



Current research priorities in Chronic Fatigue Syndrome / Myalgic Encephalomyelitis (CFS/ME): disease mechanisms, a diagnostic test and specific treatments

Jonathan R Kerr, Peter Christian, Andrea Hodgetts, Paul R Langford, Lakshmi D Devanur, Robert Petty, Beverley Burke, Lindsey I Sinclair, Selwyn CM Richards, Jane Montgomery, Clare McDermott, Tim J Harrison, Paul Kellam, David J Nutt and Stephen T Holgate

J. Clin. Pathol. published online 25 Aug 2006;
doi:10.1136/jcp.2006.042374

Updated information and services can be found at:

<http://jcp.bmjournals.com/cgi/content/abstract/jcp.2006.042374v1>

These include:

Rapid responses

You can respond to this article at:

<http://jcp.bmjournals.com/cgi/eletter-submit/jcp.2006.042374v1>

Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article

Notes

Online First contains unedited articles in manuscript form that have been peer reviewed and accepted for publication but have not yet appeared in the paper journal (edited, typeset versions may be posted when available prior to final publication). Online First articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Online First articles must include the digital object identifier (DOIs) and date of initial publication.

To order reprints of this article go to:
<http://www.bmjournals.com/cgi/reprintform>

To subscribe to *Journal of Clinical Pathology* go to:
<http://www.bmjournals.com/subscriptions/>

Current research priorities in Chronic Fatigue Syndrome / Myalgic Encephalomyelitis (CFS/ME): disease mechanisms, a diagnostic test and specific treatments

JR Kerr,¹ P Christian,² A Hodgetts,² PR Langford,² LD Devanur,¹ R Petty,¹ B Burke,¹ LI Sinclair,³ SCM Richards,⁴ J Montgomery,⁴ C McDermott,⁴ TJ Harrison,⁵ P Kellam,⁶ DJ Nutt,³ ST Holgate,⁷ and the Collaborative Clinical Study Group.*

¹Dept of Cellular & Molecular Medicine, St George's University of London; ²Dept of Paediatric Infectious Diseases, Imperial College London; ³Psychopharmacology Unit, University of Bristol; ⁴Dorset CFS Service, Poole Hospital, Dorset; Depts of ⁵Medicine and ⁶Infection, University College London; ⁷MRC Dept of Immunopharmacology, University of Southampton.

***Collaborative Clinical Study Group**

D Honeybourne (Birmingham Heartlands Hospital), AP Smith (Cardiff University), M Thomas (Cardiff University), JG Ayres (University of Aberdeen), J Main (Imperial College London), T Daymond (University of Sunderland), A Bansal (St Helier Hospital, Surrey), BK Puri (Hammersmith Hospital), R Morgan (Imperial College London), RC Peveler (University of Southampton), JS Axford (St George's University of London), W Weir (Harley Street, London), D Enlander (New York CFS Association, Fifth Avenue, New York, NY), JK Chia (ID Med, Torrance, CA).

Correspondence to: Dr Jonathan R Kerr, Sir Joseph Hotung Senior Lecturer in Inflammation, Dept of Cellular & Molecular Medicine, St George's University of London, Cranmer Terrace, London SW17 0RE, United Kingdom. Tel: 0208 725 5276. Fax: 0208 725 5260. Email: jkerr@sgul.ac.uk.

Abstract

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 in order to address 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 CFS patients and of those studies which have been confirmed using polymerase chain reaction (PCR), it is clear that the most predominant functional theme is that of immunity and defense. However, we do not yet know the precise gene signature and metabolic pathways involved. Currently, this is being addressed using a microarray representing 47,000 human genes and variants, massive parallel signature sequencing (MPSS) and real-time PCR. It will be important to ensure that once a gene signature has been identified, that 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 (SELDI-TOF) mass spectrometry following a pilot study in which putative biomarkers were identified. And, finally, clinical trials are being planned; novel treatments which we believe are important to trial in CFS patients are interferon- β and one of the anti-tumour necrosis factor- α drugs.

Introduction

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 and 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 (CMO), 'A report of the CFS/ME Working Group'.² Epidemiological studies have revealed that many CFS patients give a history of an illness consistent with virus infection which precedes the development of fatigue,³ and CFS has been shown to follow acute infection with various infectious agents. CFS patients 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 development of new, specific treatments, which are not available at present. The members of this group were also concerned at the trivialisation of CFS and the labelling of patients as sufferers of psychiatric, psychological or somatoform disease. To address the problem, a pilot study was performed to see if there was any evidence that the white blood cells of CFS patients 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 upon the pilot study. Currently, the total support is approximately £1million from the CFS Research Foundation, UK, 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 in the area of CFS.

The principal goals are to gain a clear understanding of those genes which are associated only or mainly with CFS, and also to identify protein biomarkers in the serum of CFS patients which can be used to develop a test designed to assist doctors in the clinical diagnosis of CFS in hospitals and clinics. In addition to these, and based on those genes that have been shown to be associated with CFS, clinical trials will be performed of new and established

pharmaceutical drugs in CFS patients in order to identify one or more treatments which 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 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 blood cells has become a standard methodological approach to study of 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 found to be expressed in the white blood cells and as it has been shown by various groups that the white blood cells of CFS patients exhibit reproducible alterations in gene expression as compared with normal controls (Table 1).⁴⁻⁸ Unfortunately, some studies on gene expression in CFS suffer the serious flaw of not confirming microarray analyses with real-time PCR.^{5,9-20} The genes identified by such studies using unconfirmed microarray data cannot be relied upon due to the known lack of specificity in microarray analyses, and so interpretation of these studies is extremely difficult. Considering PCR-confirmed studies only,⁴⁻⁸ (Kerr et al, unpublished), the genes identified in CFS suggest a complex picture but most prominent within which is 'immunity and defense'. 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 blood cells was examined in 25 CFS patients and 25 age and sex-matched normal blood donors for gene expression using a microarray representing 9,522 human genes. After confirmation of the results using taqman real-time polymerase chain reaction (PCR), 16 genes were shown to be expressed at very different levels in the cases compared with the controls. These genes were involved in several processes, including immunity and defense, the mitochondrion, and transcriptional and translational regulation. Although this study provides proof that CFS patients exhibit significant 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 of these studies has not yet been achieved; namely to identify with complete certainty those genes whose over- or under-expression occurs in patients with CFS, but not in either normal persons or in 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 significant 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 NCBI genomics databases (as of December 2005), and suggests the involvement of novel sequences in CFS. We have taken this phenomenon very 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 utilises microbeads which 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 genes are detected and precise gene copy

numbers generated for each. Our strategy is to identify genes which are significantly 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 2 study the genes in our CFS-associated gene signature will be tested for in many more CFS patients, patients whose disease fits criteria for CFS except for duration of disease (for example, 3-6 months duration of illness), normal controls with a degree of fatigue on the day of sampling, and disease controls (for example, 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 3 study a small subset of CFS patients will be examined who are typical in terms of their disease phenotype (or symptoms) and CFS-associated gene signature, at 13 time points over one year at intervals of one 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 which occur in CFS.

The MPSS signature sequences have also been used to indicate virus infections in our patients as compared with controls, and currently 28 possible viral candidates are being tested for in the white blood 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 suffering from other diseases. This work is being done using a technique called Surface-Enhanced, Laser-Desorption and Ionisation – Time of Flight (SELDI-TOF) mass spectrometry (www.ciphergen.com).

In this technique, minute amounts of serum are spotted onto 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. Based on the time of flight, the mass / charge (m/z) ratio for each molecule is determined. The method is able 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) which can then be used as biomarkers in a diagnostic test, assuming they are shown to be specific to patients with CFS.

Protein Biomarker Pilot Study

We have performed a pilot study of this approach at Imperial College London which has identified statistically significant protein biomarkers in the blood of CFS patients (P Christian, A Hodgetts, P Langford, JR Kerr). In this study, serum samples from 30 cases of CFS and 30 normal blood donors (age and sex-matched) were examined. Each serum was tested using CM10 and Q10 chips using 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 NP20 chips. This resulted in collection of 6 fractions containing eluants of flow-through with pH9, pH7, pH5, pH4, pH3 and acid-organic solvent. These fractions were analysed using the SELDI-PC NP20 arrays and the spectra were analysed using Ciphergen Express Data Manager (CEDM) software. Biomarkers were found which differentiated the groups and some of these were found to be reproducible (Figure 1), thus

confirming the hypothesis that such differences occur between patients with CFS and normal persons. Larger-scale studies must now take place to confirm and further detail these promising results. This work is currently being performed using adult and paediatric blood samples, as a collaboration between Imperial College London and St George's University of London.

This work is being performed quite separately from the gene expression work. 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 blood cell transcript level and the respective serum protein level. In view of this, these studies are performed independently of each other, but on the same populations in order to clarify this relationship.

Clinical trials of pharmaceutical drugs in patients with CFS

Knowledge of how a disease is caused leads directly to design and utilisation of treatments which correct the abnormal processes and, hopefully, lead 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 'biologic' treatments are now available for immune-mediated diseases.

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

Interferon- β (IFN- β) is involved in the regulation of humoral immune responses and immune responses against virus infections. IFN- β increases expression of HLA class I antigens and blocks the expression of HLA class 2 antigens. IFN- β stimulates the activity of NK cells, which are accepted to be inefficient in CFS patients. IFN- β selectively inhibits the expression of some mitochondrial genes which are implicated by gene studies in CFS patients,⁴ (Kerr et al, unpublished). IFN- β inhibits the proliferation of a number of cancer cell lines.²¹ Evidence for T cell activation has been documented in CFS patients,⁴ (Kerr et al, unpublished). Virus infection is known to trigger CFS and various studies suggest that ongoing virus infection is a feature of CFS. Finally, IFN- β is a licensed treatment for multiple sclerosis (MS) in which it has resulted in reduction of fatigue. It is thought that the pathogenesis of fatigue in MS may be cytokine-mediated,²² as has been demonstrated in the CFS. A trial of IFN- β has not been performed previously in CFS patients. As CFS patients are unusually sensitive to drugs and chemicals, a reduced dose may need to be used to avoid side effects.

The TNF- α inhibitors are another group which 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 TNF- α inhibitor (Etanercept) has been used with significant benefit in the treatment of 6 CFS patients in a pilot study (Lamprecht et al, 2001). 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 perform a larger clinical trial of etanercept in CFS patients.

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.

Acknowledgements

We thank the CFS Research Foundation (www.cfsrf.com) for funding.

References

1. Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. *Ann Intern Med.* 1994 Dec 15;121(12):953-9.
2. Report of the CFS/ME Working Group. Department of Health, January 2002. (<<http://www.doh.gov.uk/cmo/cfsmereport/>>).
3. Afari N, Buchwald D. Chronic fatigue syndrome: a review. *Am J Psychiatry.* 2003 Feb;160(2):221-36. Review.
4. Kaushik N, Fear D, Richards SC, McDermott CR, Nuwaysir EF, Kellam P, Harrison TJ, Wilkinson RJ, Tyrrell DA, Holgate ST, Kerr JR. Gene expression in peripheral blood mononuclear cells from patients with chronic fatigue syndrome. *J Clin Pathol.* 2005 Aug;58(8):826-32.
5. Vernon SD, Unger ER, Dimulescu IM, Rajeevan M, Reeves WC. Utility of the blood for gene expression profiling and biomarker discovery in chronic fatigue syndrome. *Dis Markers.* 2002;18(4):193-9.
6. Powell R, Ren J, Lewith G, Barclay W, Holgate S, Almond J. Identification of novel expressed sequences, up-regulated in the leucocytes of chronic fatigue syndrome patients. *Clin Exp Allergy.* 2003 Oct;33(10):1450-6.
7. Grans H, Nilsson P, Evengard B. Gene expression profiling in the chronic fatigue syndrome. *J Intern Med.* 2005;258(4):388-90.
8. Grans H, Nilsson M, Dahlman-Wright K, Evengard B. Reduced levels of oestrogen receptor {beta} mRNA in Swedish patients with chronic fatigue syndrome. *J Clin Pathol.* 2006 May 26; [Epub ahead of print].
9. Whistler T, Unger ER, Nisenbaum R, Vernon SD. Integration of gene expression, clinical, and epidemiologic data to characterize Chronic Fatigue Syndrome. *J Transl Med.* 2003 Dec 1;1(1):10.
10. Whistler T, Jones JF, Unger ER, Vernon SD. Exercise responsive genes measured in peripheral blood of women with chronic fatigue syndrome and matched control subjects. *BMC Physiol* 2005;24;5(1):5.
11. Whistler T, Taylor R, Craddock RC, Broderick G, Klimas N, Unger ER. Gene expression correlates of unexplained fatigue. *Pharmacogenomics* 2006;7(3):395-405.
12. Gow JW, Cannon C, Behan WMH, Herzyk P, Keir S, Riboldi-Tunnicliffe G, Behan PO, Chaudhuri A. Whole-Genome (33,000 genes) Affymetrix DNA Microarray Analysis of Gene Expression in Chronic Fatigue Syndrome. International Conference on Fatigue Science, Karuizawa, Japan. February 2005. Abstract S7-03/P2-19.
13. Carmel L, Efroni S, White PD, Aslakson E, Vollmer-Conna U, Rajeevan MS. Gene expression profile of empirically delineated classes of unexplained

- chronic fatigue. *Pharmacogenomics* 2006;7(3):375-86.
14. Broderick G, Craddock RC, Whistler T, Taylor R, Klimas N, Unger ER. Identifying illness parameters in fatiguing syndromes using classical projection methods. *Pharmacogenomics* 2006;7(3):407-19.
 15. Craddock RC, Taylor R, Broderick G, Whistler T, Klimas N, Unger ER. Exploration of statistical dependence between illness parameters using the entropy correlation coefficient. *Pharmacogenomics* 2006;7(3):421-8.
 16. Fang H, Xie Q, Boneva R, Fostel J, Perkins R, Tong W. Gene expression profile exploration of a large dataset on chronic fatigue syndrome. *Pharmacogenomics* 2006;7(3):429-40.
 17. Fostel J, Boneva R, Lloyd A. Exploration of the gene expression correlates of chronic unexplained fatigue using factor analysis. *Pharmacogenomics* 2006;7(3):441-54.
 18. Lin SM, Devakumar J, Kibbe WA. Improved prediction of treatment response using microarrays and existing biological knowledge. *Pharmacogenomics* 2006;7(3):495-501.
 19. Waltman P, Pearlman A, Mishra B. Interpreter of maladies: redescription mining applied to biomedical data analysis. *Pharmacogenomics* 2006;7(3):503-9. Review.
 20. Shoemaker J. Statistical challenges with gene expression studies. *Pharmacogenomics* 2006;7(3):511-9.
 21. Arnason BG. Long-term experience with interferon beta-1b (Betaferon) in multiple sclerosis. *J Neurol.* 2005 Sep;252 Suppl 3:iii28-iii33.
 22. Heesen C, Nawrath L, Reich C, Bauer N, Schulz KH, Gold SM. Fatigue in multiple sclerosis: an example of cytokine mediated sickness behaviour? *JNNP* 2006;77:34-9.
 23. Lamprecht K, et al. Pilot study of etanercept treatment in patients with Chronic Fatigue Syndrome. Meeting of the American Association of Chronic Fatigue Syndrome (AACFS), Seattle, 2001.
[Http://www.cfsresearch.org/cfs/conferences/14.htm](http://www.cfsresearch.org/cfs/conferences/14.htm)

Table 1. Gene expression studies in CFS. Only the microarray results of studies shown in grey have been confirmed using PCR.

Author & year	Ref. No.	No. CFS cases	No. 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 & defence
Powell et al, 2003	6	7	4	Differential display	Yes	To identify gene expression correlates of CFS	Immunity & defence
Whistler et al, 2003	9	23	0	Microarray (3,800 genes)	No	To identify gene expression correlates of CFS phenotypes	Not applicable
Whistler et al, 2005	10	5	5	Microarray (3,800 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 (9,522 genes)	Yes	To identify gene expression correlates of CFS	Immunity & defence
Gow et al, 2005	12	8	7	Microarray (33,000 genes)	No	To identify gene expression correlates of CFS	Immunity & defence
Grans et al, 2006	8	30	36	Not applicable	Yes	To determine oestrogen receptor (ER β) levels	Reduced ER β 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	11	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) & MPSS*	Yes	To identify gene expression correlates of CFS	Immunity & defence

*MPSS, massive parallel signature sequencing.

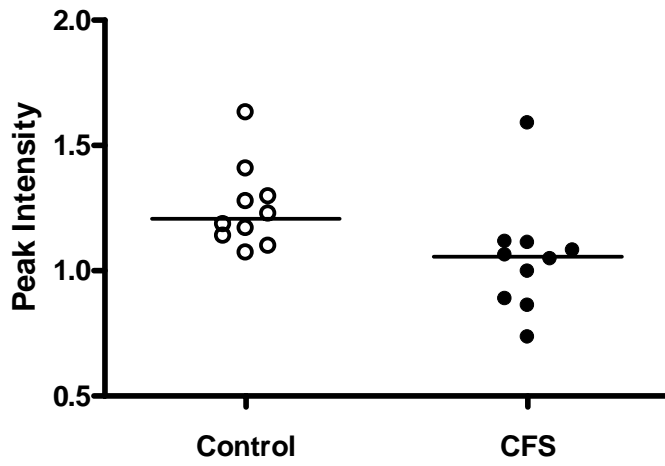


Figure 1. Potential biomarker of CFS at 17899 Da which eluted in the acid/organic wash from the anionic exchange fractionation. $p = 0.0052$

“The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive licence (or non-exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd and its Licensees to permit this article (if accepted) to be published in JCP and any other BMJPG products to exploit all subsidiary rights, as set out in our licence.”