A Cartography of Differential Gene Methylation in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: Different Network Roles in the Protein-Protein Interactions Network Play Different, Biologically Relevant, Roles

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Abstract: Myalgic Encephalomyelitis, also known as Chronic Fatigue Syndrome (ME/CFS), is a debilitating illness characterised by severe fatigue and associated with immune dysfunction. Previous studies of DNA methylation (epigenetic changes that can affect the gene transcription) have found evidence of changes in immune cells for ME/CFS. However these studies have been limited by their small sample size, precluding the ability to detect changes to methylation of smaller magnitude. Therefore, to achieve a larger sample size and detect small changes to DNA methylation, we aggregate three comparable datasets and analyse them in unison. We find 10,824 differentially methylated genes, with a very small average change. We then turn our attention to the network structure of the Protein-Protein interaction, which we built from the currently known interactions of relevant proteins, and localising the network cartography framework, we identify 184 hub genes. A distinct structuring emerges, with different hub types playing differing, meaningful, biological roles. Supporting previous theories about ME/CFS, Gene ontology enrichment analysis of these hubs reveal that they are involved in immune system processes, including response to TGF-β and LPS, as well as mitochondrial functioning. We also show that dopaminergic signalling may potentially contribute to immune pathology in ME/CFS. Our results demonstrate the potentiality of network cartographic approaches in shedding light on the epigenetic contribution to the immune dysregulation of ME/CFS.

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Introduction

Chronic Fatigue Syndrome, also known as Myalgic Encephalomyelitis (ME/CFS), is a devastating and heterogeneous disease characterised by severe fatigue that is made worse by exertion (Carruthers et al., 2011; Fukuda et al., 1994). The disease probably results from immune dysregulation (Cortes Rivera et al., 2019; Komaroff, 2019; Missailidis et al., 2019; Morris and Maes, 2013), in response to previous viral infection, such as Sars-Cov-2 (Covid-19) (Komaroff et al., 2021). The symptoms of Long Covid are also similar to ME/CFS, but it is too early to fully delineate the relationship between these two illnesses (Jason et al., 2021; Wong and Weitzer, 2021). None the less, it is now more important than ever to understand the pathophysiology of ME/CFS, and its potential implications on Long Covid.

Despite the huge impact ME/CFS has on the lives of its sufferers, efforts to find stable immune abnormalities have been largely unsuccessful. This is especially true of the investigations of cytokines, which often have conflicting results (Blundell et al., 2015; Corbitt et al., 2019). However these variations are perhaps unsurprising as cytokines function in an autocrine and paracrine manner, so their levels in the blood do not necessarily represent levels of inflammation elsewhere (Vanelzakker et al., 2019). There

have also been varying perturbations to the T cell populations found in ME/CFS, as well as B cells and natural killer (NK) cells (Ekua Weba Brenu et al., 2014; Cliff et al., 2019; Curriu et al., 2013). The source of these varying changes is unclear. Still, it is likely that the pathophysiology of ME/CFS is dominated by the effects of this inflammation (Cortes Rivera et al., 2019; Komaroff, 2017; Morris and Maes, 2013).

DNA methylation is an epigenetic modification to DNA strands which can affect gene transcription (Moore et al., 2013). Methylation can be inherited but can also be moderated by environmental circumstances during a person's life (Martin and Fry, 2018; Matosin et al., 2017). As a result, if there are methylation changes in ME/CFS immune cells, these may function differently to that of healthy controls, even in response to habitual levels of signalling molecules (such as cytokines). Attempts at finding methylation changes in immune cells of ME/CFS have been moderately successful (E. Brenu et al., 2014; De Vega et al., 2017, 2014; Helliwell et al., 2020; Herrera et al., 2018; Trivedi et al., 2018; Vega et al., 2018). Previous studies have been often limited by small patient samples, precluding the detection of small changes in gene methylation. Data acquisition through clinical procedure is, indeed, a delicate, time and resource consuming, task. Yet, the volume of publicly available datasets is rapidly growing.

Here, we hypothesise the presence, in the cells of patients with ME/CFS, of a large number of slightly differentially methylated genes (DMGs) potentially missed by studies with smaller sample sizes. To test this, we firstly merged and reconciled three highly comparable studies with publicly available data (De Vega et al., 2017, 2014; Herrera et al., 2018). Secondly, we built a Protein-Protein interaction network, looking for known interactions of proteins corresponding to differentially methylated genes in the STRING database (Szklarczyk et al., 2021, 2019). Then, adapting the notion of network cartography (Guimerà and Amaral, 2005) to our scenario, we identified network *hubs* (genes playing a central role between the DMGs). Finally, we conducted a Gene Ontology (GO) enrichment analysis of these genes.

Our data enrichment and network analysis shed light on the origins of ME/CFS immune pathology. In particular, we found that the hub proteins, 0.017% of the differentially methylated genes, interact with over half of the Protein-Protein interaction network. Moreover, the different hub types play distinct, and biological relevant, roles in ME/CFS pathology as is evidenced by their network properties and by the results of the GO enrichment analysis. Overall, we found that terms associated to response to cytokine, lipopolysaccharide (LPS) and transforming growth factor beta (TGFβ) feature in the GO analysis, as well as terms related to dopaminergic processes. This suggests that dopaminergic deregulation may underlie some immune dysfunction in ME/CFS. It is not yet clear whether long covid will share dysregulated responses to these molecules, however dopaminergic processes may play a role in long covid as dopamine has already been shown to play a role in COVID-19 itself Nataf (2020a)

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Materials and methods

We selected and merged three different, but comparable, datasets (De Vega et al., 2017, 2014; Herrera et al., 2018) and gathered a combined list of probes. Using METAL (Willer et al., 2010) we filtered for significant probes and produced a list of Differentially Methylated Genes (Maksimovic et al., 2021). Then, starting from these DMGs, we built a Protein-Protein interaction networks assembling known interactions in humans present in the STRING database (Szklarczyk et al., 2021, 2019). We adapted Guimerà and Amaral (2005)'s network cartography methodology to characterise nodes' roles in the Protein-Protein network. identifying different types of central nodes (*hub* types). Then, we used TopGO (Alexa et al., 2006; Alexa, 2021) to discover enriched Gene Ontology terms, and we performed cluster analysis (using GoFigure! (Reijnders and Waterhouse, 2021)).

To better understand the role played by the hubs, we computed the distribution of minimum distances between each node in the network and the set of hubs. We repeatead the same analysis for each set of hub types.

We contrasted our results with those found randomly sampling an equally sized set of genes. For these randomly selected genes we assessed distance distributions, and we searched for significant Gene Ontology terms.

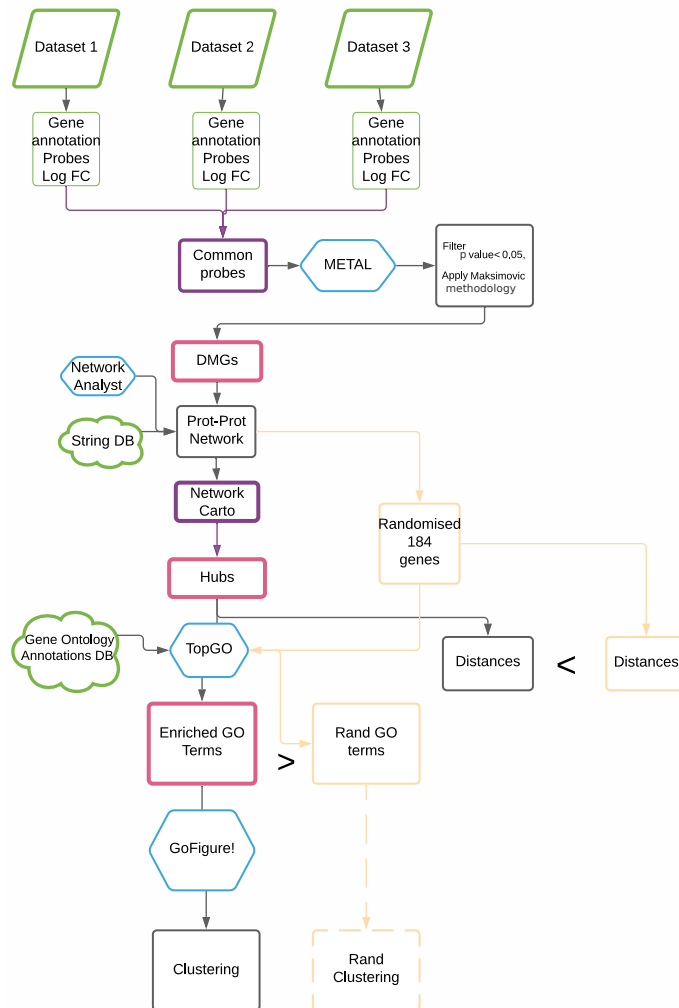We offer a schematic overview of our analytic workflow in fig. 1.

Figure 1 A schematic view of our analytical pipeline. In green the external data sources we used in our analysis. In purple the novel steps we introduce, namely: the merging of three different datasets and the localisation of Guimerà and Amaral (2005) network analytic framework. In pink the outcomes of our analysis (a list of differential methylated genes, a classification of genes in differen network hub types, and a list of enriched Gene Ontology networks). In azure some of the software we used. In yellow the analytic pipeline for the validation (random) data: as explained in the results section, we could not perform clustering on the validation GO terms because not enough terms we significant.

2.1. Data From NCBI's Gene Expression Omnibus website (GEO, (Barrett et al., 2012; Edgar et al., 2002)), we obtained three datasets covering gene methylation of peripheral blood mononuclear cells (PBMCs) in ME/CFS (De Vega et al., 2017, 2014; Herrera et al., 2018) (accession numbers GSE93266, GSE59489, and GSE156792 respectively). All three datasets were produced using the platform GPL13534, Illumina HumanMethylation450 BeadChip. The data is analysed using the GEO2R service (Barrett et al., 2012) provided by GEO. The processed data provided by the authors of the original studies is analysed by GEO2R and each probe is given a log fold change (LogFC) and p-value. We performed p-value adjustment via false discovery rate (FDR) as suggested by Benjamini and Hochberg (1995). These datafiles are then imported into R.

We identified the probes that were assessed in all three studies. Each probe, for each study, was annotated with an effect direction based on the sign of log fold change (LogFC) and given a weight based on the sample size of the relevant study. We imported probe identifier, adjusted p-values, effect direction and study weight in METAL (Willer et al., 2010) and performed a sample size based analysis. We filtered for probes with a p-value less than 0.05, which we annotated with associated gene names and gene regions as found in the original datasets. We discarded probes not related to a gene or associated to a contradictory effect direction across studies. Analysis were performed in R (Team, 2021).

2.2. Probe annotation and gene expression Starting from the identified significant probes, we proceeded to identify genes of interest for six gene regions: namely, TSS200, TSS1500, Body, 3'UTR, 5'UTR, and 1st exon. We considered for analysis all the genes present in probe with a splice variant corresponding to one of the six regions.

We approximated gene methylation level in each region covered by our study following the work of Maksimovic et al. (2021). Methylation in different gene regions results in differing effects on expression (Moore et al., 2013; Varley et al., 2013). The most consistent relationship is an inverse relationship between promoter associated regions and gene expression (Martino and Saffery, 2015; Moore et al., 2013). This may also apply to the 5'UTR and 1st exon regions (Brenet et al., 2011; Moore et al., 2013; Varley et al., 2013). In the gene body and 3'UTR, the relationship with gene expression is more complex but may be positive (Martino and Saffery, 2015; Maussion et al., 2014; McGuire et al., 2019; Yang et al., 2014). We calculated average LogFC for each analysed probe as the mean of the LogFCs over each study per probe. Let G_R be the total (weighted) LogFC of a gene G over a certain region R, that is, the sum of the (weighted) LogFCs over all the probes' in region R annotated to that gene. For the inverse regions, $G_R < 0$ may mean upregulated gene expression whilst in the positive regions $G_R > 0$ may mean upregulated gene expression. More details in the Supplementary Material.

Therefore, we classified genes with G_R greater than zero as potentially upregulated and genes with G_R less than zero as potentially down regulated. For upregulated genes, we added the sum of G_R in inverse regions per gene and subtracted the sum of the G_R of the positive regions; gene regions with a G_R suggesting down regulation would cancel out a degree of the G_R suggesting upregulation. For upregulated genes we defined their total LogFC as $G_t = \sum_\oplus G_R - \sum_\ominus G_R$ where $\ominus$ and $\oplus$ denote the inverse and positive regions respectively. Then, we discarded any upregulated gene with a negative total logFC, $G_t < 0$, . Similarly, for downregulated genes we defined their total LogFC as $G_t = \sum_\ominus G_R - \sum_\oplus G_R$ and we discarded any gene with a positive total logFC, $G_t > 0$.

2.3. Protein protein interaction network We built a protein-protein interaction network (PPIN) from upregulated and downregulated genes, using Network Analyst (Zhou et al., 2019) and the STRING database (Szklarczyk et al., 2021, 2019). The network was constructed such that each node represents a gene, and an edge connecting two nodes represents a known protein-protein interaction between the proteins encoded by the genes. The protein-protein interactions came from the STRING database and required a confidence score of at least 900. The confidence score is calculated by STRING and based on the quality of each source of evidence for an interaction (Von Mering, 2004). The only genes considered for the network are the differentially methylated genes previously identified.

In this way, we obtained a zero-order network (that is, we considered only direct interactions) of which we picked the giant connected component, comprising 4770 node (which we will refer to as "the network" in the following).

Our method for identifying hub proteins builds upon the notion of network cartography, introduced by Guimerà and Amaral (2005).
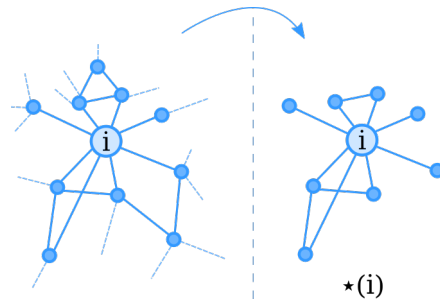
Figure 2 For any node i in our network, we denote $\star(i)$ the network induced by the first-order neighbours of i. That is, the set of nodes of the network $\star(i)$ is given by i and the all nodes at most one step away from i; the edges are given by all the edges in the original network between any two node in the set of nodes of $\star(i)$. The figure shows which nodes and edges from the original network (left) are preserved in the induced network (right).

We defined the normalised degree, Z_i, of a node i in the the network as its scaled and centralised degree: $Z_i = \frac{D_i - \overline{D}}{\sigma(D)}$, where D_i is the degree of the node i, and $\overline{D}$ and $\sigma(D)$ are the the average and the standard deviation of the degrees of all nodes in the network respectively.

We denote $\star(i)$ be the network induced by the first-order neighbours of i (that is, i, the nodes at most one step away from i, and all the interactions between these, see fig. 2). We denote $\overline{D_{\star(i)}}$ the average node degree over $\star(i)$. Then, we define the participation coefficient of i, P_i, as $P_i = 1 - \frac{\overline{D_{\star(i)}}}{D_i}$. This index provides information about the connectivity of a node i with respect to its local community. Notice that, due to the constraints imposed by the sparsity of the network reconstruction, we substituted the community identification step adopted in (Guimerà and Amaral, 2005) with a local averaging over the neighbourhood.

Using the normalised degree and the participation score of each node, each gene was assigned a role based on the cutoffs suggested by Guimerà and Amaral (2005). Specifically, nodes with a normalised degree score $Z_i \geq 2.5$ were designated as hubs. These nodes were further split into three role groups based on participation scores:

- **city hubs** are the nodes with $P_i \leq 0.3$: their neighbours area all highly connected to each other;

- **suburban hubs** are the nodes such that $0.3 \leq P_i \leq 0.75$: they are connected to more than one large cluster;

- **transit hubs** are the nodes with $0.75 \leq P_i$: their neighbours are in general not part of large clusters.

Using Graphs (James Fairbanks and Karpinski, 2021) in Julia (Bezanson et al., 2017), we computed the distribution of minimum distances from each node in the network to any of the nodes in the set of all hubs. We did the same for the sets of city hubs, suburban hubs, and transit hubs.

2.4. Gene Ontology enrichment analysis We performed gene ontology (GO) enrichment analysis using the bioconductor package topGO (Alexa et al., 2006; Alexa, 2021) in R (Team, 2021). We used a list of the hub genes abnormally methylated in ME/CFS, with a background list of all genes assayed by the 450k array so to identify significantly enriched GO terms. We used the Gene Ontology Biological Processes (GObp) dataset (Ashburner et al., 2000; "The gene ontology resource: Enriching a GOld mine," 2021) and an elimination algorithm provided by topGO (Alexa et al., 2006; Alexa, 2021; Ashburner et al., 2000; "The gene ontology resource: Enriching a GOld mine," 2021), with a threshold of at least 3 genes being annotated to a GObp term for inclusion in the analysis. Raw p-values provided by topGO were corrected for false discovery rate. With the same methodology, we analysed the genes of each hub type separately.

Using GO-Figure! (Reijnders and Waterhouse, 2021), we visualised the statistically significant GO terms from all hubs together as well as each hub type separately; assessed semantic similarity; and identified clusters in the semantic space, considering a similarity index cutoff (si) of $si = 0.45$, $si = 0.5$, and $si = 0.7$. GO-Figure! (Reijnders and Waterhouse, 2021) assigned cluster names based on a list of terms manually selected from the most general terms for each possible process within the full list of GO Terms (see Supplementary Material).
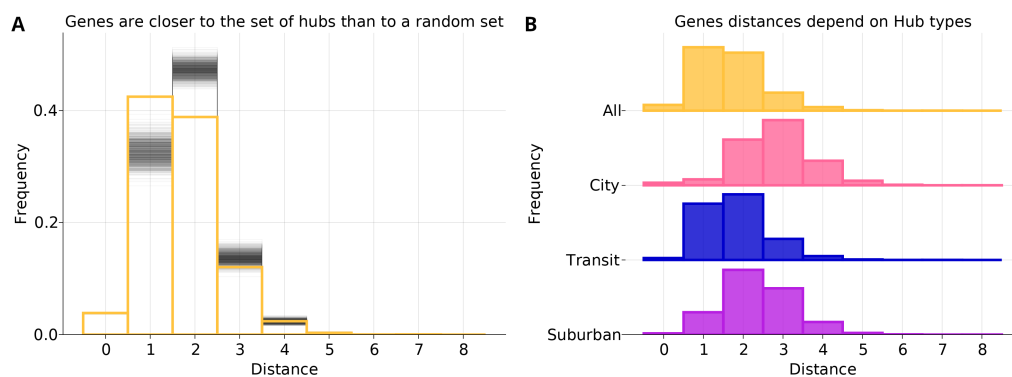
A Genes are closer to the set of hubs than to a random set
B Genes distances depend on Hub types

Figure 3 **(A)** the distribution of minimum distances from each node in the network to the set of all hubs; in yellow the distribution for the 184 hubs, in gray the distribution for each of the random replicates of 184 genes. **(B)** the distribution of minimum distances from each node in the network to each of the sets of specific hub types.

2.5. Validation To validate our results, we compared them to those we would get by sampling at random equally sized set of the genes from the DMGs. For each random sample we repeated the same analysis as above. We ran a total of 200 repetitions for the GO enrichment and 1000 repetions for the distances distribution analysis.

2.5.1 Reproducibility Elaborated data and scripts can be found in a public repository github.com/officinadata/CFS_Proteomic.

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Results

3.1. Probes and genes Combining the three datasets, we obtained a total of 478,165 probes to analyse. Filtering for statistical significance at a level of $\alpha = 0.05$, we reduced the set to 42,439 probes, of which 32,318 associated with a gene. We computed total methylation change and loocked for evidence of up or down regulation, finding 10,824 genes differentially methylated (5,532 genes up regulated and 5,292 down regulated). Of these, we discarded 4,354 genes with a small absolute average LogFC ($|G_s| < 0.01$).

Summary	Total	Down Regulated	Up Regulated
Number of genes	10824	5292	5532
Average estimated LogFC (abs)	0.103	-0.095	0.111
Median LogFC (abs)	0.053	-0.053	0.053
Highest LogFC (abs)	9.62	-9.662	3.304
Average Num Probes	3.41	3.39	3.43
Highest Num Probes	84	84	50

3.2. Hub Identification We built a zero-order network from the 4770 genes with $|G_s| > 0.01$. The protein protein interactions (PPIs) network contained 40811 edges (interactions between two proteins) coded by genes. We computed Z and P_i scores for each node in the network, and filtered as hubs those 184 proteins with a $Z \geq 2.5$. We found that 63 of the associated genes to be downregulated and 121 upregulated. We classified hubs based on their P_i scores into 61 Transit hubs, 40 suburban hubs, and 83 City hubs.

The edges connected to the 184 hubs include over half of all the edges in the network, and the set of hubs and their first neighbours includes almost half of all genes in the network (2220).

We found that the genes in the network are significantly closer to the set of all hubs than random, as see in fig. 3 (A) there are significantly more nodes one step away from the set and significantly less nodes two steps away than in a random selection of a same sized set of nodes. This was accentuated for the set of the 61 Transit hubs, whilst the set of City and Suburban hubs tend to be further away from the rest of the networks, as visible in fig. 3 (B).

Downregulated proteins are predominantly Transit hubs (43%), whilst Transit hubs are 28% of the up-regulated proteins. The proportions are reversed for City hubs (52% of upregulated and 32% of down-regulated proteins).

3.3. Gene Ontology enrichment Analysing the 184 hub genes, we identified 341 GO biological process (GObp) significantly enriched. Of these, 131 terms are associated with more than 50 genes. Briefly, the terms of note include ones related to lipopolysaccharide (LPS), transforming growth factor β ($TGF\beta$), cytokine production, reactive oxygen species (ROS), circadian rhythms, gene expression, G-protein coupled receptor (GPCRs), dopaminergic signalling, inflammatory response, and MAPK cascade.

We found 82 were significant terms for the City hubs, 24 terms for the suburban hubs, and 386 terms for the Transit hubs.

Dopamine is the only specific chemical signalling molecule appearing for both City and Suburban hubs. GPCR, phospholipase C, adenylate cyclase, and cytosolic calcium ion related terms also appear in both lists. RNA and viral processes appear in both Transit and Suburban hubs GO terms. LPS, circadian rhythms, inflammatory response, dopaminergic processes, and MAPK related signalling are common themes in the City and Transit hubs lists. The only chemical to appear in all 3 lists is dopamine. Dopamine appears in 5 terms in the City hubs list, and 1 term each in suburban and Transit hubs lists. No theme runs through all 3 type of hubs.

3.3.1 City hubs The terms enriched in City hubs are mainly related to signalling processes (44 terms). Of these, 16 are related to GPCRs, 9 of which are related to adenylate cyclase modulating receptors. We found 11 terms related to monoaminergic processes (5 related to dopaminergic processes). Glutaminergic, GABAergic, acetylcholinergic, purinergic and opioidergic terms also feature in small numbers, once again mainly to do with GPCRs. Calcium signalling and regulation of cytosolic calcium ion gradient terms appear as well, with 6 terms. In regards to immune system processes, inflammatory response, lipopolysaccharide signalling, chemokine signalling, and positive regulation of inflammation terms are enriched. Monocyte, neutrophil, dendritic cell, and general, cell chemotaxis also appear in the list.

3.3.2 Suburban hubs The Suburban Hubs featured 4 terms related to GPCRs, including both activating and inhibiting GPCRs coupled to adenylate cyclase. cAMP biosynthesis, protein kinase A and phospholipase C activity also occur, along with RNA transcription processes. We also found terms related to dopamine receptor signalling pathway and to viral transcription.

3.3.3 Transit hubs Transit hubs had the most terms related to immune processes. We found 16 terms related to cytokine signalling, 4 to B cells and 5 terms to T cells respectively, 3 each related to macrophages, leukocytes generally, and LPS. Interestingly viral process, regulation of defense response to virus, and negative regulation of NF-kB are also enriched, as well as 6 terms related to the MAPK cascade. We found terms related to growth factor and hormonal signalling but not to GPCRs. In total, 53 GO Terms include the word "signal." Also of note, 9 terms related to RNA processes and 7 included the word DNA, of which 11 terms related to transcription. Histone deacyelation also featured twice, as well as both positive and negative regulation of gene expression, and histone phosphorylation. Circadian rhythmic processes appeared, including circadian regulation of gene expression. Positive regulation of reactive oxygen species production, cellular response to reactive oxygen species, and cellular response to hydrogen peroxide also appeared in the list. Finally, the transit hubs also had multiple energy production or mitochondria-related terms, including negative regulation of mitochondrial membrane potential and negative regulation of glycolytic process.

We performed clustering analysis for the 131 terms enriched from the hubs and identified 20 different groups at a similarity index cut off (*si*) of 0.45 and 31 groups at *si* = 0.7. These can be seen in fig. 4. As seen in the figure, at both *si* levels, clusters have been associated predominantly to immune processes, and in lesser measure to dopamine was also selected by and GPCR signalling pathway.

In the following table we provide the identities of each cluster at the two *si* levels.
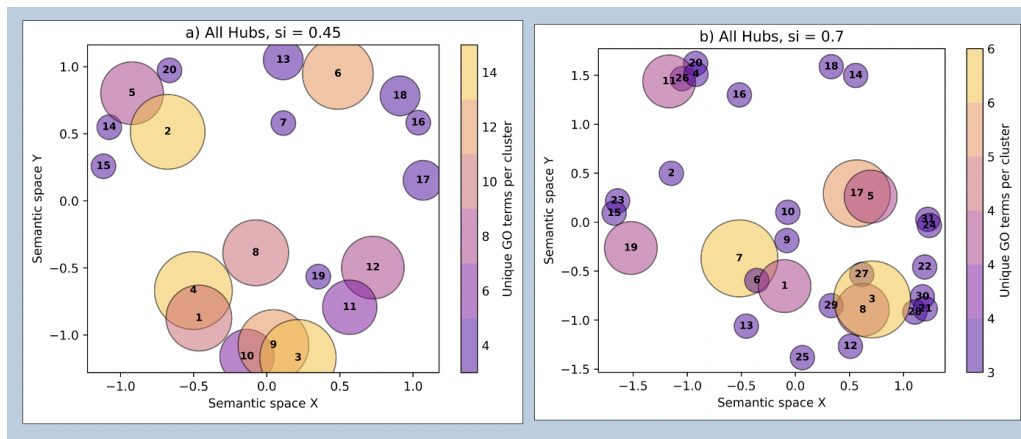
Figure 4 Clustering of GO Terms. (**a**): The clustering of 131 GO Terms by GO-Figure! at si = 0.45. Ordered by highest number of associated genes to lowest number of associated genes. Identity of the clusters in text. (**b**): clustering of the same terms by GO-Figure! at si = 0.7. Ordered by clusters with the highest number of associated genes to clusters with the lowest number of associated genes. Identity of the cluster in text.

cut off	Terms
si = 0.45	1. G protein-coupled receptor signaling pathway, 2. cellular response to dopamine, 3. positive regulation of cell population proliferation, 4. cytokine-mediated signaling pathway, 5. immune response, 6. sensory perception of pain, 7. cell-cell signaling, 8. positive regulation of cytosolic calcium ion concentration, 9. positive regulation of ERKl and ERK2 cascade, 10. positive regulation of cell migration, 11. negative regulation of transcription by RNA polymerase II, 12. regulation of cytokine production involved in inflammatory response, 13. cell chemotaxis, 14. inflammatory response, 15. wound healing, 16. Aging, 17. locomotory behavior, 18. endocrine system development, 19. positive regulation of DNA-binding transcription factor activity, 20. response to hypoxia
si = 0.7	1. G protein-coupled receptor signaling pathway, 2. cell-cell signaling, 3. positive regulation of cell population proliferation, 4. cellular response to dopamine, 5. cytokine-mediated signaling pathway, 6. adenylate cyclase-activating G protein-coupled receptor signaling pathway, 7. regulation of cytokine production involved in inflammatory response, 8. positive regulation of apoptotic process, 9. negative regulation of transcription by RNA polymerase II 10. positive regulation of cytosolic calcium ion concentration, 11. response to lipopolysaccharide, 12. positive regulation of cell migration, 13. regulation of potassium ion transport, 14. leukocyte migration, 15. blood circulation, 16. wound healing, 17. ephrin receptor signaling pathway, 18. cell chemotaxis, 19. locomotory behavior, 20. response to cAMP, 21. positive regulation of ERKl and ERK2 cascade, 22. regulation of small GTPase mediated signal transduction, 23. cognition, 24. Fe-gamma receptor signaling pathway involved in phagocytosis, 25. regulation of hormone secretion, 26. cellular response to reactive oxygen species, 27. positive regulation of cell growth, 28. positive regulation of protein kinase B signaling, 29. positive regulation of epithelial to mesenchymal transition, 30. positive regulation of phosphatidylinositol 3-kinase signaling, 31. stimulatory C-type lectin receptor signaling pathway.

For the list of cluster members, see supplementary content.

Clustering of the individual hub types lists revealed a distinct structure to the terms annotated to each type, see fig. 5. The City hubs reduced to 14 clusters, with very little overlap. Manual analysis of the terms in each cluster revealed sets of processes which are related but may be better represented by a general term rather than the terms chosen by GO-Figure!. However, the terms themselves clustered
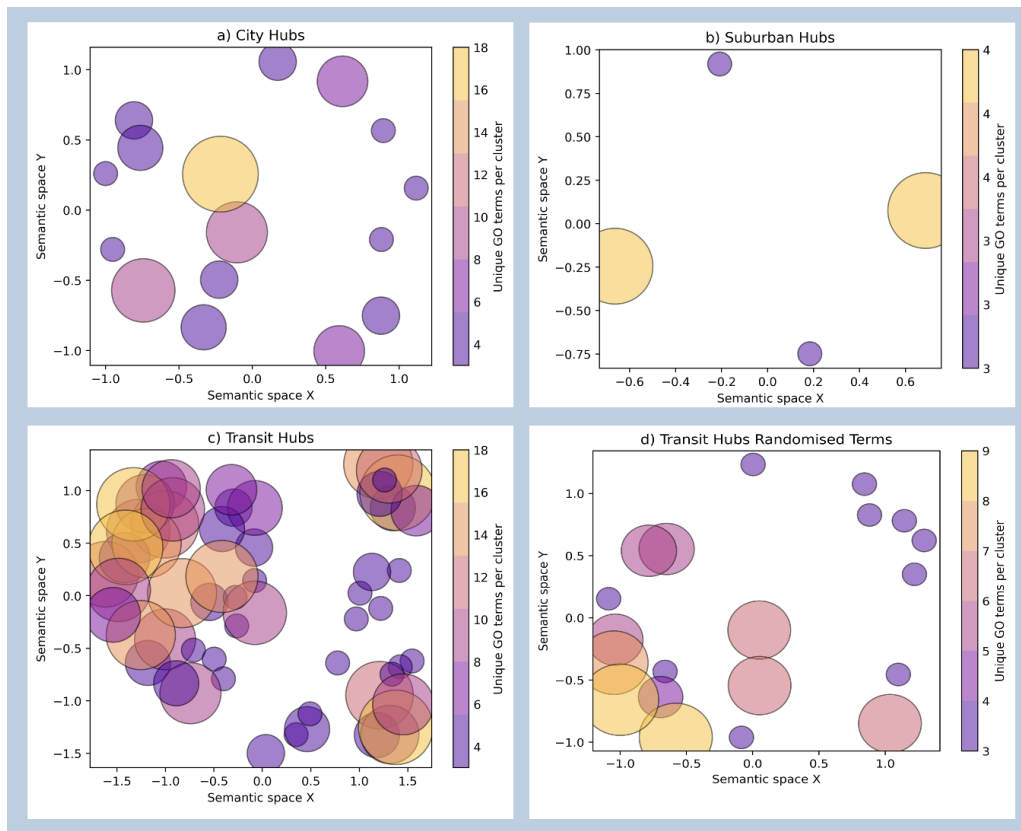
Figure 5 Clustering of GO Terms for Hubs. (**a**): The clustering of 82 GO terms enriched from the list of City hubs created on GO-Figure! (**b**): clustering of the 24 terms by GO-Figure! related to suburban hubs. (**c**): clustering of the 386 Transit hub GO terms. (**d**): clustering of 82 randomly selected Transit hub GO-terms for comparison to City hubs.

into immune, signal transduction and chemical signalling processes. The suburban hubs clustered into 3 clusters of little relation. Most crucially, the Transit hubs clustered into 46 individual clusters with a huge degree of overlap. This may suggest that the Transit hubs do indeed represent genes involved in many biological processes which are not necessarily highly related to each other. Overall we found that the GO Terms resultant from the City hubs are more closely related than those of the Transit hubs.

Validating our results against random replications, we found that for 197 of 200 Gene Ontology validation resulted in no statistically significant enriched GO terms at $\alpha = 0.05$ (both for the set of all hubs and for each hub type hub set). All of the three analyses that had some significant GO terms had only one significant GO term in one analysis, therefore no clustering could be performed.

4 Discussion

For the first time we analysed the data from these three studies together, within a network analytic framework. The three studies we selected share the same platform, the same diagnostic criteria, and the same cell type. Thus, we considered the datasets highly compatible. The combined data includes 207 participants: almost double the size of the next largest methylation study of peripheral blood mononuclear cells (PBMCs) in ME/CFS (109 participants (Herrera et al., 2018)). This large sample size gives us more power to detect small logFCs. Two of the original studies (De Vega et al., 2017, 2014) identified 826 and 5,544 DMGs respectively. The other original study does not report how many genes were differentially methylated as it was looking for associations between methylation and single nucleotide polymorphisms (SNPs) (Herrera et al., 2018). Another study on methylation in ME/CFS found 17,296 differentially methylated probes, annotated to 6,368 DMGs (Trivedi et al., 2018). This suggests that whilst we did find 10,824 DMGs, it is not inconsistent with previous reports from smaller sample sizes. Hence, as we hypothesised, we found a large number of DMGs in ME/CFS PBMCs, often with a small LogFC.

4.1. Limitations It is important to note that peripheral methylation does not necessarily represent methylation in the central nervous system (Non and Thayer, 2015; Walton et al., 2016). Because of the cell type analysed, the results discussed here focus on implications for the immune system. As we used publicly available data, we can not directly correlate the changes seen in methylation with symptomology or gene expression data of the same patient population. The protein-protein interaction networks are intrinsically biased toward protein interactions that have already been studied. Likewise, the array platform used may be biased towards known cancer related genes as it was originally invented with these genes in mind (Barker et al., 2018; Non and Thayer, 2015). Thus, the hubs we identified may be biased towards those proteins already well characterised and may be missing some less studied proteins.

Moreover, whilst the gene expression can be indicated by the logFC methylation, the latter is not a perfect proxy: other factors may affect gene expression downstream of gene methylation. The relationship between non promoter methylation and gene expression is complex. Different studies produced contrasting results about the effect of gene body methylation, finding both positive and inverse relationships with gene expression, potentially depending on the methylation of other gene regions (Damgacioglu et al., 2019; De Almeida et al., 2019; Jjingo et al., 2012; Moore et al., 2013; Tang et al., 2017; Yang et al., 2014). Furthermore, the relationship may even be non-linear (Jjingo et al., 2012). While encouraging results have already been shown (Damgacioglu et al., 2019; Levy et al., 2020; Silva et al., 2021; Zhong et al., 2019), additional work needs to be done to robustly relate methylation changes with changes in gene expression. Moreover, histone aceylation and microRNA (miRNA) changes have been found in ME/CFS and these will change the transcription of genes (Almenar-Pérez et al., 2020; Ekua W. Brenu et al., 2014; Brenu et al., 2012; Jason et al., 2011; Petty et al., 2016).

4.2. Interpreting the set enriched terms The results of the Gene Ontology enrichment analysis of the hub protein subtypes pivot around four themes touching on wider epigenetic changes, immune system processes, signalling pathways, and dopaminergic process.

4.2.1 Methylation changes underlying wider epigenetic changes Our results strengthen the hypothesis that epigenetics may play a role in ME/CFS (Almenar-Pérez et al., 2019; Cortes Rivera et al., 2019; Vega et al., 2018). However, the methylation changes in ME/CFS may partially mediate the wider epigenetic changes found in ME/CFS. Considering the list of GO terms for transit hubs, we identified 6 abnormal pathways related to the regulation of miRNA production and transcription. Furthermore, Almenar-Pérez et al. (2020) found 8 upregulated miRNA in PBMCs of ME/CFS patients; our analysis independently identifies 5 of these genes as upregulated. Other studies have analysed miRNA expression in ME/CFS (Almenar-Pérez et al., 2020; Ekua W. Brenu et al., 2014; Brenu et al., 2012; Nepotchatykh et al., 2020; Petty et al., 2016) and one has attempted to integrate differential methylation with miRNA expression (Almenar-Pérez et al., 2019). We consider plausible that a degree of the miRNA abnormalities found in ME/CFS may be caused by differential methylation of their genes. Additionally, histone acetylation pathways also appear in the list of enriched terms from transit hubs. One study in ME/CFS patients found elevated levels of HDAC2 and HDAC3, suggestive of decreased gene transcription (Jason et al., 2011). Another suggests that hypoacetylation caused by upregulated expression of the HDAC family may contribute to post exertional malaise (McGregor et al., 2019), a key feature of ME/CFS symptomology (*Beyond myalgic encephalomyelitis/chronic fatigue syndrome: Redefining an illness.*, 2015; Fukuda et al., 1994). We found methylation changes suggestive of upregulation of multiple HDAC genes, including HDAC3. Thus, the widespread methylation changes we found may contribute to the other epigenetic abnormalities previously reported.

4.2.2 Responses to perturbed immune system processes In terms of immune system processes, a few GO terms stood out. Response to LPS and LPS mediated signalling appeared in the list of GO terms for both transit and city hubs. In fact, increased antibodies to LPS (Maes et al., 2012, 2007) and elevated blood levels of LPS (Giloteaux et al., 2016) have been reported in ME/CFS: this has been postulated to contribute to immune activation both centrally and peripherally (Morris and Maes, 2013). Another key molecule is reactive oxygen species (ROS). Terms related to response to ROS appeared in the transit hubs list, as well as positive regulation of their metabolism. ROS are suggested to contribute to ME/CFS as they are a crucial aspect of oxidative stress (OS) (Cortes Rivera et al., 2019; Jammes et al., 2012, 2005; A. Komaroff and Cho, 2011; Morris et al., 2019). OS related damage (DAMPs) may initiate and maintain an immune response whilst ROS may also disrupt mitochondrial functioning leading to reduced

ATP production (Cortes Rivera et al., 2019; A. L. Komaroff and Cho, 2011; Morris and Maes, 2013). Unsurprisingly, we also found terms related to cytokine response. Finally, terms related to viral response appeared in the transit and suburban hubs enrichment. This is important because much work has been done exploring the association between ME/CFS and viruses (Bansal et al., 2012; Rasa et al., 2018). If viral responses are dysregulated by methylation changes, patients with ME/CFS may react differently to viruses than a healthy control would: relatively small changes to levels of O&NS, virus or LPS could be creating significant immune responses in an unpredictable manner.

Additionally, the GO terms relating to mitochondrial membrane potential and glycolytic activity are intriguing. There have been some findings of lowered mitochondrial membrane potential in patients with ME/CFS (Mandarano et al., 2020; Missailidis et al., 2020a, 2020b) as well as lowered glycolytic activities (Mandarano et al., 2020; Nguyen et al., 2019; Tomas et al., 2020), though the latter has been less consistent (Missailidis et al., 2021).

4.2.3 *Disruption of signalling pathways* Many GO terms were related to signalling processes. As discussed in the results section, the city hubs and suburban hubs had a high degree of GPCR associated processes enriched. These included a wide range of monoaminergic processes, including 5 dopaminergic processes. This may suggest that signalling by monoamines contributes to immune dysregulation in ME/CFS. Monoaminergic contribution to ME/CFS is not new, we present for the first time evidence for a direct implication in immune dysfunction. The finding of dysregulated acetylcholinergic and opioidergic signalling also supports suggestions that these chemicals may contribute to ME/CFS immune dysregulation (Anderson and Maes, 2020; Marshall-Gradisnik et al., 2016; Tanaka et al., 2003; Wirth and Scheibenbogen, 2020). The same can be said of adrenergic signalling (Johnston et al., 2016; Loebel et al., 2016; Scheibenbogen et al., 2018; White et al., 2012). Calcium signalling also appeared in the GO terms list: it has been suggested that abnormalities in calcium signalling may contribute to natural killer (NK) cell hypofunction in ME/CFS (Cabanas et al., 2019; Eaton-Fitch et al., 2019; Nguyen et al., 2017). Considering the widely reported presence of autoantibodies to GPCRs in ME/CFS (Loebel et al., 2016; Tanaka et al., 2003; Wirth and Scheibenbogen, 2020), it is likely that methylation changes compound the effect of already disrupted GPCRs. In the transit hubs, terms related to immune signalling were present. Specifically, signalling mediated by both B and T cell receptors, cytokines generally, IL-6, TGF-b, and LPS. This may suggest dysregulated response to antigens and cytokines. As TGF-b was found to be the only consistently upregulated cytokine in one systematic review (Corbitt et al., 2019), it seems likely that TGF-b may underlie some of the immune dysregulation if its signalling mediation is also disrupted. In sum, signalling by neurotransmitters, immune receptors, and cytokines may be abnormal and may contribute to the immune pathophysiology of ME/CFS.

4.2.4 *Dopamine* Dopaminergic processes were the only processes to feature in the results for the GO enrichment analysis of all hub types. The fatigue in ME/CFS seems likely to be partially dopaminergic centrally, considering the efficacy of multiple dopaminergic agents in ME/CFS (Blockmans et al., 2006; Blockmans and Persoons, 2016; Crosby et al., 2021; Goodnick et al., 1992; Kaiser, 2015). This would also fit with the high rates of Attention Deficit Hyperactivity Disorder (ADHD) in ME/CFS (Sáez-Francàs et al., 2012), and monoamine metabolite levels in cerebrospinal fluid (Demitrack et al., 1992). Moreover, levels of both general and mental fatigue in ME/CFS have been inversely correlated with basal ganglia activation as assessed by fMRI, potentially secondary to dopaminergic deficits (Miller et al., 2014). Numerous other neuroimaging studies also support this notion (see (Almutairi et al., 2020; Cook et al., 2017; Gay et al., 2016; Josev et al., 2019; Manca et al., 2021; Shan et al., 2020; Shan et al., 2018; Tanaka et al., 2006; Van Der Schaaf et al., 2018, 2017; Wortinger et al., 2017; Wortinger et al., 2016)). Furthermore, Carandini et al. (2021) found an inverse correlation between levels of mental fatigue and DA tract abnormalities in MS. Carandini et al. (2021) suggested that this is evidence of dopaminergic abnormalities underlying mental fatigue, as hypothesised by Dobryakova et al. (2015). Research in animals, and in patients given cytokine therapies, has demonstrated that peripheral inflammation can lead to central dopaminergic hypofunction (Anisman et al., 1996; Felger et al., 2013; Felger and Miller, 2012; Lee et al., 2021). Hence it can be postulated that the immune dysregulation and inflammation in the periphery may lead to central dopaminergic deficits, which then may lead to some of the experience of fatigue in ME/CFS. It is important to note that this does not dismiss the impact of other mediators, such as impaired mitochondrial functioning (Holden et al., 2020; Mandarano et al., 2020; Tomas et al., 2017). Dopaminergic hypo-functioning would simply make the fatigue caused by peripheral processes feel significantly worse for the patients involved.

Our results suggest that dopaminergic signalling may also be abnormal in an immune context. The

potential discovery of a dopaminergic element to ME/CFS immune dysregulation is consistent with its involvement in other autoimmune diseases including multiple sclerosis (Pacheco et al., 2014; Vidal and Pacheco, 2020a, 2020b). The effects of dopaminergic signalling in the immune system are wide, varied, and beyond the scope of this article. For review see (Arreola et al., 2016; Castorina et al., 2020; Feng and Lu, 2021). However, it is worth noting that signalling through the dopaminergic receptors D2 and D3, both of which are hub proteins and potentially hyper-expressed, may have relevant immune effects. D2-like receptor agonists have been shown to bias T cells towards a Th2 type differentiation (Huang et al., 2010). Furthermore, D2 receptor activation is thought to inhibit natural killer cell activation and cytotoxicity (Capellino et al., 2020; Zhao et al., 2013). Of note considering the consistently found decreased NK cell cytotoxicity (Brenu et al., 2011; Fletcher et al., 2010; Huth et al., 2016; Levine et al., 1998; Maher et al., 2005; Marshall-Gradisnik et al., 2016) as well as a shift towards a Th2 response (Brenu et al., 2011; Broderick et al., 2010; Skowera et al., 2004; Torres-Harding et al., 2008) in ME/CFS.

It is worth noticing that suggestive evidences propose a role for dopamine in Covid-19 pathophysiology (Attademo and Bernardini, 2021b; Berber and Doluca, 2021; Nataf, 2020b). However, dopaminergic antagonists in ME/CFS would may have sedating effect. Hence, dopaminergic agents in ME/CFS may constitute the object of careful study, considering immune parameters, symptom perception, and side effects.

4.3. Different hubs play a different biological role The participation coefficient is a combination of the clustering coefficient (Chalancon et al., 2013; Wasserman and Faust, 1994; Watts and Strogatz, 1998) and the original participation coefficient defined by Guimerà and Amaral (2005). However, whilst the clustering coefficient can be used to find modules of a network (Chalancon et al., 2013; Li et al., 2009; Nascimento, 2014; Zaki et al., 2013), and Guimerà and Amaral (2005)'s participation coefficient is based on modules, we use a combination of the two and a local averaging over network neighbours to overcome modularisation of an incomplete network. Our adapted participation coefficient highlights how much interaction there is between a group of neighbours: if a node has a high participation coefficient, its neighbours are sparsely connected, and the gene in question participates in many distinct biological processes. This is the case for transit hubs which GO enrichment terms are more numerous and cluster into smaller, more overlapping, clusters than either the terms for city hubs or suburban hubs, as we can see in fig. 5. The genes classified as transit hubs are commonly involved in signal transduction. Suggestively, whilst a few cytokine genes also appeared, transit hubs were associated to many immunity response terms.

City hubs, $P_i < 0.3$, are located in densely connected neighbourhood: therefore, their associated genes take part in well connected processes. By nature of their participation coefficient, they form functional units to a certain extent, which may interact with other units to a lesser degree. Hence an abnormality in a city hub may result in larger functional impact due to multiplying cascading effects (Shin et al., 2014), although this may be regulated by interaction strengths (Gaiarsa and Guimarães, 2019; Guimarães et al., 2018). From a biological perspective, this may suggest utility of drugs that modulate the signalling pathways seen from the GO enrichment. This can be seen in their clustering, where the clusters overlap less and contain more terms than the transit hubs.

The neighbours of suburban hubs may have varying degrees of connectivity between and within town groups of neighbours. However, whilst these hubs and their neighbours are not as interconnected as city hubs, there is still some degree of connectivity between their neighbours. These hubs almost seem to be the "go-betweens" of the city and transit hubs, as the terms enriched for them include both GPCR signalling and viral processes, not contained by the transit hubs and city hubs respectively.

4.4. Conclusions Our result suggest that small, widespread, changes in gene methylation may mediate some of the immune system pathology seen in ME/CFS. If Covid-19 does indeed lead to ME/CFS as Komaroff et al. (2021) are suggesting, then methylation profiling a large sample of patients would be beneficial in understanding its pathogenesis. Efforts to understand the cause of methylation changes in ME/CFS, and Long Covid if applicable, should also occur. Moreover, analysing relationships between the DMGs is necessary: few signalling pathways that activate key effectors, their receptors are probably present in the hubs of this study. If these proteins do exist, they may lead to the discovery of druggable targets for ME/CFS immune dysregulation.

Our results further support the presence of methylation changes contributing to the immune dysregulation that characterises ME/CFS. They also suggest that responses to LPS, cytokines, O&NS, and viruses function differently to what is seen in healthy controls, exacerbating immune abnormalities beyond

what would be expected otherwise. Finally, signalling by multiple endogenous chemicals is also perturbed and may affect immune activation. Depending on whether Long Covid is related to ME/CFS, our findings may be of interest for consideration when studying treatment implications.

References

Alexa A, Rahnenfuhrer J, Lengauer T. 2006. Improved scoring of functional groups from gene expression data by decorrelating GO graph structure. *Bioinformatics* **22**:1600–1607. doi:10.1093/bioinformatics/btl140

Alexa R Adrian. 2021. topGO: Enrichment analysis for gene ontology. doi:10.18129/B9.bioc.topGO

Almenar-Pérez E, Ovejero T, Sánchez-Fito T, Espejo JA, Nathanson L, Oltra E. 2019. Epigenetic components of myalgic encephalomyelitis/chronic fatigue syndrome uncover potential transposable element activation. *Clinical Therapeutics* **41**:675–698. doi:10.1016/j.clinthera.2019.02.012

Almenar-Pérez E, Sarría L, Nathanson L, Oltra E. 2020. Assessing diagnostic value of microRNAs from peripheral blood mononuclear cells and extracellular vesicles in myalgic encephalomyelitis/chronic fatigue syndrome. *Scientific Reports* **10**. doi:10.1038/s41598-020-58506-5

Almutairi B, Langley C, Crawley E, Thai NJ. 2020. Using structural and functional MRI as a neuroimaging technique to investigate chronic fatigue syndrome/myalgic encephalopathy: A systematic review. *BMJ Open* **10**:e031672. doi:10.1136/bmjopen-2019-031672

Anderson G, Maes M. 2020. Mitochondria and immunity in chronic fatigue syndrome. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **103**:109976. doi:10.1016/j.pnpbp.2020.109976

Anisman H, Kokkinidis L, Merali Z. 1996. Interleukin-2 decreases accumbal dopamine efflux and responding for rewarding lateral hypothalamic stimulation. *Brain Research* **731**:1–11. doi:10.1016/0006-8993(96)00460-x

Arreola R, Alvarez-Herrera S, Pérez-Sánchez G, Becerril-Villanueva E, Cruz-Fuentes C, Flores-Gutierrez EO, Garcés-Alvarez ME, De La Cruz-Aguilera DL, Medina-Rivero E, Hurtado-Alvarado G, Quintero-Fabián S, Pavón L. 2016. Immunomodulatory effects mediated by dopamine. *Journal of Immunology Research* **2016**:1–31. doi:10.1155/2016/3160486

Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G. 2000. Gene ontology: Tool for the unification of biology. *Nature Genetics* **25**:25–29. doi:10.1038/75556

Attademo L, Bernardini F. 2021b. Are dopamine and serotonin involved in COVID-19 pathophysiology? *The European journal of psychiatry* **35**:62–63. doi:10.1016/j.ejpsy.2020.10.004

Attademo L, Bernardini F. 2021a. Are dopamine and serotonin involved in COVID-19 pathophysiology? *The European journal of psychiatry* **35**:62–63. doi:10.1016/j.ejpsy.2020.10.004

Bansal AS, Bradley AS, Bishop KN, Kiani-Alikhan S, Ford B. 2012. Chronic fatigue syndrome, the immune system and viral infection. *Brain, Behavior, and Immunity* **26**:24–31. doi:10.1016/j.bbi.2011.06.016

Barker ED, Walton E, Cecil CAM. 2018. Annual research review: DNA methylation as a mediator in the association between risk exposure and child and adolescent psychopathology. *Journal of Child Psychology and Psychiatry* **59**:303–322. doi:10.1111/jcpp.12782

Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, Marshall KA, Phillippy KH, Sherman PM, Holko M, Yefanov A, Lee H, Zhang N, Robertson CL, Serova N, Davis S, Soboleva A. 2012. NCBI GEO: Archive for functional genomics data sets—update. *Nucleic Acids Research* **41**:D991–D995. doi:10.1093/nar/gks1193

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)* **57**:289–300. doi:https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Berber B, Doluca O. 2021. A comprehensive drug repurposing study for COVID19 treatment: Novel putative dihydroorotate dehydrogenase inhibitors show association to serotonin–dopamine receptors. *Briefings in Bioinformatics* **22**:1023–1037. doi:10.1093/bib/bbaa379

Beyond myalgic encephalomyelitis/chronic fatigue syndrome: Redefining an illness. 2015. National Academies Press (US).

Bezanson J, Edelman A, Karpinski S, Shah VB. 2017. Julia: A fresh approach to numerical computing. *SIAM review* **59**:65–98.

Blockmans D, Persoons P. 2016. Long-term methylphenidate intake in chronic fatigue syndrome. *Acta Clinica Belgica* **71**:407–414. doi:http://dx.doi.org/10.1080/17843286.2016.1200816

Blockmans D, Persoons P, Van Houdenhove B, Bobbaers H. 2006. Does methylphenidate reduce the symptoms of chronic fatigue syndrome? *The American Journal of Medicine* **119**:167.e23–167.e30. doi:10.1016/j.amjmed.2005.07.047

Blundell S, Ray KK, Buckland M, White PD. 2015. Chronic fatigue syndrome and circulating cytokines: A systematic review. *Brain, Behavior, and Immunity* **50**:186–195. doi:10.1016/j.bbi.2015.07.004

Brenet F, Moh M, Funk P, Feierstein E, Viale AJ, Socci ND, Scandura JM. 2011. DNA methylation of the first exon is tightly linked to transcriptional silencing. *PLoS ONE* **6**:e14524. doi:10.1371/journal.pone.0014524

Brenu E, Staines D, Marshall-Gradisnik S. 2014. Methylation profile of CD4+ t cells in chronic fatigue syndrome/myalgic encephalomyelitis. *J Clin Cell Immunol* **5**:2.

Brenu Ekua W, Ashton KJ, Batovska J, Staines DR, Marshall-Gradisnik SM. 2014. High-throughput sequencing of plasma MicroRNA in chronic fatigue syndrome/myalgic encephalomyelitis. *PLoS ONE* **9**:e102783. doi:10.1371/journal.pone.0102783

Brenu EW, Ashton KJ, Van Driel M, Staines DR, Peterson D, Atkinson GM, Marshall-Gradisnik SM. 2012. Cytotoxic lymphocyte microRNAs as prospective biomarkers for chronic fatigue syndrome/myalgic encephalomyelitis. *Journal of Affective Disorders* **141**:261–269. doi:10.1016/j.jad.2012.03.037

Brenu Ekua Weba, Huth TK, Hardcastle SL, Fuller K, Kaur M, Johnston S, Ramos SB, Staines DR, Marshall-Gradisnik SM. 2014. Role of adaptive and innate immune cells in chronic fatigue syndrome/myalgic encephalomyelitis. *International Immunology* **26**:233–242. doi:10.1093/intimm/dxt068

Brenu EW, Van Driel ML, Staines DR, Ashton KJ, Ramos SB, Keane J, Klimas NG, Marshall-Gradisnik SM. 2011. Immunological abnormalities as potential biomarkers in chronic fatigue syndrome/myalgic encephalomyelitis. *Journal of Translational Medicine* **9**:81. doi:10.1186/1479-5876-9-81

Broderick G, Fuite J, Kreitz A, Vernon SD, Klimas N, Fletcher MA. 2010. A formal analysis of cytokine networks in chronic fatigue syndrome. *Brain Behav Immun* **24**:1209–17. doi:10.1016/j.bbi.2010.04.012

Cabanas H, Muraki K, Balinas C, Eaton-Fitch N, Staines D, Marshall-Gradisnik S. 2019. Validation of impaired transient receptor potential melastatin 3 ion channel activity in natural killer cells from chronic fatigue syndrome/ myalgic encephalomyelitis patients. *Molecular Medicine* **25**. doi:10.1186/s10020-019-0083-4

Capellino S, Claus M, Watzl C. 2020. Regulation of natural killer cell activity by glucocorticoids, serotonin, dopamine, and epinephrine. *Cellular & Molecular Immunology* **17**:705–711. doi:10.1038/s41423-020-0477-9

Carandini T, Mancini M, Bogdan I, Rae CL, Barritt AW, Sethi A, Harrison N, Rashid W, Scarpini E, Galimberti D, Bozzali M, Cercignani M. 2021. Disruption of brainstem monoaminergic fibre tracts in multiple sclerosis as a putative mechanism for cognitive fatigue: A fixel-based analysis. *NeuroImage: Clinical* **30**:102587. doi:10.1016/j.nicl.2021.102587

Carruthers BM, Van De Sande MI, De Meirleir KL, Klimas NG, Broderick G, Mitchell T, Staines D, Powles ACP, Speight N, Vallings R, Bateman L, Baumgarten-Austrheim B, Bell DS, Carlo-Stella N, Chia J, Darragh A, Jo D, Lewis D, Light AR, Marshall-Gradisbik S, Mena I, Mikovits JA, Miwa K, Murovska M, Pall ML, Stevens S. 2011. Myalgic encephalomyelitis: International consensus criteria. *Journal of Internal Medicine* **270**:327–338. doi:10.1111/j.1365-2796.2011.02428.x

Castorina A, Thomas Broome S, Louangaphay K, Keay K, Leggio G, Musumeci G. 2020. Dopamine: An immune transmitter. *Neural Regeneration Research* **15**:2173. doi:10.4103/1673-5374.284976

Chalancon G, Kruse K, Babu MM. 2013. Clustering coefficient In: Dubitzky W, Wolkenhauer O, Cho K-H, Yokota H, editors. Encyclopedia of Systems Biology. New York, NY: Springer New York. pp. 422–424. doi:10.1007/978-1-4419-9863-7_1239

Cliff JM, King EC, Lee J-S, Sepúlveda N, Wolf A-S, Kingdon C, Bowman E, Dockrell HM, Nacul L, Lacerda E, Riley EM. 2019. Cellular immune function in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). *Frontiers in Immunology* **10**. doi:10.3389/fimmu.2019.00796

Cook DB, Light AR, Light KC, Broderick G, Shields MR, Dougherty RJ, Meyer JD, VanRiper S, Stegner AJ, Ellingson LD, Vernon SD. 2017. Neural consequences of post-exertion malaise in myalgic encephalomyelitis/chronic fatigue syndrome. *Brain, behavior, and immunity* **62**:87–99. doi:10.1016/j.bbi.2017.02.009

Corbitt M, Eaton-Fitch N, Staines D, Cabanas H, Marshall-Gradisnik S. 2019. A systematic review of cytokines in chronic fatigue syndrome/myalgic encephalomyelitis/systemic exertion intolerance disease (CFS/ME/SEID). *BMC Neurology* **19**. doi:http://dx.doi.org/10.1186/s12883-019-1433-0

Cortes Rivera M, Mastronardi C, Silva-Aldana C, Arcos-Burgos M, Lidbury B. 2019. Myalgic encephalomyelitis/chronic fatigue syndrome: A comprehensive review. *Diagnostics* **9**:91. doi:10.3390/diagnostics9030091

Crosby LD, Kalanidhi S, Bonilla A, Subramanian A, Ballon JS, Bonilla H. 2021. Off label use of aripiprazole shows promise as a treatment for myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS): A retrospective study of 101 patients treated with a low dose of aripiprazole. *Journal of Translational Medicine* **19**. doi:10.1186/s12967-021-02721-9

Curriu M, Carrillo J, Massanella M, Rigau J, Alegre J, Puig J, Garcia-Quintana AM, Castro-Marrero J, Negredo E, Clotet B, Cabrera C, Blanco J. 2013. Screening NK-, b- and t-cell phenotype and function in patients suffering from chronic fatigue syndrome. *Journal of Translational Medicine* **11**:68. doi:10.1186/1479-5876-11-68

Damgacioglu H, Celik E, Celik N. 2019. Estimating gene expression from high-dimensional DNA methylation levels in cancer data: A bimodal unsupervised dimension reduction algorithm. *Computers & Industrial Engineering* **130**:348–357. doi:10.1016/j.cie.2019.02.038

De Almeida BP, Apolónio JD, Binnie A, Castelo-Branco P. 2019. Roadmap of DNA methylation in breast cancer identifies novel prognostic biomarkers. *BMC Cancer* **19**. doi:10.1186/s12885-019-5403-0

De Vega WC, Herrera S, Vernon SD, McGowan PO. 2017. Epigenetic modifications and glucocorticoid sensitivity in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). *BMC Medical Genomics* **10**. doi:10.1186/s12920-017-0248-3

De Vega WC, Vernon SD, McGowan PO. 2014. DNA methylation modifications associated with chronic fatigue syndrome. *PLoS ONE* **9**:e104757. doi:10.1371/journal.pone.0104757

Demitrack MA, Gold PW, Dale JK, Krahn DD, Kling MA, Straus SE. 1992. Plasma and cerebrospinal fluid monoamine metabolism in patients with chronic fatigue syndrome: Preliminary findings. *Biological Psychiatry* **32**:1065–1077. doi:10.1016/0006-3223(92)90187-5

Dobryakova E, Genova HM, Deluca J, Wylie GR. 2015. The dopamine imbalance hypothesis of fatigue in multiple sclerosis and other neurological disorders. *Frontiers in Neurology* **6**. doi:10.3389/fneur.2015.00052

Eaton-Fitch N, Du Preez S, Cabanas H, Staines D, Marshall-Gradisnik S. 2019. A systematic review of natural killer cells profile and cytotoxic function in myalgic encephalomyelitis/chronic fatigue syndrome. *Systematic Reviews* **8**. doi:10.1186/s13643-019-1202-6

Edgar R, Domrachev M, Lash AE. 2002. Gene expression omnibus: NCBI gene expression and hybridization array data repository. *Nucleic acids research* **30**:207–210.

Felger JC, Miller AH. 2012. Cytokine effects on the basal ganglia and dopamine function: The subcortical source of inflammatory malaise. *Frontiers in Neuroendocrinology* **33**:315–327. doi:https://doi.org/10.1016/j.yfrne.2012.09.003

Felger JC, Mun J, Kimmel HL, Nye JA, Drake DF, Hernandez CR, Freeman AA, Rye DB, Goodman MM, Howell LL, Miller AH. 2013. Chronic interferon- decreases dopamine 2 receptor binding and striatal dopamine release in association with anhedonia-like behavior in nonhuman primates. *Neuropsychopharmacology* **38**:2179–2187. doi:10.1038/npp.2013.115

Feng Y, Lu Y. 2021. Immunomodulatory effects of dopamine in inflammatory diseases. *Frontiers in Immunology* **12**. doi:10.3389/fimmu.2021.663102

Fletcher MA, Zeng XR, Maher K, Levis S, Hurwitz B, Antoni M, Broderick G, Klimas NG. 2010. Biomarkers in chronic fatigue syndrome: Evaluation of natural killer cell function and dipeptidyl peptidase IV/CD26. *PLoS ONE* **5**:e10817. doi:10.1371/journal.pone.0010817

Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. 1994. The chronic fatigue syndrome: A comprehensive approach to its definition and study. International chronic fatigue syndrome study group. *Ann Intern Med* **121**:953–9. doi:10.7326/0003-4819-121-12-199412150-00009

Gaiarsa MP, Guimarães PR. 2019. Interaction strength promotes robustness against cascading effects in mutualistic networks. *Scientific reports* **9**:1–7.

Gay CW, Robinson ME, Lai S, O'Shea A, Craggs JG, Price DD, Staud R. 2016. Abnormal resting-state functional connectivity in patients with chronic fatigue syndrome: Results of seed and data-driven analyses. *Brain Connectivity* **6**:48–56. doi:10.1089/brain.2015.0366

Giloteaux L, Goodrich JK, Walters WA, Levine SM, Ley RE, Hanson MR. 2016. Reduced diversity and altered composition of the gut microbiome in individuals with myalgic encephalomyelitis/chronic fatigue syndrome. *Microbiome* **4**. doi:10.1186/s40168-016-0171-4

Goodnick PJ, Sandoval R, Brickman A, Klimas NG. 1992. Bupropion treatment of fluoxetine-resistant chronic fatigue syndrome. *Biological Psychiatry* **32**:834–838. doi:10.1016/0006-3223(92)90087-g

Guimarães PR, Pires MM, Cantor M, Coltri PP. 2018. Interaction paths promote module integration and network-level robustness of spliceosome to cascading effects. *Scientific reports* **8**:1–11.

Guimerà R, Amaral LAN. 2005. Cartography of complex networks: Modules and universal roles. *Journal of Statistical Mechanics: Theory and Experiment* **2005**:P02001. doi:10.1088/1742-5468/2005/02/p02001

Helliwell AM, Sweetman EC, Stockwell PA, Edgar CD, Chatterjee A, Tate WP. 2020. Changes in DNA methylation profiles of myalgic encephalomyelitis/chronic fatigue syndrome patients reflect systemic dysfunctions. *Clinical Epigenetics* **12**. doi:10.1186/s13148-020-00960-z

Herrera S, De Vega WC, Ashbrook D, Vernon SD, McGowan PO. 2018. Genome-epigenome interactions associated with myalgic encephalomyelitis/chronic fatigue syndrome. *Epigenetics* **13**:1174–1190. doi:10.1080/15592294.2018.1549769

Holden S, Maksoud R, Eaton-Fitch N, Cabanas H, Staines D, Marshall-Gradisnik S. 2020. A systematic review of mitochondrial abnormalities in myalgic encephalomyelitis/chronic fatigue syndrome/systemic exertion intolerance disease. *Journal of Translational Medicine* **18**. doi:10.1186/s12967-020-02452-3

Huang Y, Qiu AW, Peng YP, Liu Y, Huang HW, Qiu YH. 2010. Roles of dopamine receptor subtypes in mediating modulation of t lymphocyte function. *Neuro Endocrinol Lett* **31**:782–91.

Huth TK, Staines D, Marshall-Gradisnik S. 2016. ERK1/2, MEK1/2 and p38 downstream signalling molecules impaired in CD56dimCD16+ and CD56brightCD16dim/- natural killer cells in chronic fatigue syndrome/myalgic encephalomyelitis patients. *Journal of Translational Medicine* **14**. doi:10.1186/s12967-016-0859-z

James Fairbanks SS Mathieu Besançon, Karpinski S. 2021. JuliaGraphs/graphs.jl: An optimized graphs package for the julia programming language.

Jammes Y, Steinberg JG, Delliaux S. 2012. Chronic fatigue syndrome: Acute infection and history of physical activity affect resting levels and response to exercise of plasma oxidant/antioxidant status and heat shock proteins. *Journal of Internal Medicine* **272**:74–84. doi:10.1111/j.1365-2796.2011.02488.x

Jammes Y, Steinberg JG, Mambrini O, Bregeon F, Delliaux S. 2005. Chronic fatigue syndrome: Assessment of increased oxidative stress and altered muscle excitability in response to incremental exercise. *Journal of Internal Medicine* **257**:299–310. doi:10.1111/j.1365-2796.2005.01452.x

Jason LA, Islam MF, Conroy K, Cotler J, Torres C, Johnson M, Mabie B. 2021. COVID-19 symptoms over time: Comparing long-haulers to ME/CFS. *Fatigue: Biomedicine, Health & Behavior* **9**:59–68. doi:10.1080/21641846.2021.1922140

Jason L, Sorenson M, Sebally K, Alkazemi D, Lerch A, Porter N, Kubow S. 2011. Increased HDAC in association with decreased plasma cortisol in older adults with chronic fatigue syndrome. *Brain, Behavior, and Immunity* **25**:1544–1547. doi:10.1016/j.bbi.2011.04.007

Jjingo D, Conley AB, Yi SV, Lunyak VV, Jordan IK. 2012. On the presence and role of human gene-body DNA methylation. *Oncotarget* **3**:462–474. doi:10.18632/oncotarget.497

Johnston S, Staines D, Klein A, Marshall-Gradisnik S. 2016. A targeted genome association study examining transient receptor potential ion channels, acetylcholine receptors, and adrenergic receptors in

chronic fatigue syndrome/myalgic encephalomyelitis. *BMC Medical Genetics* **17**. doi:10.1186/s12881-016-0342-y

Josev EK, Malpas CB, Seal ML, Scheinberg A, Lubitz L, Rowe K, Knight SJ. 2019. Resting-state functional connectivity, cognition, and fatigue in response to cognitive exertion: A novel study in adolescents with chronic fatigue syndrome. *Brain Imaging and Behavior* 1–16. doi:http://dx.doi.org/10.1007/s11682-019-00119-2

Kaiser JD. 2015. A prospective, proof-of-concept investigation of KPAX002 in chronic fatigue syndrome. *International journal of clinical and experimental medicine* **8**:11064–11074.

Komaroff A, Cho T. 2011. Role of infection and neurologic dysfunction in chronic fatigue syndrome. *Seminars in Neurology* **31**:325–337. doi:10.1055/s-0031-1287654

Komaroff AL. 2019. Advances in understanding the pathophysiology of chronic fatigue syndrome. *JAMA* **322**:499. doi:10.1001/jama.2019.8312

Komaroff AL. 2017. Inflammation correlates with symptoms in chronic fatigue syndrome. *Proceedings of the National Academy of Sciences* **114**:8914–8916. doi:10.1073/pnas.1712475114

Komaroff AL, Bateman L, Coalition USMCC. 2021. Will COVID-19 lead to myalgic encephalomyelitis/chronic fatigue syndrome? *Frontiers in Medicine* **7**. doi:10.3389/fmed.2020.606824

Komaroff AL, Cho TA. 2011. Role of infection and neurologic dysfunction in chronic fatigue syndrome. *Semin Neurol* **31**:325–37. doi:10.1055/s-0031-1287654

Lee WK, Kim Y, Jang H, Sim JH, Choi HJ, Shin Y, Choi JJ. 2021. Exogenous transforming growth factor- in brain-induced symptoms of central fatigue and suppressed dopamine production in mice. *International Journal of Molecular Sciences* **22**:2580. doi:10.3390/ijms22052580

Levine PH, Whiteside TL, Friberg D, Bryant J, Colclough G, Herberman RB. 1998. Dysfunction of natural killer activity in a family with chronic fatigue syndrome. *Clin Immunol Immunopathol* **88**:96–104. doi:10.1006/clin.1998.4554

Levy JJ, Titus AJ, Petersen CL, Chen Y, Salas LA, Christensen BC. 2020. MethylNet: An automated and modular deep learning approach for DNA methylation analysis. *BMC Bioinformatics* **21**. doi:10.1186/s12859-020-3443-8

Li M, Wang J, Chen J, Pan Y. 2009. Hierarchical organization of functional modules in weighted protein interaction networks using clustering coefficient. Springer Berlin Heidelberg. pp. 75–86. doi:10.1007/978-3-642-01551-9_8

Loebel M, Grabowski P, Heidecke H, Bauer S, Hanitsch LG, Wittke K, Meisel C, Reinke P, Volk H-D, Fluge Ø, Mella O, Scheibenbogen C. 2016. Antibodies to adrenergic and muscarinic cholinergic receptors in patients with chronic fatigue syndrome. *Brain, Behavior, and Immunity* **52**:32–39. doi:10.1016/j.bbi.2015.09.013

Maes M, Mihaylova I, Leunis J-C. 2007. Increased serum IgA and IgM against LPS of enterobacteria in chronic fatigue syndrome (CFS): Indication for the involvement of gram-negative enterobacteria in the etiology of CFS and for the presence of an increased gut–intestinal permeability. *Journal of Affective Disorders* **99**:237–240. doi:https://doi.org/10.1016/j.jad.2006.08.021

Maes M, Twisk FNM, Kubera M, Ringel K, Leunis J-C, Geffard M. 2012. Increased IgA responses to the LPS of commensal bacteria is associated with inflammation and activation of cell-mediated immunity in chronic fatigue syndrome. *Journal of Affective Disorders* **136**:909–917. doi:10.1016/j.jad.2011.09.010

Maher KJ, Klimas NG, Fletcher MA. 2005. Chronic fatigue syndrome is associated with diminished intracellular perforin. *Clinical and Experimental Immunology* **0**:050927060953002. doi:10.1111/j.1365-2249.2005.02935.x

Maksimovic J, Oshlack A, Phipson B. 2021. Gene set enrichment analysis for genome-wide DNA methylation data. *Genome Biology* **22**. doi:10.1186/s13059-021-02388-x

Manca R, Khan K, Mitolo M, De Marco M, Grieveson L, Varley R, Wilkinson ID, Venneri A. 2021. Modulatory effects of cognitive exertion on regional functional connectivity of the salience network in women with ME/CFS: A pilot study. *Journal of the Neurological Sciences* **422**:117326. doi:https://doi.org/10.1016/j.jns.2021.117326

Mandarano AH, Maya J, Giloteaux L, Peterson DL, Maynard M, Gottschalk CG, Hanson MR. 2020. Myalgic encephalomyelitis/chronic fatigue syndrome patients exhibit altered t cell metabolism and cytokine associations. *Journal of Clinical Investigation* **130**:1491–1505. doi:10.1172/jci132185

Marshall-Gradisnik S, Huth T, Chacko A, Smith P, Staines D, Johnston S. 2016. Natural killer cells and single nucleotide polymorphisms of specific ion channels and receptor genes in myalgic encephalomyelitis/chronic fatigue syndrome. *The Application of Clinical Genetics* 39. doi:10.2147/tacg.s99405

Martin EM, Fry RC. 2018. Environmental influences on the epigenome: Exposure- associated DNA methylation in human populations. *Annual Review of Public Health* **39**:309–333. doi:10.1146/annurev-publhealth-040617-014629

Martino D, Saffery R. 2015. Characteristics of DNA methylation and gene expression in regulatory features on the infinium 450k beadchip. doi:10.1101/032862

Matosin N, Cruceanu C, Binder EB. 2017. Preclinical and clinical evidence of DNA methylation changes in response to trauma and chronic stress. *Chronic Stress* **1**:247054701771076. doi:10.1177/2470547017710764

Maussion G, Yang J, Suderman M, Diallo A, Nagy C, Arnovitz M, Mechawar N, Turecki G. 2014. Functional DNA methylation in a transcript specific 3'UTR region of TrkB associates with suicide. *Epigenetics* **9**:1061–1070. doi:10.4161/epi.29068

McGregor NR, Armstrong CW, Lewis DP, Gooley PR. 2019. Post-exertional malaise is associated with hypermetabolism, hypoacetylation and purine metabolism deregulation in ME/CFS cases. *Diagnostics* **9**:70. doi:10.3390/diagnostics9030070

McGuire MH, Herbrich SM, Dasari SK, Wu SY, Wang Y, Rupaimoole R, Lopez-Berestein G, Baggerly KA, Sood AK. 2019. Pan-cancer genomic analysis links 3'UTR DNA methylation with increased gene expression in t cells. *EBioMedicine* **43**:127–137. doi:10.1016/j.ebiom.2019.04.045

Miller AH, Jones JF, Drake DF, Tian H, Unger ER, Pagnoni G. 2014. Decreased basal ganglia activation in subjects with chronic fatigue syndrome: Association with symptoms of fatigue. *PLoS ONE* **9**:e98156. doi:10.1371/journal.pone.0098156

Missailidis D, Annesley SJ, Allan CY, Sanislav O, Lidbury BA, Lewis DP, Fisher PR. 2020a. An isolated complex v inefficiency and dysregulated mitochondrial function in immortalized lymphocytes from ME/CFS patients. *International Journal of Molecular Sciences* **21**:1074. doi:10.3390/ijms21031074

Missailidis D, Annesley SJ, Fisher PR. 2019. Pathological mechanisms underlying myalgic encephalomyelitis/chronic fatigue syndrome. *Diagnostics* **9**:80. doi:10.3390/diagnostics9030080

Missailidis D, Sanislav O, Allan CY, Annesley SJ, Fisher PR. 2020b. Cell-based blood biomarkers for myalgic encephalomyelitis/chronic fatigue syndrome. *International Journal of Molecular Sciences* **21**:1142. doi:10.3390/ijms21031142

Missailidis D, Sanislav O, Allan CY, Smith PK, Annesley SJ, Fisher PR. 2021. Dysregulated provision of oxidisable substrates to the mitochondria in ME/CFS lymphoblasts. *International Journal of Molecular Sciences* **22**:2046. doi:10.3390/ijms22042046

Moore LD, Le T, Fan G. 2013. DNA methylation and its basic function. *Neuropsychopharmacology* **38**:23–38. doi:10.1038/npp.2012.112

Morris G, Maes M. 2013. A neuro-immune model of myalgic encephalomyelitis/chronic fatigue syndrome. *Metab Brain Dis* **28**:523–40. doi:10.1007/s11011-012-9324-8

Morris G, Maes M, Berk M, Puri BK. 2019. Myalgic encephalomyelitis or chronic fatigue syndrome: How could the illness develop? *Metabolic Brain Disease* **34**:385–415. doi:10.1007/s11011-019-0388-6

Nascimento MCV. 2014. Community detection in networks via a spectral heuristic based on the clustering coefficient. *Discrete Applied Mathematics* **176**:89–99. doi:10.1016/j.dam.2013.09.017

Nataf S. 2020b. An alteration of the dopamine synthetic pathway is possibly involved in the pathophysiiology of COVID-19. *Journal of Medical Virology* **92**:1743–1744. doi:10.1002/jmv.25826

Nataf S. 2020a. An alteration of the dopamine synthetic pathway is possibly involved in the pathophysiology of COVID-19. *Journal of Medical Virology* **92**:1743–1744. doi:10.1002/jmv.25826

Nepotchatykh E, Elremaly W, Caraus I, Godbout C, Leveau C, Chalder L, Beaudin C, Kanamaru E, Kosovskaia R, Lauzon S, Maillet Y, Franco A, Lascau-Coman V, Bouhanik S, Gaitan YP, Li D, Moreau

A. 2020. Profile of circulating microRNAs in myalgic encephalomyelitis and their relation to symptom severity, and disease pathophysiology. *Scientific Reports* **10**. doi:10.1038/s41598-020-76438-y

Nguyen T, Johnston S, Clarke L, Smith P, Staines D, Marshall-Gradisnik S. 2017. Impaired calcium mobilization in natural killer cells from chronic fatigue syndrome/myalgic encephalomyelitis patients is associated with transient receptor potential melastatin 3 ion channels. *Clinical & Experimental Immunology* **187**:284–293. doi:10.1111/cei.12882

Nguyen T, Staines D, Johnston S, Marshall-Gradisnik S. 2019. Reduced glycolytic reserve in isolated natural killer cells from myalgic encephalomyelitis/ chronic fatigue syndrome patients: A preliminary investigation. *Asian Pac J Allergy Immunol* **37**:102–108. doi:10.12932/ap-011117-0188

Non AL, Thayer ZM. 2015. Epigenetics for anthropologists: An introduction to methods. *American Journal of Human Biology* **27**:295–303. doi:10.1002/ajhb.22679

Pacheco R, Contreras F, Zouali M. 2014. The dopaminergic system in autoimmune diseases. *Frontiers in Immunology* **5**. doi:10.3389/fimmu.2014.00117

Petty RD, McCarthy NE, Le Dieu R, Kerr JR. 2016. MicroRNAs hsa-miR-99b, hsa-miR-330, hsa-miR-126 and hsa-miR-30c: Potential diagnostic biomarkers in natural killer (NK) cells of patients with chronic fatigue syndrome (CFS)/ myalgic encephalomyelitis (ME). *PLOS ONE* **11**:e0150904. doi:10.1371/journal.pone.0150904

Rasa S, Nora-Krukle Z, Henning N, Eliassen E, Shikova E, Harrer T, Scheibenbogen C, Murovska M, Prusty BK. 2018. Chronic viral infections in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). *Journal of Translational Medicine* **16**. doi:10.1186/s12967-018-1644-y

Reijnders MJMF, Waterhouse RM. 2021. Summary visualizations of gene ontology terms with GO-figure! *Frontiers in Bioinformatics* **1**. doi:10.3389/fbinf.2021.638255

Sáez-Francàs N, Alegre J, Calvo N, Antonio Ramos-Quiroga J, Ruiz E, Hernández-Vara J, Casas M. 2012. Attention-deficit hyperactivity disorder in chronic fatigue syndrome patients. *Psychiatry Research* **200**:748–753. doi:https://doi.org/10.1016/j.psychres.2012.04.041

Scheibenbogen C, Loebel M, Freitag H, Krueger A, Bauer S, Antelmann M, Doehner W, Scherbakov N, Heidecke H, Reinke P, Volk H-D, Grabowski P. 2018. Immunoadsorption to remove ß2 adrenergic receptor antibodies in chronic fatigue syndrome CFS/ME. *PLOS ONE* **13**:e0193672. doi:10.1371/journal.pone.0193672

Shan ZY, Barnden LR, Kwiatek RA, Bhuta S, Hermens DF, Lagopoulos J. 2020. Neuroimaging characteristics of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS): A systematic review. *Journal of Translational Medicine* **18**. doi:10.1186/s12967-020-02506-6

Shan ZY, Finegan K, Bhuta S, Ireland T, Staines DR, Marshall-Gradisnik SM, Barnden LR. 2018. Brain function characteristics of chronic fatigue syndrome: A task fMRI study. *NeuroImage: Clinical* **19**:279–286. doi:10.1016/j.nicl.2018.04.025

Shin D-H, Qian D, Zhang J. 2014. Cascading effects in interdependent networks. *Ieee Network* **28**:82–87.

Silva TC, Young JI, Martin ER, Chen X, Wang L. 2021. MethReg: Estimating the regulatory potential of DNA methylation in gene transcription. doi:10.1101/2021.02.18.431696

Skowera A, Cleare A, Blair D, Bevis L, Wessely SC, Peakman M. 2004. High levels of type 2 cytokine-producing cells in chronic fatigue syndrome. *Clinical and Experimental Immunology* **135**:294–302. doi:10.1111/j.1365-2249.2004.02354.x

Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT, Morris JH, Bork P, Jensen LJ, Christian. 2019. STRING v11: Protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Research* **47**:D607–D613. doi:10.1093/nar/gky1131

Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, Doncheva NT, Legeay M, Fang T, Bork P, Jensen LJ, Christian. 2021. The STRING database in 2021: Customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Research* **49**:D605–D612. doi:10.1093/nar/gkaa1074

Tanaka M, Sadato N, Okada T, Mizuno K, Sasabe T, Tanabe HC, Saito DN, Onoe H, Kuratsune H, Watanabe Y. 2006. Reduced responsiveness is an essential feature of chronic fatigue syndrome: A fMRI study. *BMC Neurology* **6**. doi:10.1186/1471-2377-6-9

Tanaka S, Kuratsune H, Hidaka Y, Hakariya Y, Tatsumi KI, Takano T, Kanakura Y, Amino N. 2003. Autoantibodies against muscarinic cholinergic receptor in chronic fatigue syndrome. *Int J Mol Med* **12**:225–30.

Tang B, Zhou Y, Wang C-M, Huang THM, Jin VX. 2017. Integration of DNA methylation and gene transcription across nineteen cell types reveals cell type-specific and genomic region-dependent regulatory patterns. *Scientific Reports* **7**. doi:10.1038/s41598-017-03837-z

Team RC. 2021. R: A language and environment for statistical computing.

The gene ontology resource: Enriching a GOld mine. 2021. *Nucleic Acids Res* **49**:D325–d334. doi:10.1093/nar/gkaa1113

Tomas C, Brown A, Strassheim V, Elson J, Newton J, Manning P. 2017. Cellular bioenergetics is impaired in patients with chronic fatigue syndrome. *PLOS ONE* **12**:e0186802. doi:10.1371/journal.pone.0186802

Tomas C, Elson JL, Strassheim V, Newton JL, Walker M. 2020. The effect of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) severity on cellular bioenergetic function. *PLoS One* **15**:e0231136. doi:10.1371/journal.pone.0231136

Torres-Harding S, Sorenson M, Jason LA, Maher K, Fletcher MA. 2008. Evidence for t-helper 2 shift and association with illness parameters in chronic fatigue syndrome (CFS). *Bull IACFS ME* **16**:19–33.

Trivedi MS, Oltra E, Sarria L, Rose N, Beljanski V, Fletcher MA, Klimas NG, Nathanson L. 2018. Identification of myalgic encephalomyelitis/chronic fatigue syndrome-associated DNA methylation patterns. *PLOS ONE* **13**:e0201066. doi:10.1371/journal.pone.0201066

Van Der Schaaf ME, De Lange FP, Schmits IC, Geurts DEM, Roelofs K, Van Der Meer JWM, Toni I, Knoop H. 2017. Prefrontal structure varies as a function of pain symptoms in chronic fatigue syndrome. *Biological Psychiatry* **81**:358–365. doi:10.1016/j.biopsych.2016.07.016

Van Der Schaaf ME, Roelofs K, De Lange FP, Geurts DEM, Van Der Meer JWM, Knoop H, Toni I. 2018. Fatigue is associated with altered monitoring and preparation of physical effort in patients with chronic fatigue syndrome. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging* **3**:392–404. doi:10.1016/j.bpsc.2018.01.015

Vanelzakker MB, Brumfield SA, Lara Mejia PS. 2019. Neuroinflammation and cytokines in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS): A critical review of research methods. *Frontiers in Neurology* **9**. doi:10.3389/fneur.2018.01033

Varley KE, Gertz J, Bowling KM, Parker SL, Reddy TE, Pauli-Behn F, Cross MK, Williams BA, Stamatoyannopoulos JA, Crawford GE, Absher DM, Wold BJ, Myers RM. 2013. Dynamic DNA methylation across diverse human cell lines and tissues. *Genome Research* **23**:555–567. doi:10.1101/gr.147942.112

Vega WC de, Erdman L, Vernon SD, Goldenberg A, McGowan PO. 2018. Integration of DNA methylation & health scores identifies subtypes in myalgic encephalomyelitis/chronic fatigue syndrome. *Epigenomics* **10**:539-539-557. doi:http://dx.doi.org/10.2217/epi-2017-0150

Vidal PM, Pacheco R. 2020a. The cross-talk between the dopaminergic and the immune system involved in schizophrenia. *Frontiers in Pharmacology* **11**. doi:10.3389/fphar.2020.00394

Vidal PM, Pacheco R. 2020b. Targeting the dopaminergic system in autoimmunity. *Journal of Neuroimmune Pharmacology* **15**:57–73. doi:10.1007/s11481-019-09834-5

Von Mering C. 2004. STRING: Known and predicted protein-protein associations, integrated and transferred across organisms. *Nucleic Acids Research* **33**:D433–D437. doi:10.1093/nar/gki005

Walton E, Hass J, Liu J, Roffman JL, Bernardoni F, Roessner V, Kirsch M, Schackert G, Calhoun V, Ehrlich S. 2016. Correspondence of DNA methylation between blood and brain tissue and its application to schizophrenia research. *Schizophrenia Bulletin* **42**:406–414. doi:10.1093/schbul/sbv074

Wasserman S, Faust K. 1994. Social network analysis: Methods and applications, Social network analysis: Methods and applications. New York, NY, US: Cambridge University Press. doi:10.1017/CBO9780511815478

Watts DJ, Strogatz SH. 1998. Collective dynamics of "small-world" networks. *Nature* **393**:440–442. doi:10.1038/30918

White AT, Light AR, Hughen RW, Vanhaitsma TA, Light KC. 2012. Differences in metabolite-detecting, adrenergic, and immune gene expression after moderate exercise in patients with chronic fatigue syndrome, patients with multiple sclerosis, and healthy controls. *Psychosomatic Medicine* **74**:46–54. doi:10.1097/psy.0b013e31824152ed

Willer CJ, Li Y, Abecasis GR. 2010. METAL: Fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* **26**:2190–2191. doi:10.1093/bioinformatics/btq340

Wirth K, Scheibenbogen C. 2020. A unifying hypothesis of the pathophysiology of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS): Recognitions from the finding of autoantibodies against ß2-adrenergic receptors. *Autoimmunity Reviews* **19**:102527. doi:10.1016/j.autrev.2020.102527

Wong TL, Weitzer DJ. 2021. Long COVID and myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS)—a systemic review and comparison of clinical presentation and symptomatology. *Medicina* **57**:418. doi:10.3390/medicina57050418

Wortinger LA, Endestad T, Melinder AMD, Ø MG, Sevenius A, Wyller VB. 2016. Aberrant resting-state functional connectivity in the salience network of adolescent chronic fatigue syndrome. *PLoS ONE* **11**. doi:10.1371/journal.pone.0159351

Wortinger LA, Glenne Øie M, Endestad T, Bruun Wyller V. 2017. Altered right anterior insular connectivity and loss of associated functions in adolescent chronic fatigue syndrome. *PLOS ONE* **12**:e0184325. doi:10.1371/journal.pone.0184325

Yang X, Han H, Daniel, Fides, Peter, Liang G. 2014. Gene body methylation can alter gene expression and is a therapeutic target in cancer. *Cancer Cell* **26**:577–590. doi:10.1016/j.ccr.2014.07.028

Zaki N, Efimov D, Berengueres J. 2013. Protein complex detection using interaction reliability assessment and weighted clustering coefficient. *BMC Bioinformatics* **14**:163. doi:10.1186/1471-2105-14-163

Zhao W, Huang Y, Liu Z, Cao B-B, Peng Y-P, Qiu Y-H. 2013. Dopamine receptors modulate cytotoxicity of natural killer cells via cAMP-PKA-CREB signaling pathway. *PLoS ONE* **8**:e65860. doi:10.1371/journal.pone.0065860

Zhong H, Kim S, Zhi D, Cui X. 2019. Predicting gene expression using DNA methylation in three human populations. *PeerJ* **7**:e6757. doi:10.7717/peerj.6757

Zhou G, Soufan O, Ewald J, Hancock REW, Basu N, Xia J. 2019. NetworkAnalyst 3.0: A visual analytics platform for comprehensive gene expression profiling and meta-analysis. *Nucleic Acids Research* **47**:W234–W241. doi:10.1093/nar/gkz240