune system in individuals with genetic predisposition to develop ME/CFS and in consequence, there could be poor CD4 T cell immunity to mitogens and other specific antigens, as it has been described in some individuals. Ultimately, we hypothesize that within ME/CFS there is a subgroup of patients with DRB1 and DQB1 alleles that could confer greater susceptibility to EBV, where immune evasion mechanisms generated by cells with latency induce immunodeficiency. Accordingly, we propose new endeavors to investigate if anti-EBV

Herpes

Intervirology

Meta-Analysis

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Human Herpesvirus 6 Infection and Risk of Chronic Fatigue Syndrome: A Systematic Review and Meta-Analysis

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Keywords
Chronic fatigue syndrome · Human herpesvirus 6 · Systematic review · Meta-analysis

Abstract
Introduction: Chronic fatigue syndrome (CFS) is a neurological disease that is accompanied by excessive fatigue or tiredness. There are several reports confirming the association between human herpesvirus 6 (HHV-6) infection and CFS illness. This systematic review and meta-analysis was

fatigue syndrome and HHV 6," "chronic fatigue syndrome and HHV-6," "chronic fatigue syndrome and HHV6," "chronic fatigue syndrome and Herpes virus 6," and "chronic fatigue syndrome and Herpesvirus6" in MEDLINE (PubMed), Web of Science, and EMBASE. ***Results:*** The literature search identified 17 studies to be included in the systematic review and 11 studies in meta-analysis. The symmetry funnel plot and Egger's test (*p* value = 0.2) identified no publication bias among studies. Moreover, the low level of I^2 revealed homogeneity across studies. ***Discussion:*** In conclusion, the asso-

Literature Review and Past Methodologies

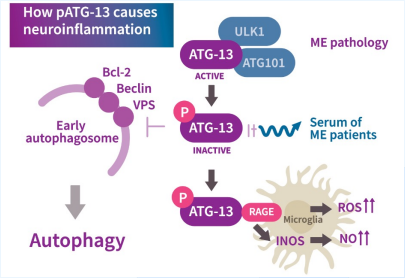
Proteomics research has identified pathways and proteins of interest.

Germain et al. (2021) identify disruptions in Ephrin-Eph signaling pathway.

Focused effects with respect to “stem cell fate, immune cell activation, immune cell trafficking, and … some disease pathogenesis.” (e.g., cancer, inflammation, etc.)

Gottschalk et al. (2022) note elevated ATG13 expression in patient serum samples.

Increased cellular stress from ROS and NO species from phosphorylation leads to autophagy and other pathologies.



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Hypothesis

What other pathways could be associated with ME/CFS?

3

Results

Volcano plot

-log$_{10}$(p-value) v. z-score difference used to evaluate up/downregulation.

Methodology

Two subsets for patient and control (*N* = 20 each).

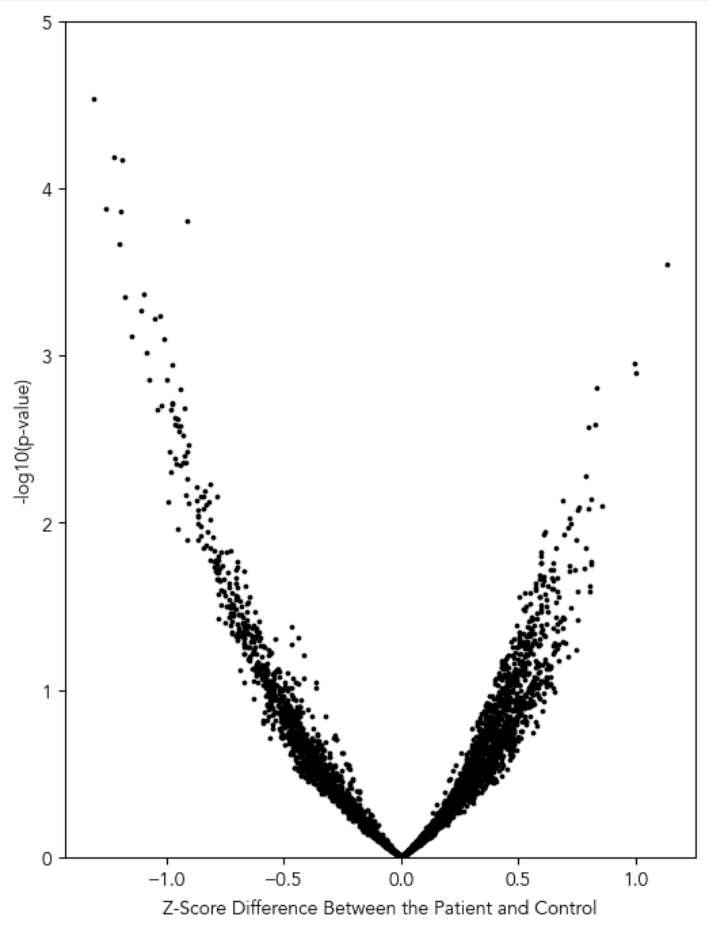
Two-tailed, equal variance Student's t-test.

Correction using Benjamini-Hochberg (α = 0.05).

Takeaways

Higher upregulated proteins in patients.

Greater number of statistically significant downregulated proteins for control.



Enrichment analysis

We identified two pathways of interest that are overexpressed in patients.

Methodology

Average patient abundances by gene.

Identify top correlations and obtain gene name.

Get top 100 genes correlated for Enrichr analysis.

Takeaways

LCAT and FTL frequently appeared in correlations.

LCAT: Phospholipase D signaling pathway ($p < 0.02$)

FTL: Complement/coagulation cascades ($p < 10^{-11}$)

Term	Overlap	p-val (adj.)
Phospholipase D signaling pathway	6/148	0.017
Pathogenic E. coli infection	5/197	0.192
Platelet activation	4/124	0.192

ADCY1, ADCY2, ADCY3, ADCY4, ADCY5, ADCY6, ADCY7, ADCY8, ADCY9, AGPAT1, AGPAT2, AGPAT3, AGPAT4, AGPAT5, AGT, AGTR1, AKT1, AKT2, AKT3, ARF1, ARF6, AVP, AVPR1A, AVPR1B, AVPR2, CXCL8, CXCR1, CXCR2, CYTH1, CYTH2, CYTH3, **CYTH4**, DGKA, DGKB, DGKD, DGKE, DGKG, DGKH, DGKI, DGKK, DGKQ, DGKZ, DNM1, DNM2, DNM3, **EGF**, EGFR, **F2**, F2R, FCER1A, FCER1G, **FYN**, GAB1, GAB2, GNA12, GNA13, GNAS, GRB2, GRM1, GRM2, GRM3, GRM4, GRM5, GRM6, GRM7, GRM8, **HRAS**, IGH, INS, INSR, JMJD7-PLA2G4B, KIT, **KITLG**, KRAS, LPAR1, LPAR2, LPAR3, LPAR4, LPAR5, LPAR6, MAP2K1, MAP2K2, MAPK1, MAPK3, MRAS, MS4A2, MTOR, NRAS, PDGFA, PDGFB, PDGFC, PDGFD, PDGFRA, PDGFRB, PIK3CA, PIK3CB, PIK3CD, PIK3CG, PIK3R1, PIK3R2, PIK3R3, PIK3R5, PIK3R6, PIP5K1A, PIP5K1B, PIP5K1C, PLA2G4A, PLA2G4B, PLA2G4C, PLA2G4D, PLA2G4E, PLA2G4F, PLCB1, PLCB2, PLCB3, PLCB4, PLCG1, PLCG2, PLD1, PLD2, PLPP1, PLPP2, PLPP3, PRKCA, PTGFR, PTK2B, PTPN11, RAF1, RALA, RALB, RALGDS, RAPGEF3, RAPGEF4, RHEB, RHOA, RRAS, RRAS2, SHC1, SHC2, SHC3, SHC4, SOS1, SOS2, SPHK1, SPHK2, SYK, TSC1, TSC2

Table 1. Top 3 pathways associated with LCAT gene. List of genes related to top pathway (overexpressed in blue).

Term	Overlap	p-val (adj.)
Complement and coagulation cascades	12/85	1.96E-12
Coronavirus disease	6/232	0.066
Staphylococcus aureus infection	4/95	0.066

A2M, BDKRB1, BDKRB2, C1QA, C1QB, C1QC, C1R, C1S, C2, C3, C3AR1, C4A, C4B, **C4BPA**, C4BPB, **C5**, C5AR1, **C6**, C7, C8A, C8B, C8G, C9, CD46, CD55, CD59, **CFB**, CFD, CFH, **CFHR1**, CFHR2, CFHR3, CFHR4, CFHR5, **CFI**, CLU, CPB2, CR1, CR1L, CR2, **F10**, **F11**, F12, **F13A1**, **F13B**, F2, F2R, F2RL2, F2RL3, F3, F5, F7, F8, **F9**, **FGA**, FGB, FGG, ITGAM, ITGAX, ITGB2, KLKB1, KNG1, MASP1, MASP2, MBL2, PLAT, PLAU, PLAUR, PLG, PROC, PROCR, PROS1, SERPINA1, SERPINA5, SERPINB2, SERPINC1, SERPIND1, SERPINE1, SERPINE2, SERPINF2, SERPING1, TFPI, THBD, VSIG4, VTN, VWF

Table 2. Top 3 pathways associated with FTL gene. List of genes related to top pathway (overexpressed in blue).

Heat map

Z-scored normalized data were plotted to evaluate clusters and abundances.

Methodology

Collapsed duplicates by row average, then column.

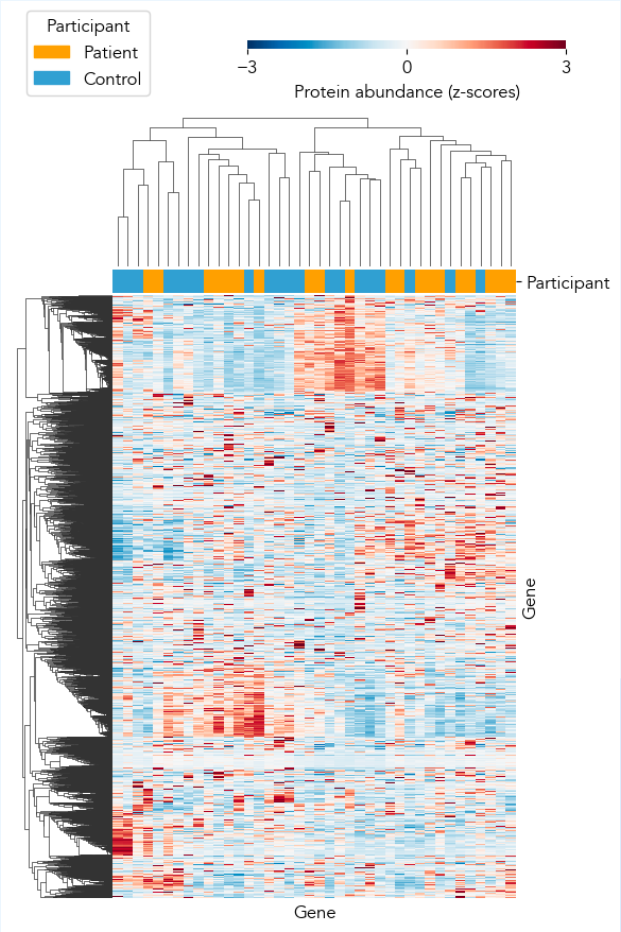
Z-score normalization.

Average linkage, Pearson correlation for clustering.

Takeaways

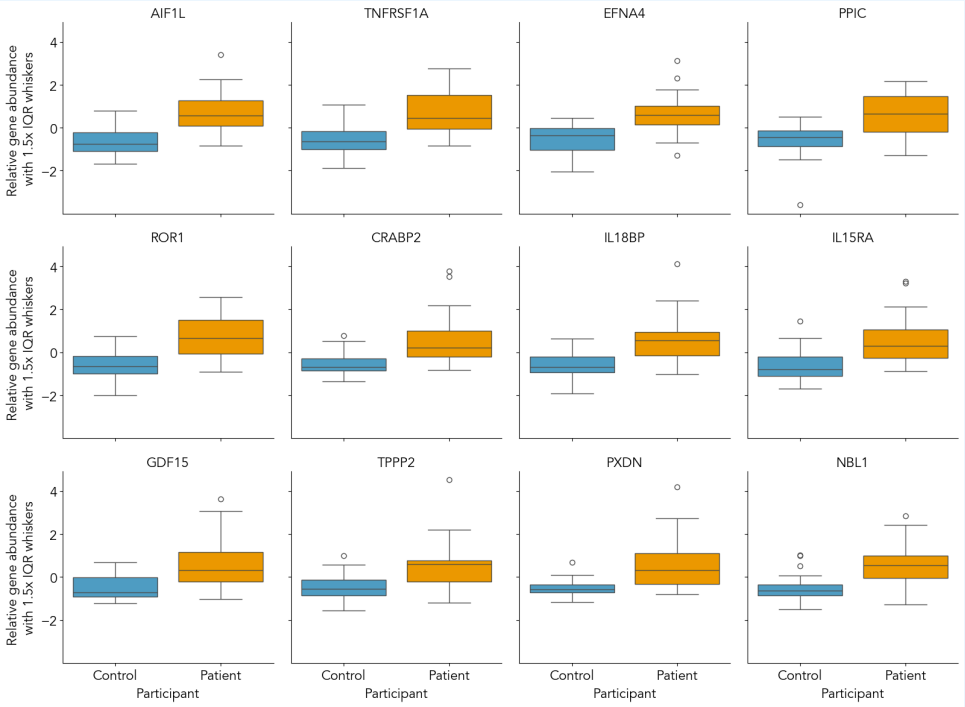
Interesting clusters exist in some areas, which may correspond to a pathway of overexpression.

Noticeable overexpressed genes in a few areas.



Targeted Analysis

Box plots of 12 overexpressed genes.



Methodologies

Row average and sort.

Takeaway

The most overexpressed gene is AIF1L (actin-binding promoting gene).

Follow up would include analysis of *under*-expressed genes and associated pathways.

4

Conclusions

Discussion

Two overexpressed pathways are frameworks for additional research.

Phospholipase D

(from lit. review) Gottschalk et al. (2022) note elevated ATG13 expression in patient serum samples.

ATG13 is downstream of the mTOR (mammalian Target of Rapamycin), which is downstream of PLD.

Pharmaceutical interventions can target these upstream receptors as potential treatment options.

Complement and Coagulation

Limited research was found in conjunction with ME/CFS.

Theory: Thrombocytopenia reported in patients with EBV infections. Post-infection ME/CFS is well-documented. Patients in sample could have existing effects and is indicated as such.

This finding indicates the potential for multiple subtypes of ME/CFS, which add to the collection of potential biomarkers.

References

Castro-Marrero, J., Zacares, M., Almenar-Pérez, E., Alegre-Martín, J., & Oltra, E. (2021). Complement Component C1q as a Potential Diagnostic Tool for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome Subtyping. *Journal of Clinical Medicine*, *10*(18), 4171. https://doi.org/10.3390/jcm10184171

Germain, A., Levine, S. M., & Hanson, M. R. (2021). In-Depth Analysis of the Plasma Proteome in ME/CFS Exposes Disrupted Ephrin-Eph and Immune System Signaling. *Proteomes*, *9*(1), Article 1. https://doi.org/10.3390/proteomes9010006

Gottschalk, G., Peterson, D., Knox, K., Maynard, M., Whelan, R. J., & Roy, A. (2022). Elevated ATG13 in serum of patients with ME/CFS stimulates oxidative stress response in microglial cells via activation of receptor for advanced glycation end products (RAGE). *Molecular and Cellular Neuroscience*, *120*, 103731. https://doi.org/10.1016/j.mcn.2022.103731

Mozhgani, S.-H., Rajabi, F., Qurbani, M., Erfani, Y., Yaslianifard, S., Moosavi, A., Pourrostami, K., Baradaran Bagheri, A., Soleimani, A., Behzadian, F., Safavi, M., & Rezaei, F. (2021). Human Herpesvirus 6 Infection and Risk of Chronic Fatigue Syndrome: A Systematic Review and Meta-Analysis. *Intervirology*, *65*(1), 49–57. https://doi.org/10.1159/000517930

Ruiz-Pablos, M., Paiva, B., Montero-Mateo, R., Garcia, N., & Zabaleta, A. (2021). Epstein-Barr Virus and the Origin of Myalgic Encephalomyelitis or Chronic Fatigue Syndrome. *Frontiers in Immunology*, *12*, 656797. https://doi.org/10.3389/fimmu.2021.656797

Sheikh, K., & Belluck, P. (2022, May 21). What We Know About Long Covid So Far. *The New York Times*. https://www.nytimes.com/2022/05/21/well/long-covid-symptoms-treatment.html

Q&A