Gene Profiling of Patients with Chronic Fatigue Syndrome/Myalgic Encephalomyelitis

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Chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) is a multisystem disease, the pathogenesis of which remains undetermined. Following two microarray studies, we reported the differential expression of 88 human genes in patients with CFS; 85 of these genes were upregulated and 3 were downregulated. The top functional categories of these 88 genes were hematologic disease and function, immunologic disease and function, cancer, cell death, immune response, and infection. Clustering of quantitative polymerase chain reaction data from CFS/ME patients revealed seven subtypes with distinct differences in Short Form (SF)-36 scores, clinical phenotypes, and severity. Gene signatures in each subtype implicate five human genes as possible targets for specific therapy. Development of a diagnostic test for subtype status is now a priority. The possibility that these subtypes represent individual host responses to particular microbial infections is being investigated and may provide another route to specific therapies for CFS patients.

Introduction

Chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) is a disease characterized by severe and debilitating fatigue, sleep abnormalities, impaired memory and concentration, and musculoskeletal pain [1]. In the Western world, the population prevalence is estimated to be of the order of 0.5% [2,3]. Research studies have identified various features relevant to the pathogenesis of CFS/ME, such as viral infection; immune abnormalities and immune activation; exposure to toxins, chemicals, and pesticides; stress; hypotension; lymphocyte abnormalities; and neuroendocrine dysfunction. However, the precise underlying disease mechanisms and means by which these abnormalities interrelate in CFS/ME patients remain to be clarified [4,5].

Human Gene Expression in CFS/ME

Various studies have analyzed the gene expression in peripheral blood of patients with CFS/ME, and in all of these patients, genes of immunity and defense are prominent [6–10,11•,12,13•,14–17]. Unfortunately, in several of these studies, quantitative polymerase chain reaction (qPCR) confirmation was not carried out, so the results may be unreliable [6,8–10,14–17]. The genes identified in those studies utilizing PCR confirmation suggest a complex pathogenesis [7,11•,12,13•].

Pilot study of gene expression in CFS

In 2001, we began a pilot study of gene expression in the peripheral blood of CFS patients [13•]. We enrolled 25 CFS cases diagnosed according to the Fukuda criteria [1] and 25 normal blood donors matched for age, sex, and geographic location using a single-color microarray representing 9522 human genes. Genes showing differential expression (using a cutoff fold-difference of expression ≤ 1.5 and a P value of 0.001) were further analyzed using TaqMan (Applied Biosystems, Foster City, CA) real-time PCR in fresh samples. This approach identified 16 human genes as being differentially expressed, 15 of which were upregulated (*ABCD4*, *PRKCL1*, *MRPL23*, *CD2BP2*, *GSN*, *NTE*, *POLR2G*, *PEX16*, *EIF2B4*, *EIF4G1*, *ANAPC11*, *PDCD2*, *KHSRP*, *BRMS1*, *GABARAPL1*) and one was downregulated (*IL-10RA*) (Table 1). 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. This study confirmed our hypothesis that CFS patients exhibit reproducible alterations in gene regulation [13•]. However, the scope of this study was limited due to the restricted size of the microarray used.

Definitive study of gene expression in CFS

We undertook a comprehensive study to determine the precise abnormalities of gene expression that occur in patients with CFS/ME [18••]. We analyzed gene expression in peripheral blood from 25 CFS/ME patients diagnosed according to the Fukuda diagnostic criteria [1] and 50 normal blood donors using the Affymetrix (Santa Clara, CA) U133+2 microarray using a cutoff fold-difference of expression 2.5 or higher. There were 182 genes that showed differential expression using this analysis, and these were then tested in 55 CFS/ME patients and 75 normal blood donors using TaqMan real-time PCR. There were 82 genes confirmed as being differentially expressed in CFS patients.

Next, we attempted to identify additional transcription factor (TF) genes, which may be important given the involvement of these 82 human genes. Promoter sequences for each of the 82 genes were analyzed for overrepresentation of transcription factor binding sites [19]. This approach identified overrepresentation of 13 TF binding sites among these genes consisting of 12 human TFs (*REPIN1, SP1, ETS1, GABPA, GTF3A, EGR1, EGR2, EGR3, NFKB1, NHLH1, EGR4, REST*) and one viral TF (*BRLF1*). The involvement of seven of these TFs in CFS was subsequently confirmed (Table 1).

Therefore, overall, differential expression was confirmed for 88 genes, 85 of which were upregulated and 3 of which were downregulated. (As *NFKB1* was present in both the original list as well as the TF list, the total was 82 plus 6, or 88.) Highly represented functions were hematologic disease, immunologic disease, cancer, cell death, immune response, and infection [18••].

CFS/ME subtypes

We then clustered the relative quantity values from qPCR experiments for the 88 genes in the 55 CFS patients for which real-time PCR was available to identify patient subtypes with similar gene expression profiles. Clustering of real-time quantitative PCR (RQ) values from CFS/ME patients identified 7 subtypes consisting of 2, 5, 2, 19, 7, 14, and 3, CFS/ME patients, respectively. Clustering of mean fold-difference values (mean of CFS/ME subtype RQ divided by mean of normal RQ) for the 88 CFS/ME-associated genes revealed a distinct profile of gene expression in each subtype and clustering of genes with similar profiles of expression in the different subtypes. Absolute fold-difference values for each gene in each subtype are shown in Figure 1, which emphasizes the predominance of upregulation for most genes in all CFS/ME subtypes. Analysis of mean age and sex ratios for each subtype reveals that subtypes 3, 5, and 7 are made up of female patients only; subtype 2 is predominantly male patients; and the remaining subtypes are composed of both. Age differences were less clearly demarcated. The clinical phenotype was distinct between subtypes; subtypes 1 and 7 were the most severe, followed sequentially by subtypes 2, 4, 5, and 6/3. Analysis of variance testing revealed significant differences between groups for the Short Form (SF)-36 total score ($P = 0.016$), social functioning ($P = 0.03$), and emotional role ($P = 0.003$), whereas the difference between groups approached significance for general health ($P = 0.08$) and mental health ($P = 0.08$) (Fig. 1). After adjusting for multiple comparisons, significant associations were found between specific groups and clinical phenotypes. Subtype 7 had the most pain, lowest SF-36 scores (along with subtype 1), and most severe individual symptoms (including swollen glands, sore throat, headaches); subtype 1 had the worst cognition and mental health score and poor sleep, despite having the least pain; subtype 4 had moderate neurocognitive function and cognitive defects combined with moderate levels of bodily pain and sleep problems; subtype 5 had the best mental health but poor neurocognitive function, gastrointestinal complaints, and the most marked muscle weakness and postexertional malaise; and subtype 2 had marked postexertional malaise, muscle pain, and joint pain but poor mental health. Subtypes 4 and 6 were predominant in Dorset, England; subtype 4 was predominant in London and New York, and subtype 5 was predominant in Bristol, England [18••,20••].

We have repeated this work using 62 new and previously untested patients and have been able to demonstrate the same gene expression abnormalities along with the existence of subtypes with marked clinical differences (Kerr, unpublished data).

The existence of subtypes had already been proposed for CFS [21,22] and may explain the difficulty in identifying consistent and reproducible abnormalities in CFS/ME patients. This is a problem that has confounded researchers for decades and undermines the validity of the disease in the eyes of the medical community. It has been suggested that subtypes could exist on the basis of triggering factors, presence of minor symptoms, or underlying processes [21,22]. However, although the existence of subtypes is widely accepted, there has been little agreement on the criteria that should be used to distinguish one subtype from another. In this regard, a subtyping system based on the presence of "minor" symptoms [22], although possible, did not produce subtypes with marked differences in disease severity, unlike the genomic subtypes. In light of this, our results are particularly intriguing and suggest a possible and reproducible means of CFS/ME subtype discrimination. However, this approach must be tested and possibly modified before it can be accepted as a valid and clinically applicable means of subtype discrimination.

Possible weakness of using microarrays

Another development is the suggestion that standard microarrays may not be entirely adequate, as their design depends on prior knowledge of the gene sequences that are looked for in the samples. The study of Powell et al. [7] is particularly interesting in this regard because it is the only published study of relevant size that used an entirely

Table 1. CFS/ME-associated genes and transcription factors identified through analysis microarrays, real-time PCR, and transcription-factor binding sites

Gene symbol	Gene name	Genbank Accession #	TaqMan assay identification #	Fold difference (CFS/normal)	*P* value
*ABCD4**	ATP-binding cassette, sub-family D (ALD), member 4	NM_020323	Hs00245340_m1	2.08	0.028
ACTR3	ARP3 actin-related protein 3 homolog (yeast)	NM_005721	Hs00828586_m1	1.42	0.004
AKAP10	A-kinase (PRKA) anchor protein 10	NM_007202	Hs00183673_m1	1.54	0.001
*ANAPC11**	APC11 anaphase promoting complex subunit 11 homolog (yeast)	NM_016476	Hs00212858_m1	3.32	0.000
ANAPC5	Anaphase promoting complex subunit 5	NM_016237	Hs00212120_m1	2.36	0.000
APP	Amyloid beta (A4) precursor protein	NM_201413	Hs00169098_m1	2.5	0.000
ARL4C	ADP-ribosylation factor-like 4C	NM_005737	Hs00255039_s1	2.96	0.000
ARPC5	Actin-related protein 2/3 complex, subunit 5	NM_005717	Hs00271722_m1	3.23	0.000
ARSD	Arylsulfatase D	NM_001669	Hs00534692_m1	1.98	0.001
ATP6V1C1	ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C1	NM_001695	Hs00184625_m1	2.03	0.000
BCOR	BCL6 co-repressor	NM_017745	Hs00372369_m1	1.6	0.010
BMP2K	BMP2 inducible kinase	NM_198892	Hs00214079_m1	1.3	0.014
*BRMS1**	Breast cancer metastasis suppressor 1	NM_015399	Hs00363036_m1	2.68	0.001
*CD2BP2**	CD2 (cytoplasmic tail) binding protein 2	NM_006110	Hs00272036_m1	1.8	0.000
CD47	CD47 molecule	NM_198793	Hs00179953_m1	2.2	0.000
CEP350	Centrosomal protein 350 kDa	NM_014810	Hs00402774_m1	2.02	0.005
CITED2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain 2	NM_006079	Hs00366696_m1	2.39	0.000
CMTM6	CKLF-like MARVEL transmembrane domain containing 6	NM_017801	Hs00215083_m1	1.41	0.012
CREBBP	CREB binding protein (Rubinstein-Taybi syndrome)	NM_004380	Hs00231733_m1	1.43	0.016
CRK	V-crk sarcoma virus CT10 oncogene homolog (avian)	NM_016823	Hs00180418_m1	2.51	0.000
CTBP1	C-terminal binding protein 1	NM_001328	Hs00179922_m1	1.45	0.062
CXCR4	Chemokine (C-X-C motif) receptor 4	NM_003467	Hs00607978_s1	1.67	0.000
EBI2	Epstein-Barr virus induced gene 2 (lymphocyte-specific GPCR)	NM_004951	Hs00270639_s1	3.44	0.001
EGR1†	Early growth response 1	NM_001955	Hs00152928_m1	2.82	0.015
EGR3†	Early growth response 3	NM_004421	Hs00231780_m1	1.92	0.017
*EIF2B4**	Eukaryotic translation initiation factor 2B, subunit 4 delta, 67 kDa	NM_172195	Hs00248984_m1	2.06	0.025
EIF3S10	Eukaryotic translation initiation factor 3, subunit 10 theta, 150/170 kDa	NM_003750	Hs00186707_m1	3.58	0.003
*EIF4G1**	Eukaryotic translation initiation factor 4A, isoform 2	NM_198241	Hs00191933_m1	3.05	0.003

*Genes identified in pilot study.
†Transcription factors identified by analysis of promoter regions and real-time PCR.
CFS/ME—chronic fatigue syndrome/myalgic encephalomyelitis; PCR—polymerase chain reaction.

Table 1. CFS/ME-associated genes and transcription factors identified through analysis microarrays, real-time PCR, and transcription-factor binding sites ***(Continued)***

Gene symbol	Gene name	Genbank Accession #	TaqMan assay identification #	Fold difference (CFS/normal)	*P* value
EIF4G3	Eukaryotic translation initiation factor 4 gamma, 3	NM_003760	Hs00186804_m1	1.67	0.000
ETS1†	V-ets erythroblastosis virus E26 oncogene homolog 1 (avian)	NM_005238	Hs00901425_m1	2.11	0.000
FAM126B	Family with sequence similarity 126, member B	NM_173822	Hs00545158_m1	1.64	0.003
FNTA	Farnesyltransferase, CAAX box, alpha	NM_002027	Hs00357739_m1	2.18	0.000
*GABARAPL1**	GABA(A) receptor-associated protein like 1	NM_031412	Hs00744468_s1	5.64	0.000
GABPA†	GA binding protein transcription factor, alpha subunit 60 kDa	NM_002031	Hs00745591_s1	8.06	0.000
GCN1L1	GCN1 general control of amino-acid synthesis 1-like 1 (yeast)	NM_006836	Hs00412445_m1	2.05	0.001
GLTSCR2	Flioma tumor suppressor candidate region gene 2	NM_015710	Hs00414236_m1	1.24	0.026
GNAS	GNAS complex locus	NM_080425	Hs00255603_m1	1.7	0.000
*GSN**	Felsolin (amyloidosis, Finnish type)	NM_198252	Hs00609276_m1	2.93	0.000
GTF2A2	Feneral transcription factor IIA, 2, 12 kDa	NM_004492	Hs00362112_m1	1.79	0.03
HIF1A	Hypoxia-inducible factor 1, alpha subunit	NM_001530	Hs00153153_m1	0.81	0.016
IFNAR1	Interferon (alpha, beta and omega) receptor 1	NM_000629	Hs00265057_m1	1.76	0.001
*IL10RA**	Interleukin 10 receptor, alpha	NM_001558	Hs00387004_m1	1.73	0.000
IL6R	Interleukin 6 receptor	NM_000565	Hs00794121_m1	1.19	0.06
IL6ST	Interleukin 6 signal transducer	NM_002184	Hs00174360_m1	1.8	0.002
IL7R	Interleukin 7 receptor	NM_002185	Hs00233682_m1	0.82	0.032
JAK1	Janus kinase 1 (a protein tyrosine kinase)	NM_002227	Hs00233820_m1	1.91	0.000
*KHSRP**	KH-type splicing regulatory protein (FUSE binding protein 2)	NM_003685	Hs00269352_m1	1.67	0.000
MAPK9	Mitogen-activated protein kinase 9	NM_139070	Hs00177102_m1	1.4	0.045
METTL3	Methyltransferase like 3	NM_019852	Hs00219820_m1	2.06	0.000
*MRPL23**	Mitochondrial ribosomal protein L23	NM_021134	Hs00221699_m1	2.06	0.001
MRPS6	Mitochondrial ribosomal protein S6	NM_032476	Hs00606808_m1	1.53	0.025
MRRF	Mitochondrial ribosome recycling factor	NM_138777	Hs00751845_s1	8.91	0.000
MSN†	Moesin	NM_002444	Hs00792607_mH	1.33	0.002
MTMR6	Myotubularin related protein 6	NM_004685	Hs00395064_m1	1.71	0.003
NFKB1†	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)	NM_003998	Hs00231653_m1	1.59	0.000
NHLH1†	Nescient helix loop helix 1	NM_005589	Hs00271582_s1	11.51	0.001

*Genes identified in pilot study.
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Gene symbol	Gene name	Genbank Accession #	TaqMan assay identification #	Fold difference (CFS/normal)	*P* value
NR1D2	Nuclear receptor subfamily 1, group D, member 2	NM_005126	Hs00233309_m1	2.44	0.001
*NTE**	Neuropathy target esterase	NM_006702	Hs00198648_m1	1.7	0.04
NUFIP2	Nuclear fragile X mental retardation protein interacting protein 2	NM_020772	Hs00325168_m1	1.5	0.000
PAPOLA	Poly(A) polymerase alpha	NM_032632	Hs00413685_m1	1.32	0.002
*PDCD2**	Programmed cell death 2	NM_002598	Hs00751277_sH	6.76	0.010
PDCD6	Programmed cell death 6	NM_013232	Hs00737034_m1	1.74	0.000
*PEX16**	Peroxisomal biogenesis factor 16	NM_004813	Hs00191337_m1	1.74	0.003
PGM2	Phosphoglucomutase 2	NM_018290	Hs00217619_m1	2.17	0.000
PIK3R1	Phosphoinositide-3-kinase, regulatory subunit 1 (p85 alpha)	NM_181523	Hs00236128_m1	0.68	0.025
*PKN1**	Protein kinase N1	NM_213560	Hs00177028_m1	1.56	0.000
*POLR2G**	Polymerase (RNA) II (DNA directed) polypeptide G	NM_002696	Hs00275738_m1	2.58	0.008
PPP2R5C	Protein phosphatase 2, regulatory subunit B (B56), gamma isoform	NM_002719	Hs00604902_m1	1.38	0.022
PRKAA1	Protein kinase, AMP-activated, alpha 1 catalytic subunit	NM_006251	Hs01562315_m1	1.72	0.001
PRKAR1A	Protein kinase, cAMP-dependent, regulatory, type I, alpha	NM_002734	Hs00267597_m1	2.63	0.000
PUM2	Pumilio homolog 2 (*Drosophila*)	NM_015317	Hs00209677_m1	1.39	0.001
RAP2C	RAP2C, member of RAS oncogene family	NM_021183	Hs00221801_m1	2.1	0.015
REPIN1[†]	Replication initiator 1	NM_013400	Hs00274221_s1	3.62	0.000
RNF141	Ring finger protein 141	NM_16422	Hs00212656_m1	2.37	0.000
SELENBP1	Selenium binding protein 1	NM_003944	Hs00187625_m1	1.92	0.002
SFXN1	Sideroflexin 1	NM_022754	Hs00224259_m1	1.6	0.022
SHPRH	SNF2 histone linker PHD RING helicase	NM_173082	Hs00542737_m1	1.77	0.05
SNAP23	Synaptosomal-associated protein, 23 kDa	NM_003825	Hs00187075_m1	2.02	0.000
SORL1	Sortilin-related receptor, L(DLR class) A repeats-containing	NM_003105	Hs00268342_m1	1.54	0.000
SOS1	Son of sevenless homolog 1 (*Drosophila*)	NM_005633	Hs00362308_m1	2.31	0.002
TAF11	TAF11 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 28 kDa	NM_005643	Hs00194573_m1	1.87	0.05
TCF3	Transcription factor 3 (E2A immuno-globulin enhancer binding factors E12/E47)	NM_003200	Hs00413032_m1	1.44	0.023
TDP1	Tyrosyl-DNA phosphodiesterase 1	NM_018319	Hs00217832_m1	1.67	0.010
TNFRSF1A	Tumor necrosis factor receptor superfamily, member 1A	NM_001065	Hs00533560_m1	1.37	0.016

*Genes identified in pilot study.
[†]Transcription factors identified by analysis of promoter regions and real-time PCR.
CFS/ME—chronic fatigue syndrome/myalgic encephalomyelitis; PCR—polymerase chain reaction.

Table 1. CFS/ME-associated genes and transcription factors identified through analysis microarrays, real-time PCR, and transcription-factor binding sites *(Continued)*

Gene symbol	Gene name	Genbank Accession #	TaqMan assay identification #	Fold difference (CFS/normal)	*P* value
UBTF	Upstream binding transcription factor, RNA polymerase I	NM_014233	Hs00610729_g1	2.26	0.024
USP38	Ubiquitin specific peptidase 38	NM_032557	Hs00261419_m1	1.71	0.002
WAPAL	Wings apart-like homolog (*Drosophila*)	NM_015045	Hs00386162_m1	1.69	0.027
WDR26	WD repeat domain 26	NM_025160	Hs00228535_m1	2.62	0.000

*Genes identified in pilot study.
†Transcription factors identified by analysis of promoter regions and real-time PCR.
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open-ended screening method (differential display). It found that 4 of 12 PCR-confirmed, CFS-associated transcripts could not be matched to known genes in either the Celera (Rockville, MD) or National Center for Biotechnology Information genomics databases (as of December 2005), which suggests the involvement of novel sequences in CFS. In view of this, we screened CFS patients using a combination of microarrays [13•,18••] 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 mRNAs in the sample. Then, those signature sequences that have bound mRNA attached to them are sequenced while they are still attached to the bead and then used to generate precise numbers of each signature sequence present in the sample. Therefore, all mRNA sequences are detected without prior knowledge of the sequence, and precise copy numbers are generated for each signature sequence. Our strategy in using MPSS was to detect unusual and unpredicted sequences.

The MPSS study was performed using 20 CFS patients diagnosed according to Fukuda criteria and 20 normal blood donors. All total RNA samples from CFS patients were pooled and all total RNA samples from normal blood donors were pooled, resulting in two samples (one CFS and one normal). The two samples were analyzed using MPSS, and the copy numbers for each 17-nucleotide signature sequence between CFS and normal samples were compared. However, the results of MPSS testing did not add to the results of the microarray testing (Kerr, unpublished data).

Specificity of these gene abnormalities to CFS

We are currently in the process of testing disease controls (rheumatoid arthritis, osteoarthritis, and endogenous depression) for these genes in order to determine whether this gene signature is specific to CFS. This is likely to identify some genes that we have found to be differentially expressed in CFS but that are also differentially expressed in other diseases.

Stability of gene signatures (subtype status)

A small subset of patients with CFS who are typical in terms of their disease phenotype (or symptoms) and CFS-associated gene signature is being examined at 13 time points over 1 year at intervals of 1 month. Clinical symptoms and severity will be recorded, gene levels determined, and an attempt will be made to associate particular abnormalities of gene expression with the presence and severity of particular symptoms that occur in CFS. We have already tested three patients at two to four time points (over 2–7 months) and found that their CFS-associated gene signature was stable and that their subtype status did not change during this time (Kerr, unpublished data).

Development of a Diagnostic Test

Diagnostic test for CFS

We have attempted to discover protein biomarkers of CFS using a technique called surface-enhanced, laser-desorption, and ionization time-of-flight mass spectrometry (http://www.ciphergen.com) following a promising pilot study [23]. However, the results were disappointing. At around the same time, we discovered the genomic subtypes of CFS [18••], the existence of which may have confounded the biomarker discovery study, because we were, unknowingly, studying a heterogeneous population in the CFS group. Given the presence of genomic subtypes, it seems most unlikely that a single diagnostic test could now be developed for CFS. However, a diagnostic test for genomic subtype does seem feasible.

Diagnostic test for CFS genomic subtype

Assuming that the genomic subtypes are biologically meaningful, and there is no reason to doubt this at the present time, we need to develop a rapid means of subtype discrimination that can be applied to individuals. At present, in order to assign subtype status to an individual, we must test them for the 88 CFS-associated genes as part of a study including other CFS patients, because we have not yet defined the range for each gene in each subtype.

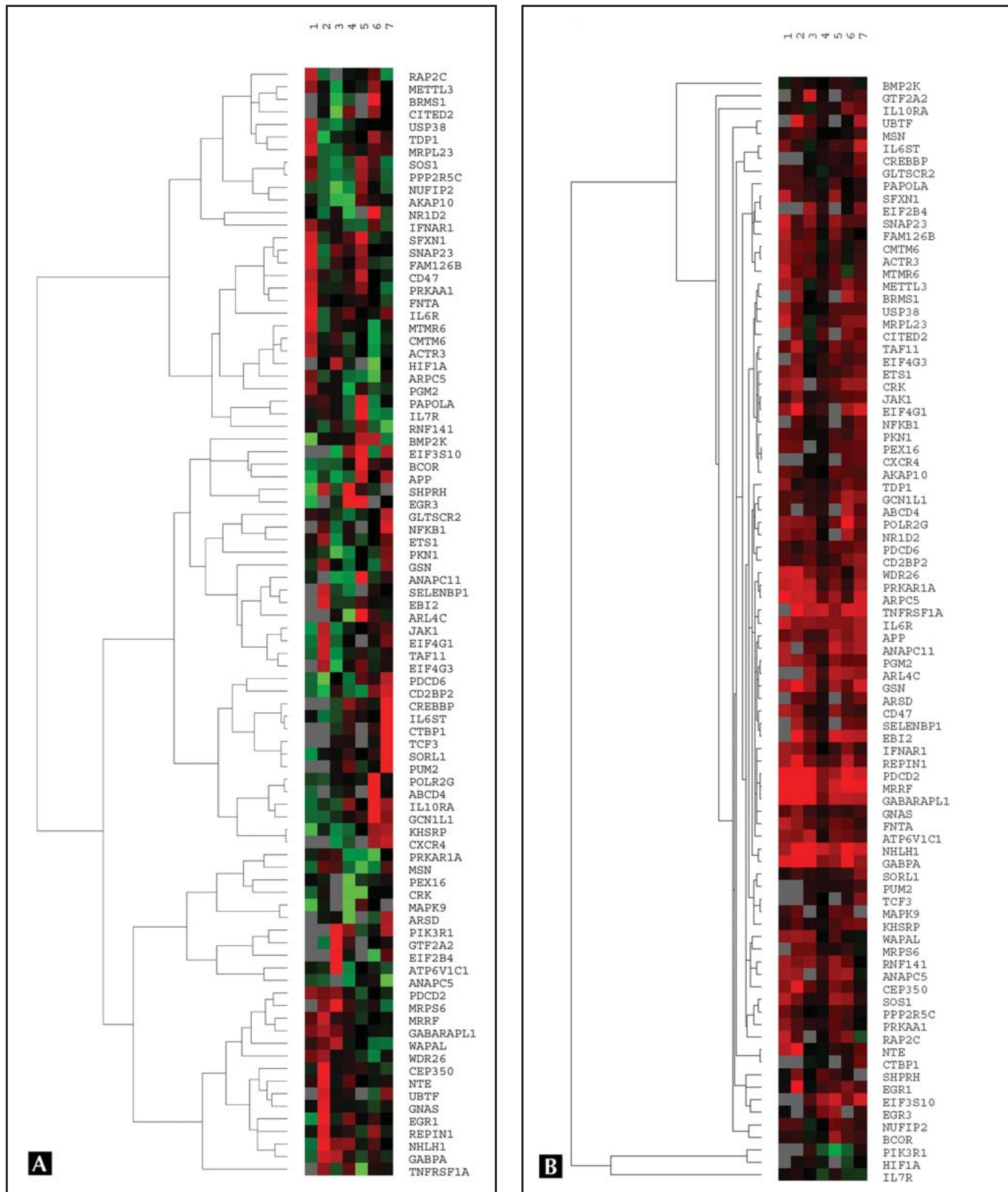

Figure 1. The clustering of normalized (**A**) and median-centered (**B**) fold-difference values (mean RQ in chronic fatigue syndrome/myalgic encephalomyelitis patients divided by mean RQ in normal controls) for each of the 88 chronic fatigue syndrome/myalgic encephalomyelitis–associated genes. RQ—real-time polymerase chain reaction. (*From* Kerr et al. [18••]; with permission.)

One possible approach would be to identify those genes that are most important for subtype discrimination and then to develop these gene expression assays for the purpose of a subtype discriminative test. Another possible approach, assuming that the CFS-associated gene signatures are stable and that they are determined by genetic predisposition,

Table 2. Treatable microbial infections in CFS

Infectious agent	Treatment that has been used for this infection with clinical benefit in CFS patients
Enteroviruses	Interferon-α, inteferon-γ [28•]
Epstein-Barr virus	Valacyclovir [34••], valganciclovir [35••]
Parvovirus B19	Intravenous immunoglobulin [33]
Chlamydia pneumoniae	Tetracyclines, macrolides [30]
Coxiella burnetii	Tetracyclines [36]

CFS—chronic fatigue syndrome.

would be to develop a subtype discrimination test based on single nucleotide polymorphisms (SNPs). If this approach was effective, it would be ideal for testing individuals, because SNP testing is robust, absolute, and reproducible, unlike the relative nature of gene expression testing. We are currently testing all of our subtyped CFS patients for a large number of SNPs located within the 88 CFS-associated genes to discover subtype-specific SNP combinations that could be developed for subtype-discriminative tests.

The Role of Microbial Infections in CFS

Novel microbial infections in CFS

The signature sequences that were found to be differentially expressed between CFS and normal samples in the MPSS study were blasted against the virus database, producing a list of 55 novel mammalian viruses with possible involvement in CFS. PCR primers were designed for nine of these and were used to screen the same cDNA samples. However, all of these PCRs were negative (Kerr, unpublished data).

Identification of the Epstein-Barr virus (EBV) *BRLF1* gene, (encoding the EBV R transactivator) during the analysis of promoter regions of CFS-associated genes to identify overrepresentation of transcription factor binding sites was quite surprising, as antibodies to the related Z transactivator (encoded by the *BZLF1* gene) have previously been detected in a large proportion of CFS patients [24]. However, *BRLF1* mRNA was not detected in either CFS patients or controls (Kerr, unpublished data).

Role of known microbial triggers in particular genomic subtypes of CFS

We hypothesize that particular genomic CFS subtypes represent a specific host response to particular microbial infections that are responsible for triggering and perpetuating the disease in a majority of patients. This is supported by epidemiologic studies showing the importance of infections and the fact that we have found typical CFS-associated human gene signatures in several CFS patients whose disease was triggered several years previously by laboratory-documented acute infection with either EBV or *Coxiella burnetii* [18••,20••] (Kerr, unpublished data).

We are currently looking for evidence of past and current infection with five treatable microbial infections that are well known to trigger and perpetuate CFS. These infectious agents are EBV [25], parvovirus B19 [26], enterovirus [27,28•,29••], *Chlamydia pneumoniae* [30], and *Coxiella burnetii* [31].

Use of Existing and Novel Drugs in CFS/ME

Modulation of human genes

Within the CFS gene signature, there were five human genes that are known to be targeted by one or more existing drugs designed or intended for use in other diseases. Based on the expression levels of these five genes, these drugs may be predicted to be beneficial for particular CFS subtypes. These genes, corresponding drugs, and CFS subtypes are as follows: *APP*, bapineuzumab (an anti–Alzheimer's disease drug), and subtypes 1, 2, 3, 4, 5, 6, and 7; *CXCR4*, JM1300 (originally developed as an anti-HIV drug), and subtypes 5 and 6; *FNTA*, lonafarnib and tipifarnib (anticancer drugs), and subtypes 1, 2, 3, 5, and 6; *IL6ST*, tocilizumab (anti–rheumatoid arthritis drug), and subtypes 1, 2, 5, 6, and 7; *TNF*, golimumab, adalimumab, etanercept, certolizumab pegol, infliximab (anti–rheumatoid arthritis drugs), and subtype 2.

One tumor necrosis factor-α inhibitor (etanercept) has been used with considerable benefit in the treatment of six patients with CFS in a pilot study [32]. Unfortunately, this trial was not published as a paper but only as a meeting abstract. The use of tumor necrosis factor-α inhibitors in CFS is strongly supported by scientific data on the immune responses in CFS, epidemiologic data, and now data from gene expression studies [18••]. It is an urgent priority to repeat this work and carry out a larger clinical trial of etanercept in patients with CFS. We had hoped to undertake trials of the use of etanercept and interferon-β in CFS patients, but were not successful in obtaining funding because of concerns that the adverse effects of interferon-β may mimic CFS.

Inhibition of microbial metabolism

There are several infectious agents that are known to trigger and perpetuate CFS that have also been targeted with antimicrobial therapy. In some of these instances, there has been clear evidence of clinical benefit or cure in infected CFS patients, for example, CFS with evidence of infection with enteroviruses [28•,29••], parvovirus B19 [33], EBV [34••,35••], *Chlamydia pneumoniae* [30], and *Coxiella burnetii* [36] (Table 2). We are currently conducting a study on the role of these five treatable microbial infections in particular genomic subtypes of CFS.

Conclusions

Progress is being made toward an understanding of the pathogenesis of this intriguing and devastating disease.

Once we understand the pathogenesis and have a means by which to determine subtype status and the presence of any treatable microbial infections, it is likely that the route to specific and curative treatments will quickly become clear.

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Disclosure

No potential conflict of interest relevant to this article was reported.

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