

Gene expression in peripheral blood mononuclear cells from patients with chronic fatigue syndrome

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Background: Chronic fatigue syndrome (CFS) is a multisystem disease, the pathogenesis of which remains undetermined.

Aims: To test the hypothesis that there are reproducible abnormalities of gene expression in patients with CFS compared with normal healthy persons.

Methods: To gain further insight into the pathogenesis of this disease, gene expression was analysed in peripheral blood mononuclear cells from 25 patients with CFS diagnosed according to the Centers for Disease Control criteria and 25 normal blood donors matched for age, sex, and geographical location, using a single colour microarray representing 9522 human genes. After normalisation, average difference values for each gene were compared between test and control groups using a cutoff fold difference of

expression ≥ 1.5 and a p value of 0.001. Genes showing differential expression were further analysed using Taqman real time polymerase chain reaction (PCR) in fresh samples.

Results: Analysis of microarray data revealed differential expression of 35 genes. Real time PCR confirmed differential expression in the same direction as array results for 16 of these genes, 15 of which were upregulated (ABCD4, PRKCL1, MRPL23, CD2BP2, GSN, NTE, POLR2G, PEX16, EIF2B4, EIF4G1, ANAPC11, PDCD2, KHSRP, BRMS1, and GABARAPL1) and one of which was downregulated (IL-10RA). This profile suggests T cell activation and perturbation of neuronal and mitochondrial function. Upregulation of neuropathy target esterase and eukaryotic translation initiation factor 4G1 may suggest links with organophosphate exposure and virus infection, respectively.

Conclusion: These results suggest that patients with CFS have reproducible alterations in gene regulation.

Abbreviations: CFS, chronic fatigue syndrome; NBS, National Blood Service; PBMC, peripheral blood mononuclear cells; PCR, polymerase chain reaction

Keywords: chronic fatigue syndrome; pathogenesis; gene expression; T cell activation; mitochondrion; neurone; organophosphate; virus infection; interleukin 10 receptor

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Discussion

In our present study, we studied transcript profiles from patients with CFS and from sex and age matched normal controls from the same area of South East England. The expression of 16 genes was significantly different in patients compared with controls in both microarray analysis and real time PCR. These genes may be important in the pathogenesis of CFS and can be grouped according to immune, neuronal, mitochondrial, and other functions that have particular relevance to our present knowledge of the epidemiology of CFS (table 4). Our present study has certain parallels with two published studies in this area, summarised in table 4.

T cell activation is suggested by upregulation of CD2BP2 and downregulation of IL-10RA10–12; in addition, PRKCL1 plays a role in the immune response. Genes that are active in the immune response have been found to be differentially expressed in all studies of gene expression in CFS (table 4). Furthermore, genes that are crucial for T cell activation¹⁰ have also been found to be upregulated in all three studies, namely: CD2BP2 and IL-10RA (present study); moesin and cathepsin C4; ITGA and NFATC3.5 These findings are consistent with previous work showing that patients with CFS have evidence of immune activation, such as increased numbers of activated T cells and cytotoxic T cells, and raised circulating cytokine concentrations.^{3,13–19}

A neuronal component is suggested by the upregulation of PRKCL1, NTE, GSN, GABARAPL1, KHSRP, and EIF2B4. Protein kinase C family members are implicated in various psychiatric and affective disorders, and have been implicated in previous gene studies of CFS.⁵ NTE is a target for organophosphates and chemical warfare agents, both of which may precipitate CFS,²⁰ on the basis of a neuropathy resulting from inactivation of serine esterase activity.²¹ GSN regulates cell growth and plays a role in amyloidosis (Finnish type), which may result in dysfunction of neurones, skeletal muscle, and thyroid gland.^{22,23}

GABARAPL1 is a microtubule associated anchor protein with increased expression in neuronal cells.²⁴ KHSRP facilitates splicing of the N1 exon of the SRC protooncogene in neuronal but not other cells.²⁵ EIF2B4 is a mitochondrial translation initiation factor and one of the EIF2B family, within which mutations have been shown to be associated with central nervous system hypomyelination and encephalopathy.²⁶ Powell and colleagues⁴ have reported upregulation of an EIF2B3 gene homologue (BQ580379). These findings are interesting in that abnormalities in the white matter of the frontal lobes have been found in patients with CFS using magnetic resonance imaging and have been suggested to account for the cognitive defect in CFS.²⁷ Neuronal gene involvement in CFS has also been reported by Vernon and colleagues.⁵

Mitochondrial involvement is suggested by the upregulation of EIF2B4, EIF4G1 (see above), and MRPL23. Mitochondrial gene upregulation has also been reported by Powell et al.⁴

The cell cycle is implicated by upregulation of ANAPC11, which regulates the onset of anaphase by mediation of degradation of mitotic cyclins. Powell and colleagues⁴ reported upregulation of MAD1L1, which prevents the onset of anaphase until all chromosomes are aligned at the metaphase plate.

"The upregulation of EIF4G1 identified in our present study may represent a common host response to persistent infection with several different viruses"

Transcriptional perturbation is suggested by the upregulation of POLR2G and BRMS1. Powell and colleagues⁴ reported the upregulation of genes homologous with POLR1B (BQ580386) and RCOR3 (BQ580388), which are each involved in transcriptional regulation.

Upregulated peroxisomal function is suggested by the upregulation of ABCD4 and PEX16, which may suggest enhanced defence to oxidative stress in CFS. Oxidative stress has already been suggested as a disease mechanism in CFS.^{28,29}

Persistent virus infection is a recognised feature of CFS, which is interesting in the light of our finding of upregulation of EIF4G1 transcript variant 5, a mitochondrial translation initiation factor. Whistler and colleagues⁶ have also reported this finding in patients with CFS who have rapid (?triggered by virus infection) as compared with insidious onset. EIF4G1 is a component of the protein complex, EIF4F, which is crucial in translation through its involvement in the recognition of the mRNA cap, ATP dependent unwinding of 5' terminal secondary structure, and recruitment of mRNA to the ribosome.³⁰ Various

viruses have developed strategies to divert EIF4G1 from its utilisation by the cellular machinery to facilitate production of viral proteins.³⁰ The best characterised example is that of poliovirus,^{31,32} but this has also been demonstrated to occur with coxsackie virus,³³ rhinoviruses,³⁴ rotavirus,³⁵ influenza virus,³⁶ adenovirus,³⁷ vesicular stomatitis virus,³⁸ and human immunodeficiency virus 1.³⁹ Therefore, the upregulation of EIF4G1 identified in our present study may represent a common host response to persistent infection with several different viruses. The vulnerability of EIF4G1 to virus modification may have particular importance for the development of CFS after an acute virus infection.⁴⁰

Take home messages

- * Sixteen genes were differentially expressed in patients with chronic fatigue syndrome compared with normal controls, as assessed by microarray and quantitative polymerase chain reaction
- * The involvement of genes from several disparate pathways suggests a complex pathogenesis involving T cell activation and abnormalities of neuronal and mitochondrial function
- * These results suggest possible molecular bases for the recognised contributions of organophosphate exposure and virus infection

In conclusion, we report the differential expression of 16 human genes in patients with CFS compared with normal controls. The involvement of genes from several disparate pathways suggests a complex pathogenesis involving T cell activation and abnormalities of neuronal and mitochondrial function, and suggests possible molecular bases for the recognised contributions of organophosphate exposure and virus infection, respectively.