Current research priorities in chronic fatigue syndrome/myalgic encephalomyelitis: disease mechanisms, a diagnostic test and specific treatments

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REVIEW

Current research priorities in chronic fatigue syndrome/ myalgic encephalomyelitis: disease mechanisms, a diagnostic test and specific treatments

J R Kerr, P Christian, A Hodgetts, P R Langford, L D Devanur, R Petty, B Burke, L I Sinclair, S C M Richards, J Montgomery, C R McDermott, T J Harrison, P Kellam, D J Nutt, S T Holgate, and the Collaborative Clinical Study Group*

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Chronic fatigue syndrome (CFS) is an illness characterised by disabling fatigue of at least 6 months duration, which is accompanied by various rheumatological, infectious and neuropsychiatric symptoms. A collaborative study group has been formed to deal with the current areas for development in CFS research—namely, to develop an understanding of the molecular pathogenesis of CFS, to develop a diagnostic test and to develop specific and curative treatments. Various groups have studied the gene expression in peripheral blood of patients with CFS, and from those studies that have been confirmed using polymerase chain reaction (PCR), clearly, the most predominant functional theme is that of immunity and defence. However, we do not yet know the precise gene signature and metabolic pathways involved. Currently, this is being dealt with using a microarray representing 47 000 human genes and variants, massive parallel signature sequencing and real-time PCR. It will be important to ensure that once a gene signature has been identified, it is specific to CFS and does not occur in other diseases and infections. A diagnostic test is being developed using surface-enhanced, laser-desorption and ionisation-time-of-flight mass spectrometry based on a pilot study in which putative biomarkers were identified. Finally, clinical trials are being planned; novel treatments that we believe are important to trial in patients with CFS are interferon-β and one of the anti-tumour necrosis factor-α drugs.

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Chronic fatigue syndrome (CFS) is an illness characterised by disabling fatigue of at least 6 months duration, which is accompanied by various rheumatological, infectious and neuropsychiatric symptoms.[1] The prevalence of CFS is 0.5% and it is more common in women than in men. The diagnosis is clinical, and there is no laboratory test nd no specific treatment. CFS is now accepted as a valid disease in its own right, and this, along with the urgent need to elucidate its pathogenesis and to develop strategies for diagnosis and treatment, was emphasised in the recent report to the chief medical officer.[2] Epidemiological studies have shown that many patients with CFS give a history of an illness consistent with viral infection that precedes the development of fatigue,[3] and CFS has been shown to follow acute infection with various infectious agents. Patients with CFS have been shown to have evidence of immune activation. However, despite considerable research, the causative and perpetuating disease mechanisms remain unknown.

In 2001, a collaborative study group was formed to specifically investigate the molecular pathogenesis of CFS, to develop a diagnostic test and to take this knowledge forward into the development of new, specific treatments, which are not available at present. The members of this group were also concerned about the trivialisation of CFS and the labelling of patients as having psychiatric, psychological or somatoform disease. To deal with the problem, a pilot study was carried out to see if there was any evidence that the white cells of patients with CFS exhibited a specific gene signature, as has been shown for several other immune-mediated diseases. This pilot study provided clear support for the hypothesis that abnormalities of gene regulation occur in CFS.[4] Following this, further funding was awarded by the CFS Research Foundation, Hertfordshire, UK (www.cfsrf.com) to continue with the research and to expand on the pilot study. Currently, the total support is approximately £1 million from the CFS Research Foundation, and the purpose of this review is to outline how this money is being spent, what will be gained from this research and what are the future priorities for research on CFS.

The principal goals are to gain a clear understanding of those genes that are associated only or mainly with CFS, and also to identify protein biomarkers in the serum of patients with CFS, which can be used to develop a test designed to assist doctors in the clinical diagnosis of CFS. In addition to these, and on the basis of those genes that have been shown to be associated with CFS, clinical trials of new and established pharmaceutical drugs on patients with CFS will be carried out to identify one or more treatments that will cure most cases of the disease.

Which genes occur at abnormal levels in patients with CFS?

Information generated by sequencing of the human genome along with advances in the

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Abbreviations: CFS, chronic fatigue syndrome; IFN, interferon; MPSS, massive parallel signature sequencing; PCR, polymerase chain reaction

manufacture of automated chips and data analysis has provided the potential to correlate the genome of an organism with its biological functions. Analysis of gene expression in peripheral blood white cells has become a standard approach to study the pathogenesis of many human diseases. In CFS, blood has been shown to be a good choice because it is accessible, because it has been shown that most genes are expressed in the white cells and that the white cells of patients with CFS exhibit reproducible changes in gene expression as compared with normal controls (table 1).[4–8] Unfortunately, some studies on gene expression in CFS have the serious flaw of not confirming microarray analyses with real-time polymerase chain reaction (PCR).[5] [9–20] The genes identified by such studies using unconfirmed microarray data cannot be relied on, owing to the known lack of specificity in microarray analyses, and, hence, interpretation of these studies is extremely difficult. Considering PCR-confirmed studies only[4–8] (Kerr et al, unpublished data), the genes identified in CFS suggest a complex picture, most prominent in which is ''immunity and defence''. This supports previous findings on the role of the immune system in the maintenance of this disease.

In our own pilot study,[4] total RNA in the circulating white cells was examined in 25 patients with CFS and 25 age-matched and sex-matched normal blood donors for gene expression using a microarray representing 9522 human genes. After confirmation of the results using taqman real-time PCR, 16 genes were shown to be expressed at different levels in the cases compared with the controls. These genes were involved in several processes, including immunity and defence, the mitochondrion, and transcriptional and translational regulation. Although this study proves that patients with CFS exhibit important and reproducible differences in gene expression compared with controls, the particular profile of genes identified indicates that the picture is complex.

But the ultimate goal in all these studies has not yet been achieved—namely, to identify with complete certainty those genes whose overexpression or underexpression occurs in patients with CFS, but not in either normal people or patients with other diseases. In addition, such research must be comprehensive enough to identify particular metabolic pathways that are involved in CFS. Therefore, we must use methods that look at all known genes and then be able to group the genes together so that we have knowledge of the pathways involved.

Another interesting development is the suggestion that standard microarrays may not be adequate, as their design depends on prior knowledge of the gene sequences that are looked for in the samples, as described above. The study of Powell et al[6] is particularly interesting in this regard, because it is the only published study of relevant size, to date, that used an entirely open-ended screening method (differential display) and found that 4 of 12 PCR-confirmed, CFS-associated, transcripts could not be matched to known genes in either the Celera or National Center for Biotechnology Information genomics databases (as of December 2005), and suggests the involvement of novel sequences in CFS. We have taken this phenomenon seriously and are reproducing our 2005 pilot study using a combination of both microarrays (representing 47 000 human genes and variants) and massive parallel signature sequencing (MPSS).

MPSS is a new method that precisely quantifies all mRNA species and has the potential to detect entirely new human genes, as well as viral and other genes. The method uses microbeads that are bound to signature sequences, which bind genes in the sample. Then, those signature sequences that have bound gene attached to them are sequenced while they are still attached to the bead and used to generate precise numbers of each signature sequence present in the sample. Therefore, all

Table 1 Gene expression studies in chronic fatigue syndrome

Author and year	No of CFS cases	No of normal controls	Gene expression screening method	PCR used	Purpose of study	Main functional themes implicated in pathogenesis of CFS*
Vernon et al, 2002[5]	5	17	Filter array (1764 genes)	No	To identify gene expression correlates of CFS	Immunity and defence
Powell et al, 2003[6]	**7**	**4**	**Differential display**	**Yes**	**To identify gene expression correlates of CFS**	**Immunity and defence**
Whistler et al, 2003[9]	23	0	Microarray (3800 genes)	No	To identify gene expression correlates of CFS phenotypes	Not applicable
Whistler et al, 2005[10]	5	5	Microarray (3800 genes)	No	To identify gene expression correlates of exercise	Not applicable
Grans et al, 2005[7]	**20**	**14**	**Microarray (30 000 genes)**	**Yes**	**To identify gene expression correlates of CFS**	**Not applicable**
Kaushik et al, 2005[4]	**25**	**25**	**Microarray (9522 genes)**	**Yes**	**To identify gene expression correlates of CFS**	**Immunity and defence**
Gow et al, 2005[12]	8	7	Microarray (33 000 genes)	No	To identify gene expression correlates of CFS	Immunity and defence
Grans et al, 2006[8]	**30**	**36**	**Not applicable**	**Yes**	**To determine ERβ levels**	**Reduced ERβ levels—consistent with immunomodulation**
Carmel et al, 2006[13]	40	37	Microarray (19 760 genes)	No	To identify gene expression correlates of CFS phenotypes	Not applicable
Whistler et al, 2006[10]	40	37	Microarray (19 760 genes)	No	To identify gene expression correlates of CFS phenotypes	Energy metabolism, signal transduction, cell proliferation, apoptosis
Broderick et al, 2006[14]	40	37	Microarray (19 760 genes)	No	To identify illness parameters in fatiguing illness	Not applicable
Fang et al, 2006[16]	40	37	Microarray (19 760 genes)	No	To identify gene expression correlates of CFS phenotypes	Immune response, apoptosis, ion-channel, reg. of cell growth, neuronal activity
Fostel et al, 2006[17]	40	37	Microarray (19 760 genes)	No	To identify gene expression correlates of CFS	Immune response, androgen receptors, P450, cytoskeleton, signalling
Kerr et al, unpublished	**47**	**74**	**Microarray (47 000 genes) and MPSS**	**Yes**	**To identify gene expression correlates of CFS**	**Immunity and defence**

CFS, chronic fatigue syndrome; ERβ, oestrogen receptor β; MPSS, massive parallel signature sequencing; PCR, polymerase chain reaction.
*MPSS.
Only the microarray results of studies shown in bold face have been confirmed using PCR.

genes are detected and precise gene copy numbers are generated for each. Our strategy is to identify genes that are markedly differentially expressed between CFS and normal groups in microarray and MPSS studies, and to confirm these using real-time PCR. This is critical due to the known lack of specificity of gene arrays and other such sensitive screening methods.

In a phase II study, the genes in our CFS-associated gene signature will be tested for in many more patients with CFS, patients whose disease fits the criteria for CFS except for duration of disease (eg, 3–6 months duration of illness), normal controls with a degree of fatigue on the day of sampling and disease controls (eg, rheumatoid arthritis, osteoarthritis, endogenous depression, etc). This will exclude some genes identified in the first phase, but the genes that are left can be taken to be specific to the disease process(es) in CFS.

In a phase III study, a small subset of patients with CFS will be examined, who are typical in terms of their disease phenotype (or symptoms) and CFS-associated gene signature, at 13 time points over 1 year at intervals of 1 month. Clinical symptoms and their severity will be recorded and gene levels determined, and an attempt made to associate particular abnormalities of gene expression with the presence and severity of particular symptoms that occur in CFS.

The MPSS signature sequences have also been used to indicate viral infections in our patients as compared with controls, and currently 28 possible viral candidates are being tested for in the white cells of our study subjects.

Development of a diagnostic test to be used in clinical laboratories

Progress is also being made towards identifying biomarkers in the serum of patients with CFS. A biomarker is a protein that occurs at different levels in the serum of patients as compared with normal people and patients with other diseases. This work is being carried out using a technique called surface-enhanced, laser-desorption and ionisation-time-of-flight mass spectrometry (www.ciphergen.com).

In this technique, minute amounts of serum are spotted on the surface of aluminium chips, which are then subjected to an ionisation current. This method combines chromatographic separation, achievable due to the presence of biochemically active chip surfaces, with mass spectrometry. On the basis of the time of flight, the mass:charge (m:z) ratio for each molecule is determined. The method enables us to determine the mass and relative amount of each individual molecule in complex protein mixtures. Analysis of mass spectra from cases as compared with controls identifies peaks (or proteins), the presence or absence of which can reliably distinguish between the two groups. It is these proteins (or combinations of them) that can then be used as biomarkers in a diagnostic test, assuming that they are shown to be specific to patients with CFS.

Protein biomarker pilot study

We have carried out a pilot study of this approach at Imperial College London (London, UK), which has identified significant protein biomarkers in the blood of patients with CFS (PC, AH, PRL, JRK). In this study, serum samples from 30 patients with CFS and 30 normal blood donors (age-matched and sex-matched) were examined. Each serum was tested using CM10 and Q10 chips with a matrix consisting of a saturated solution of sinapic acid in 50% acetonitrile and 0.5% trifluoroacetic acid. Pooled sera from each group (10 pools each of 3 sera for each group) were then anionically fractionated using resin by a standard protocol and analysed using normal-phase-20 chips (NP20). This resulted in a collection of six fractions containing eluants of flow-through with pH 9, pH 7, pH 5, pH 4, pH 3 and

acid–organic solvent. These fractions were analysed using the NP20 arrays, and the spectra were analysed using Ciphergen Express Data Manager software (http://ciphergen.com). Biomarkers were found that differentiated the groups, and some of these were found to be reproducible (fig 1), thus confirming the hypothesis that such differences occur between patients with CFS and normal people. Larger-scale studies are now being carried out to confirm and further detail these promising results. This work is currently being undertaken using adult and paediatric blood samples, as a collaboration between Imperial College London and St George's University of London (London, UK).

This work is being carried out separately from that on gene expression. The reason for this is that it is well recognised that genes that are differentially expressed in a particular disease state may be detected as differentially expressed at the protein level in only 30–70% of cases. Therefore, it seems that many factors may influence the relationship between the white cell transcript level and the respective serum protein level. In view of this, these studies are carried out independently of each other, but on the same populations to clarify this relationship.

Clinical trials of drugs in patients with CFS

Knowledge of how a disease is caused leads directly to design and utilisation of treatments that correct the abnormal processes and, hopefully, leads to improvement or cure of the disease. In the context of genomic research, many treatments have been designed in this way. For example, a range of so-called ''biological'' treatments are now available for immune-mediated diseases.

On the basis of the results of gene expression studies, funded by the CFS Research Foundation, a clinical trial of interferon-β (IFNβ) is planned at St George's University of London. We envisage that this will be the first of several clinical trials that are based on our gene expression findings, using the novel gene approach outlined above.

IFNβ is associated with the regulation of humoral immune responses and immune responses against viral infections. It increases expression of human leucocyte class 1 antigens and blocks the expression of human leucocyte class 2 antigens. IFNβ also stimulates the activity of natural killer cells, which are considered to be inefficient in patients with CFS. IFNβ selectively inhibits the expression of some mitochondrial genes that are implicated by gene studies in patients with CFS[4] (Kerr et al, unpublished). It inhibits the proliferation of several cancer cell lines.[21] Evidence for T-cell-activation has been documented in patients with CFS[4] (Kerr et al, unpublished). Viral infection is known to trigger CFS, and various studies suggest that ongoing viral infection is a feature of CFS. Finally, IFNβ is a licensed treatment for multiple sclerosis, helping in reduction of fatigue. The pathogenesis of fatigue in multiple sclerosis is thought to
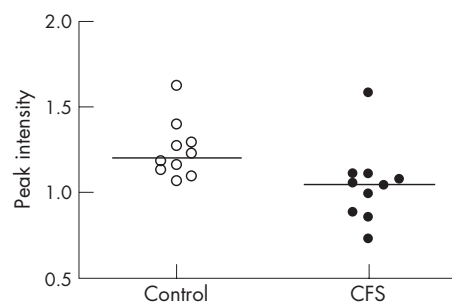

Figure 1 Potential biomarker of chronic fatigue syndrome (CFS) at 17 899 Da, which eluted in the acid–organic wash from the anionic exchange fractionation. $p = 0.005$.

be cytokine mediated,[22] as has been shown in CFS. A trial of IFNβ has not been carried out previously in patients with CFS. As patients with CFS are unusually sensitive to drugs and chemicals, a reduced dose may need to be used to avoid side effects.

The TNFα inhibitors are another group of drugs that may provide benefit in CFS. This group of drugs has been shown to lead to dramatic improvement in patients with rheumatoid arthritis, Crohn's disease, psoriasis and other diseases, including asthma. One TNFα inhibitor (etanercept) has been used with considerable benefit in the treatment of six patients with CFS in a pilot study.[23] Unfortunately, this trial was not published as a paper but only as a meeting abstract. The use of TNF-α inhibitors in CFS is strongly supported by scientific data on the immune responses in CFS, epidemiological data, and now data from gene expression studies[6] (Kerr *et al*, unpublished). It is also an urgent priority to repeat this work and carry out a larger clinical trial of etanercept in patients with CFS.

CONCLUSION
In the near future, we can expect a diagnostic test for CFS, an understanding of the mechanisms of the disease and treatments that will work in most cases of this tragic and all-too-common illness.

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