Microbial infections in eight genomic subtypes of chronic fatigue syndrome/myalgic encephalomyelitis

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ABSTRACT

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Background The authors have previously reported genomic subtypes of chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) based on expression of 88 human genes.

Aim To attempt to reproduce these findings, determine the specificity of this signature to CFS/ME, and test for associations between CFS/ME subtype and infection. Methods Expression levels of 88 human genes were determined in blood of 62 new patients with idiopathic CFS/ME (according to Fukuda criteria), six patients with Q-fever-associated CFS/ME from the Birmingham Q-fever outbreak (according to Fukuda criteria). 14 patients with endogenous depression (according to DSM-IV criteria)

and 29 normal blood donors. Results In patients with CFS/ME, differential expression was confirmed for all 88 genes. Q-CFS/ME had similar patterns of gene expression to idiopathic CFS/ME. Gene expression in patients with endogenous depression was similar to that in the normal controls, except for upregulation of five genes (APP, CREBBP, GNAS, PDCD2 and PDCD6). Clustering of combined gene data in CFS/ME patients for this and the authors' previous study (117 CFS/ME patients) revealed genomic subtypes with distinct differences in SF36 scores, clinical phenotypes, severity and geographical distribution. Antibody testing for Epstein-Barr virus, enterovirus, Coxiella burnetii and parvovirus B19 revealed evidence of subtype-specific relationships for Epstein-Barr virus and enterovirus, the two most common infectious triggers of CFS/ME. Conclusions This study confirms the involvement of these genes in CFS/ME.

INTRODUCTION

Chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) is a disease characterised 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 inter-relate in patients with CFS/ME remain to be clarified.^{4 5}

Various groups have analysed the gene expression in peripheral blood of patients with CFS/ME, and, in all of these studies, genes of immunity and defence are prominent. Following a pilot microarray study which identified 16 abnormally expressed genes in CFS/ME,⁶ we reported on a comprehensive microarray study which reveals abnormal expression of 88 human genes in patients with CFS/ME.⁷ Clustering of these data revealed seven genomic subtypes of CFS/ME with distinct differences in SF36 scores, clinical phenotypes, severity and geographical distribution.⁷ ⁸ However, remaining questions relate to reproducibility and the specificity of these gene abnormalities to CFS/ME and possible associations with infectious agents.

In this study, we set out to determine whether these findings were reproducible in fresh subjects, whether the previously reported dysregulation of these genes also occurred in drug-free patients with endogenous depression, and whether there was any relationship between particular microbial infections and CFS/ME genomic subtype. The results show that these findings are reproducible and that gene expression in patients with endogenous depression was markedly different from that in patients with CFS/ME, and was similar to that in the normal controls, in terms of these 88 human genes. Also, clustering of gene data revealed eight genomic subtypes with distinct clinical differences, and several of these had interesting associations with particular microbial infections.

METHODS

Subject enrolment, clinical characterisation and blood sampling

Patients with CFS/ME (n=62), who lived in Birmingham (n=6), Bristol (n=3), London (n=9)and New York (n=44), were diagnosed according to Fukuda diagnostic criteria for CFS/ME¹ and enrolled into the study. All had idiopathic CFS/ME except the six Birmingham patients, who had CFS/ME that had been triggered by laboratory-documented Q fever. Patients with psychiatric disease were excluded using the Minnesota International Neuropsychiatric Interview, thus ensuring that none of our CFS/ME patients had major psychiatric disease or misused alcohol or other drugs. Clinical and quantitative PCR (qPCR) data for these new patients were combined with those for 55 CFS/ME patients from a previous study,^{7 8} giving a total of 117 CFS/ME patients, who lived in Birmingham (n=6), Bristol (n=14), Leicester (n=1), London (n=12), New York (n=55) and Dorset (n=28).

Patients with endogenous depression (n=14) were enrolled from Bristol, UK, and surrounding area. These patients fulfilled Diagnostic and statistical manual of mental disorders, 4th edition (DSM-IV) criteria, had not smoked within the previous year, and had not taken antidepressants in the previous year.

Healthy normal blood donors enrolled from the Dorset National Blood Service (n=29) were used as a comparison group. Restrictions imposed by the Dorset National Blood Service on those allowed to donate blood are outlined elsewhere.⁶

For all patient groups, individuals who smoked in the previous year, who abused alcohol or other drugs, or were currently taking (or were within 3 months of taking) antibiotics, steroids, cytotoxic drugs or antidepressants were excluded from the study.

For all enrolled subjects (patients and controls), according to the recommendations of the International CFS Study Group,⁹ severity of physical and mental fatigue was assessed using the Chalder Fatigue Scale,¹⁰ level of disability was assessed using the Medical Outcomes Survey Short Form-36 (SF36), accompanying symptoms were characterised using the Somatic and Psychological Health Report, sleep abnormalities were assessed using the Pittsburgh Sleep Questionnaire, and assessment of type and severity of pain was performed using the McGill Pain Questionnaire.

Patients and controls gave informed written consent according to guidance of the Wandsworth Research Ethics Committee (approval number 05/Q0803/137). For the New York patients, approval of the local institutional review board was obtained. The human experimentation guidelines of the US Department of Health and Human Services were followed in this study.

A 2.5 ml sample of blood was taken from both CFS/ME patients and normal blood donors (as part of routine blood donation) into PAXgene tubes (PreAnalytix, Qiagen, UK), and total RNA extracted using the PAXgene blood RNA kit (PreAnalytix), according to the instructions of the manufacturer. RNA quality and amount were confirmed by micro-spectrophotometry (Nanodrop, Rockland, Delaware, USA). Total RNA samples used in this study had an absorbance ratio (A_{260}/A_{280}) of 1.9–2.0.

Quantitative PCR

qPCR (Applied Biosystems, Foster City, California, USA) was used to quantitate the amount of mRNA for 88 CFS/ME-associated human genes by the comparative method, using custom 384-well low-density arrays and the ABI PRISM 7900HT instrument (Applied Biosystems), with glyceraldehyde-3-phosphate dehydrogenase as the endogenous control gene. Experiments were performed in triplicate using the protocol described previously.⁶ ⁷ Data were displayed using SDS v2.2 software (ABI), discordant data between replicates were omitted, and results for each low-density array were calculated and loaded into ABI SDS v2.2 Enterprise Edition software.

The threshold cycle (Ct) for each test gene in each sample was compared with that for glyceraldehyde-3-phosphate dehydrogenase to calculate a Δ Ct value. Δ Ct values were then normalised to the calibrator sample to give the Δ ACt values. Relative quantities (RQ) (2^{$-\Delta\Delta$ Ct}) of each mRNA of interest were then calculated. Samples showing a difference between minimum and maximum RQ values of \geq 100 (indicating poor replicate concordance) were excluded. The t test was used to compare mean RQ values between groups. p \leq 0.05 was taken to be significant.

Clustering of qPCR-generated gene values of CFS/ME patients

Ct values for all 88 CFS/ME-associated genes in 117 CFS/ME patients were then normalised and clustered using Genesis software.¹¹ For each of the eight CFS/ME subtypes identified using this approach, mean RQ values were calculated for each gene, and used to generate fold-difference (CFS/ME/normal) values for each gene in each CFS/ME subtype. Mean fold-difference values for each gene in each CFS/ME subtype were then

clustered with and without normalisation/median centring using Cluster v2.11 software and visualised using Treeview v1.60 software.¹² The clustering algorithm in both of these software programs has been described previously.¹²

Detection of anti-microbial antibodies

IgM and IgG antibodies specific to four microbes that are well recognised to trigger CFS/ME were detected by ELISA, according to the manufacturer's instructions: Epstein-Barr virus (EBV) (viral capsid antigen (VCA) IgM and IgG, early antigen IgG and Epstein-Barr nuclear antigen (EBNA) IgG; Meridien Bioscience Inc, Cincinnati, Ohio, USA), enterovirus (all serotypes; Virion Serion, Wurzberg, Germany), parvovirus B19 (viral protein 2 IgM and IgG; Biotrin, Dublin, Ireland) and *Coxiella burnetii* (phase I and II IgG; Virion Serion).

Statistical testing

Testing of the significance of associations of gene expression levels with different patient groups was performed using a two-tailed t test. Testing of the significance of association between clinical variables and CFS/ME genomic subtype was performed using χ^2 , analysis of variance (ANOVA) and the Mann–Whitney U tests. Testing of the significance of association between microbial markers in CFS/ME and CFS/ME subtypes was performed using χ^2 analysis and ANOVA.

RESULTS

Subjects and clinical characterisation

A total of 117 patients with CFS/ME fulfilling Centers for Disease Control diagnostic criteria were used in this study. For 55, previously published data were used, while the remaining 62 had not previously been tested; for six of these, CFS/ME disease had been triggered by laboratory-documented *C burnetii* infection. In addition, 14 patients with endogenous depression and 29 normal blood donors were studied.

A summary of the clinical details of these subjects is shown in table 1. In general, all CFS/ME groups had similar profiles of symptoms and mean clinical scores, and Q-CFS/ME was phenotypically similar to the other CFS/ME cases in which the triggering factors were unknown. Patients with endogenous depression had a markedly low prevalence of numbness/tingling and tender lymphadenopathy, and less bodily pain, as indicated by the McGill Pain Questionnaire mean score, as compared with CFS/ME. Normal blood donors had very low prevalence of all symptoms, little fatigue (Chalder), pain (McGill), associated symptoms (Somatic and Psychological Health Report), normal sleep (Pittsburgh Sleep Questionnaire Index) and high SF36 total scores (table 1), as would be expected.

Quantitative PCR

qPCR was carried out using TaqMan primers/probes specific for 88 human genes that were previously found to be differentially expressed in CFS/ME patients.⁷ This analysis confirmed that most of these genes differed significantly between CFS/ME and the normal group. Of the 88 genes, 84 were found to be upregulated and four were downregulated (*HIF1A, IL7R, PAPOLA, SHPRH*), which is similar to what we reported previously.⁷ Gene expression in patients with Q-CFS/ME was also found to be markedly different from the normal group, and very similar to that found in patients with CFS/ME. Gene expression in patients with endogenous depression did not differ markedly from that in the normal group, except in the case of five genes (*APP, CREBBP, GNAS, PDCD2, PDCD6*), where significant upregulation (fold difference \geq 1.5) was found (table 2).

Variable	CFS/ME patients in previous study ⁷ (n=55)	CFS/ME patients, previously untested (n = 56)	Q-CFS/ME patients* (n=6)	All CFS/ME patients (n = 117)	Patients with endogenous depression (n=14)	Normal blood donors (n=29)
Gender (M:F)	19:36	10:46	6:0	35:82	4:10	14:15
Mean age (years)	41.6	40.25	41.5	41.3	41.36	44.6
Mean duration of disease (years:months)	3.17	2.9	5.7	3.4	0:6	NA
Symptoms/signs						
Headache	26	30	1	57	5	1
Sore throat	27	29	0	56	1	1
Poor memory/concentration	30	46	4	80	11	3
Muscle pain	37	42	6	85	5	2
Muscle weakness	36	31	5	72	2	1
Joint pain	41	52	6	99	8	1
Post-exertional malaise	47	54	5	106	9	2
Sleep problem	44	24	0	68	4	3
Gastrointestinal problems	35	36	2	73	6	1
Fainting/dizziness	25	45	5	75	8	1
Numbness/tingling	24	25	2	51	1	0
Tender lymphadenopathy	27	22	2	51	0	0
Mean scores						
Physical fatigue (Chalder)	16.13	14.36	10.83	15.15	14.00	7.69
Mental fatigue (Chalder)	8.05	7.34	6.00	7.98	7.42	4.24
McGill Pain Questionnaire	15.28	18.57	18.80	17.58	9.67	2.48
Sphere questionnaire	11.25	11.21	7.33	10.87	12.45	2.07
SF36 questionnaire	46.45	38.65	52.85	45.12	46.19	83.61
Pittsburgh Sleep Quality Index	10.22	10.00	8.17	10.01	12.25	4.28

 Table 1
 Patient information including age, sex, symptoms and questionnaire results summarising fatigue severity, pain, sleep, general function and associated symptoms for patients with CFS/ME and normal blood donors enrolled in microarray and real-time PCR studies, respectively

*These six Q-CFS/ME patients were all part of the 1989 Birmingham Q-CFS/ME outbreak cohort.

CFS/ME, chronic fatigue syndrome/myalgic encephalomyelitis; NA, not applicable; Sphere, Somatic and Psychological Health Report.

Genomic CFS/ME subtypes

Clustering of Δ Ct values for the 88 CFS/ME-associated genes in the 117 CFS/ME patients identified eight subtypes (designated A–H), consisting of 27, 6, 19, 5, 21, 13, 19 and 4 CFS/ME patients, respectively. There were three patients whose gene profile did not fit into any of these eight subtype groupings. Mean fold-difference values for each CFS/ME subtype are shown in table 3 and figure 1. Most genes in each subtype were shown to be upregulated (figure 1 and table 3).

The relationship between the subtypes of the present study and those of the previous study which examined only 55 CFS/ME patients^{7 8} may be difficult to determine. As these subtypes are derived by using clustering, which finds similar groups on the basis of gene expression values, there is no means to predict the outcome of the clustering. As there was incomplete preservation of the previous CFS/ME patient groupings in the present study, we have designated the subtypes, A–H, to distinguish them from those of the previous study, which were designated 1–7.⁷

Analysis of sex ratios for each subtype reveals that subtype D is made up of females only, subtype H is made up of equal numbers of males and females, and the remaining subtypes are made up predominantly of females.

It is particularly interesting that five of six CFS/ME patients with Q-CFS/ME clustered in the same subtype (subtype A).

The clinical phenotype was distinct between subtypes. Subtype D was the most severe, having the lowest scores for SF36 modules RP, VIT, GH, BP and total score, and the highest frequency of occurrence of muscle pain and sleep problems (see figure 2 for definitions of abbreviations for SF36 modules). Subtype B was the least severe, having the highest scores for SF36 modules RP, GH, MH and total score. Subtype B had a higher median score for the SF36-RP (physical role) than all the others combined (87.5 vs 0), p=0.04; Mann–Whitney U test). However, subtype B had the highest frequency of cognitive dysfunction, muscle weakness and post-exertional malaise. Subtype B showed a higher frequency of cognitive dysfunction than all non-subtype B patients combined (p=0.03) and showed an increased severity and duration of headache compared with all non-subtype B patients combined (p=0.02). Subtype B also had a higher median score for mental fatigue (Chalder scale) than all non-subtype B patients combined, although this did not reach significance (9.5 vs 7.0; p=0.06). Subtypes B and C had the best mental health scores, and subtypes A and F had the worst (figure 2A,B).

Subtype E had a higher median score for SF36-VIT than all the others combined (35.0 vs 15.0; p=0.05; Mann–Whitney U test). Subtype E resulted in the highest frequency of gastrointestinal problems. Patients of subtype F showed a higher frequency of increased severity of numbness/tingling compared with all non-subtype F patients combined (p=0.03). Patients of subtype H showed an increased frequency of severity of sore throat compared with all non-subtype H patients combined (p=0.01) (figure 2A,B).

As regards possible association of subtype with geographical location, there was evidence to support this, as we found previously.⁷ Predominant subtypes in each geographical location were as follows: Birmingham, subtype A; Bristol, subtype C;

Table 2 CFS/ME-associated genes and transcription factors in patients with CFS/ME, Q-fever-associated CFS/ME and endogenous depression

accession accession peak peak peak peak peak ABD2+ MAD23-SM_011 2.7 0.0 0.031 0.031 0.2 0.7 AGAPA MK 00721 Hod018857 n1 5.2 0.01 6.28 0.031 1.16 0.3 AGAPC1 MK 01723 Hod018857 n1 5.2 0.01 5.2 0.031 1.5 0.24 AMAPC13 MK 01623 Hod21250 n1 2.44 0.0003 2.3 0.01 1.5 0.22 AMAPC MK 01573 Hod22120 n1 1.42 0.0001 1.45 0.002 1.65 0.003 2.33 0.17 1.73 0.32 AMAP MK 019185 Hod25368 n1 1.65 0.01 1.45 0.03 1.77 0.14 1.18 0.23 AMAP MK 019185 Hod018455 n1 3.65 0.001 4.31 0.18 0.33 AMAP MK 019185 Hod018452 n1 3.65 0.001 1.41		GenBank		CFS/ME (n=111)		Q-CFS/ME (n=	=6)	Endogenous depression (n=14)	
ABCDV* NM J20232 Hu02r530 ml J.2.1 0.01 5.01 0.01 1.2 0.57 ARAPR NM 00222 Hu011S87285 ml 1.2.5 0.002 1.7 0.04 1.2 0.7 ARAPC11* NM 002224 Hu011S87285 ml 1.2.5 0.006 1.47 0.002 1.16 0.37 ARAPC13* NM 0157275 ml Hu012S1270 ml 2.25 0.006 1.22 0.001 1.32 0.321 ARAPC NM 015777 Hu012S500 gl 1.71 0.00001 5.25 0.0021 1.33 0.621 ARAVC NM 001584 Hu002S500 gm 1.37 0.00008 2.32 0.0071 1.38 0.421 ARAVC NM 001584 Hu002S500 gm 1.37 0.00004 2.32 0.0071 1.38 0.423 ARAVC NM 001584 Hu002S500 gm 1.38 0.0002 2.43 0.037 1.38 0.138 ARAVC NM 001583 Hu002S500 gm 1.38 <th0.0002< th=""> 4.0</th0.0002<>	Gene symbol	accession number	Taqman assay ID‡	Fold difference	p Value	Fold difference	p Value	Fold difference	p Value
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ALMAP NM 00222 HoD18372 HoD12372 N 5.22 0.01 6.28 0.031 1.18 0.337 ALMAPC11* NM 015271 HoD012120 m1 2.57 0.0001 5.28 0.001 1.52 0.321 ALMAPC NM 015771 HoD025050 m1 1.42 0.0001 5.28 0.0071 1.48 0.74 ALMAPC NM 005777 HoD025702 m1 1.52 0.0001 2.58 0.0071 1.38 0.74 ALMAP NM 001595 HoD025702 m1 2.58 0.001 1.65 0.051 1.33 0.125 BCDR NM 013939 HoD027405 m1 3.58 0.0002 2.43 0.001 1.48 0.135 BCDR NM 013939 HoD027305 m1 3.58 0.0002 2.43 0.001 1.43 0.33 CDF29 NM 0.00139 HoD027305 m1 3.58 0.0011 4.51 0.021 1.32 0.33 <td>ACTR3</td> <td>NM 005721</td> <td>Hs00828586 m1</td> <td>13.53</td> <td>0.0029</td> <td>17.77</td> <td>0.04</td> <td>1.22</td> <td>0.72</td>	ACTR3	NM 005721	Hs00828586 m1	13.53	0.0029	17.77	0.04	1.22	0.72
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ABSD NM 0.06689 H-00054862 nl 1.22 0.001 1.55 0.021 1.23 0.75 BCOR NM 0.0119455 H-000180258 nl 1.90 0.0045 2.37 0.014 1.12 0.128 BRM2X NM 0.015999 H-00323058 nl 3.18 0.0002 2.43 0.011 1.44 0.334 CDAP2Y NM 0.015739 H-00323058 nl 3.38 0.0007 2.60 0.011 1.43 0.025 CDAP2Y NM 0.016079 H-0032508 3.38 0.0007 2.60 0.011 1.38 0.026 CPESSO NM 0.016079 H-00326666 nl 5.28 0.000031 6.21 0.016 0.025 0.16 0.026 CPESSO NM 0.01380 H-00012908 1.34 0.017 - - - - - - - - - - - - - <t< td=""><td>ARPC5</td><td>NM_005717</td><td>Hs00271722_m1</td><td>3.71</td><td>0.000008</td><td>4.26</td><td>0.0047</td><td>1.46</td><td>0.49</td></t<>	ARPC5	NM_005717	Hs00271722_m1	3.71	0.000008	4.26	0.0047	1.46	0.49
Alfrey/IC1 NM, 01985 He0014825, ml 2.66 0.0009 2.03 0.021 1.23 0.028 BMD2/* NM, 019859 He002305, ml 3.08 0.011 4.27 0.007 1.09 0.28 BMD2/* NM, 019898 He002305, ml 3.08 0.00004 5.15 0.01 1.44 0.328 CD28P2* NM, 019793 He0013935, ml 3.38 0.0007 2.60 0.022 1.07 0.125 CP730 NM, 01810 He0038305, ml 5.28 0.0001 6.49 0.01 1.36 0.022 CPFEZ NM, 01801 He0038408, ml 3.61 0.014 6.31 0.025 1.61 0.021 CPFEZ NM, 01932 He0038308, ml 3.61 0.00044 1.29 0.002 1.41 0.022 1.45 0.033 1.40 0.828 CPFEZ NM, 004951 He0019822, ml 1.59 0.022 2.13 0.021 1.45 0.025 1.33 0.55	ARSD	NM_001669	Hs00534692_m1	1.62	0.001	1.65	0.05	1.07	0.133
BL/H NMI HBUIZ 409 HB LOP LJ UDMP LJ UDMP LMB UDZ BMMZX NMI 19839 H6U024005 NI 3.08 UDMDZ 2.43 UDM1 1.28 UDM2 BMMX1 NMI 060072 2.40 UDM1 1.28 UDM2 CDF2 NMI MI 160072736 min 3.83 UDM07 2.60 UDM1 1.36 0.833 CFEDZ NM 160040274 min 3.86 UDM07 4.033 UCM2 0.44 0.31 0.44 0.42 0.44 0	ATP6V1C1	NM_001695	Hs00184625_m1	2.66	0.0009	2.03	0.021	1.23	0.75
DBMC31 NML DBMC31 NML D12 D13 D14 D13 D13 D14 CD2BP2* NML D05110 HB00232026 m1 3.23 D.0007 2.60 D.001 1.44 D.334 CD2BP2* NML D6110 HB002702 m1 3.85 D.0007 2.60 D.002 1.07 D.25 CCP350 NML D601010 HB002705 m1 3.26 D.000031 6.21 D.046 1.21 D.045 CFED2 NML D605707 m1 3.61 D.014 6.31 D.046 1.21 D.025 CRK NML D03867 HB007375 m1 1.36 D.071 4.16 D.021 1.45 D.022 CRF NML D0387 HB0075925 m1 1.38 D.0070 2.813 D.007 1.55 D.134 CRF NML D0487 HB0075925 m1 1.58 D.027 2.13 D.046 1.33 D.55 CRF NML D0411 <td>BLUK</td> <td>NIVI_UT7745</td> <td>HSUU372369_m1</td> <td>1.90</td> <td>0.0045</td> <td>2.37</td> <td>0.007</td> <td>1.09</td> <td>0.28</td>	BLUK	NIVI_UT7745	HSUU372369_m1	1.90	0.0045	2.37	0.007	1.09	0.28
Jamos MM_001333 Houd230304_min 3.06 Cuade 5.15 0.01 1.44 0.334 CD47 MM_01873 HouD17955_m1 3.38 0.0007 2.60 0.001 1.44 0.334 CD47 MM_01873 HouD27206 1.35 0.001 6.49 0.01 1.36 0.002 CTED2 MM_008079 HouD2305 1.528 0.000031 6.21 0.049 1.33 0.172 CREAP MM_01380 HouD21703 1.36 0.014 6.31 0.045 1.21 0.045 CREA MM_004380 HouD31792_m1 5.13 0.071 4.16 0.022 1.63 0.071 1.55 0.13 0.021 1.65 0.13 0.657 CFRH MM_004951 HouD21706392_m1 1.16 0.0024 1.13 0.042 1.33 0.657 CFRH MM_004951 HouD21706392_m1 2.10 0.017 - - - - - - - <td< td=""><td>BIVIPZK DDMS1*</td><td>NM 015200</td><td>HSUU214079_M1</td><td>8.05</td><td>0.01</td><td>14.27</td><td>0.04</td><td>1.18</td><td>0.125</td></td<>	BIVIPZK DDMS1*	NM 015200	HSUU214079_M1	8.05	0.01	14.27	0.04	1.18	0.125
DLDB.* IML_DOI 10 INDUDUCT 1.1.2 DUBUT INT_DOI 10 IDDU 1200 CEP320 NM_014810 H5001775 1.3.3 D.0007 2.61 D.041 1.5.6 D.025 CEP320 NM_014810 H5001775 1.3.61 D.014 6.31 D.046 1.21 D.046 CMTMA NM_00587 H5001805 1.3.71 7.12 D.02 9.22 D.025 1.61 D.021 CMTMA NM_00582 H50018015 1.3.1 D.014 6.31 D.046 1.21 D.046 CRF NM_00132 H5001977 1.13.46 D.00009 28.13 D.007 1.65 D.128 CRF NM_001951 H50050776 1.1 0.46 D.0022 1.13 D.047 D.026 1.13 D.047 D.026 1.33 D.05 D.238 EB2 NM_004421 H5005077 1.1 2.10 D.0024 1.55 D.067 1.29 D.255 EF31 NM_005		NM_006110	Hs00303030_III1 Hs00272036_m1	3.00	0.0002	2.43 5.15	0.037	1.20	0.194
CPF35D NM_D14810 Hs0040777_m1 3.85 0.001 6.49 0.01 1.36 0.803 C/TE22 NM_008079 Hs005686 m1 5.28 0.000031 6.21 0.044 1.33 0.172 C/TE22 NM_008079 Hs002103737 m1 3.61 0.014 6.31 0.046 1.21 0.046 C/REBP NM_004380 Hs0021732 m1 1.51 0.071 4.16 0.02 1.40 0.683 C/RF NM_00451 Hs007992 m1 1.68 0.00044 1.29 0.001 1.68 0.683 C/RF NM_00451 Hs007972 m1 5.99 0.002 2.518 0.007 1.05 0.128 E6/R NM_00451 Hs0023780 m1 2.11 0.017 - <td< td=""><td>CD2Di 2 CD47</td><td>NM 198793</td><td>Hs00179953_m1</td><td>3 38</td><td>0.000004</td><td>2.60</td><td>0.001</td><td>1.44</td><td>0.334</td></td<>	CD2Di 2 CD47	NM 198793	Hs00179953_m1	3 38	0.000004	2.60	0.001	1.44	0.334
CHED2 NM_006079 H+00366695,m1 5.28 0.000031 6.21 0.049 1.33 0.172 CMTMB6 NM_017801 H+00251033,m1 7.02 0.02 9.82 0.025 1.61 0.045 CRK NM_016323 H+001987273,m1 7.02 0.02 9.82 0.0003 1.40 0.683 CRF NM_016323 H+001982778,s1 1.3.4 0.00004 1.29 0.0003 1.40 0.683 CRF NM_00455 H+00192023,s1 1.3.4 0.0002 2.1.6 0.011 0.8 0.683 CRR NM_00455 H+00152028,m1 2.61 0.017 - <th< td=""><td>CEP350</td><td>NM 014810</td><td>Hs00402774 m1</td><td>3.85</td><td>0.001</td><td>6.49</td><td>0.01</td><td>1.36</td><td>0.803</td></th<>	CEP350	NM 014810	Hs00402774 m1	3.85	0.001	6.49	0.01	1.36	0.803
CMTMB NM_017801 Hs00215083_m1 3.61 0.014 6.31 0.046 1.21 0.405 CREBP NM_016820 Hs0010181_m1 1.02 0.02 9.82 0.0025 1.61 0.021 CRF NM_01238 Hs0010917982_m1 5.13 0.071 4.16 0.02 1.45 0.134 CRP1 NM_00451 Hs00270539_s1 5.13 0.001 4.16 0.02 1.33 0.657 EBR NM_00461 Hs00270539_s1 5.99 0.002 2.616 0.011 0.88 0.877 EGR1 NM_00461 Hs00231780_m1 2.11 0.017 - - - - - - 1.61 0.165 EGR1 NM_00376 Hs00191833_m1 2.47 0.0007 0.34 0.035 1.16 0.165 EF463 NM_00328 Hs0095158_m1 3.19 0.001 3.757 0.055 1.09 0.761 FAM128B NM_173822 Hs0055158_m1 3.18	CITED2	NM_006079	Hs00366696_m1	5.28	0.000031	6.21	0.049	1.33	0.172
CREEBB NM_00489 Hb00231733_m1 7.02 0.02 9.82 0.025 1.61 0.021 CIW NM_016823 Hb001982_m1 1.58 0.000044 1.29 0.0003 1.40 0.683 CIPI NM_004567 Hb0007978_s1 1.34.6 0.00002 28.13 0.007 1.05 0.128 EDR NM_004555 Hb00152228_m1 1.69 0.03 0.34 0.026 1.33 0.65 EOR NM_00421 Hb0021780_m1 2.87 0.0026 1.13 0.048 0.58 0.739 EFR3 NM_003750 Hb00186707_m1 2.47 0.0026 1.13 0.048 0.58 0.739 EFR31 NM_003760 Hb0018604_m1 2.17 0.00012 3.22 0.007 1.35 0.83 EFX1 NM_00232 Hb00545158_m1 3.19 0.01 5.52 0.00 1.26 0.966 EFX4 NM_102321 Hb0074468_s1 5.27 0.00042 1.83 0.00	CMTM6	NM 017801	Hs00215083 m1	3.61	0.014	6.31	0.046	1.21	0.405
CRK NM_001328 H500189418_m1 198 0.000044 1.29 0.0003 1.40 0.683 C7BP1 NM_001328 H50017932_s1 5.13 0.071 4.16 0.002 1.45 0.128 CRCR4 NM_004951 H500270639_s1 5.99 0.002 26.16 0.011 0.88 0.887 EBR NM_004421 H50023780_m1 2.11 0.017 - <	CREBBP	NM 004380	Hs00231733 m1	7.02	0.02	9.82	0.025	1.61	0.021
CTBP1 NM_003467 Hs0076922_m1 5.13 0.071 4.16 0.02 1.45 0.134 CXGR4 NM_003467 Hs0026793_s1 5.99 0.002 26.16 0.011 0.88 0.887 EGR1 NM_001955 Hs0015228_m1 1.69 0.03 0.34 0.025 1.33 0.65 EGR3 NM_0043750 Hs0018970_m1 2.10 0.0034 1.55 0.067 1.29 0.295 EFA310 NM_003750 Hs00186804_m1 2.17 0.0007 0.34 0.055 1.69 0.761 EFA31* NM_003760 Hs00186804_m1 2.17 0.0017 2.32 0.007 1.35 0.35 EFA31 NM_00238 Hs0001425_m1 3.08 0.0007 2.30 0.001 1.24 0.256 EFA31 NM_00237 Hs0035773 pm1 3.86 0.0007 2.30 0.001 1.41 0.252 GABAAPLY NM_002031 Hs00745591_s1 5.57 0.00042 1.83	CRK	NM 016823	Hs00180418 m1	1.98	0.000044	1.29	0.0003	1.40	0.683
CXCPA NM_004457 H=00607978_s1 12.4.6 0.0009 28.13 0.007 1.05 0.128 EB/Z NM_004951 H=000270639_s1 5.99 0.002 26.16 0.011 0.88 0.6677 EGR1 NM_00421 H=00231780_m1 2.11 0.017 - - - - EZ244* NM_003750 H=0024894_m1 2.17 0.0026 1.13 0.048 0.58 0.739 EFX310 NM_003750 H=0016804_m1 2.47 0.00012 3.22 0.0079 1.35 0.83 EF461* NM_05238 H=0016804_m1 2.17 0.00012 3.22 0.007 1.24 0.036 EFA3 NM_05238 H=00164468_s1 5.77 0.030 1.24 0.255 GABARAPL* NM_0314 H=0057739_s11 3.86 0.0007 2.30 0.011 1.24 0.254 GABARAPL* NM_002031 H=00745591_s1 1.54 0.0001 1.91 0.027 1.12	CTBP1	NM_001328	Hs00179922 m1	5.13	0.071	4.16	0.02	1.45	0.134
EH2 NM_004951 Hs00270639_s1 5.99 0.02 26.16 0.01 0.88 0.687 EGR1 NM_00421 Hs00231780_m1 1.69 0.03 0.34 0.026 1.33 0.65 EGR3 NM_00421 Hs00231780_m1 2.11 0.017 - - - - EFZ44* NM_103750 Hs0016907_m1 2.17 0.0026 1.13 0.048 0.58 0.295 EF461* NM_003760 Hs0018684_m1 2.17 0.00012 3.22 0.0075 1.35 0.83 EF31 NM_00538 Hs0001425_m1 3.86 0.0001 1.91 0.52 0.33 1.26 0.906 FVTA NM_002031 Hs00745591_s1 5.27 0.00042 1.83 0.001 1.24 0.254 GABPA NM_002031 Hs00745591_s1 5.49 0.0016 5.33 0.33 1.47 0.52 GABPA NM_002031 Hs00745591_s1 5.40 0.0001 1.91	CXCR4	NM_003467	Hs00607978_s1	13.46	0.00009	28.13	0.007	1.05	0.128
EGR1 NM_001955 Hs00152282 m1 1.69 0.03 0.24 0.026 1.33 0.65 EGR3 NM_00421 Hs0013780 m1 2.11 0.017 -	EBI2	NM_004951	Hs00270639_s1	5.99	0.002	26.16	0.011	0.88	0.687
EGR3 NM_004421 Hs0021780_m1 2.11 0.017 - E/// A// ANM <td>EGR1</td> <td>NM_001955</td> <td>Hs00152928_m1</td> <td>1.69</td> <td>0.03</td> <td>0.34</td> <td>0.026</td> <td>1.33</td> <td>0.65</td>	EGR1	NM_001955	Hs00152928_m1	1.69	0.03	0.34	0.026	1.33	0.65
EIF284* NM, 172195 Hs00248984, m1 2.87 0.026 1.13 0.048 0.58 0.739 EIF3S10 NM_003750 Hs00186707,m1 2.10 0.0034 1.55 0.067 1.29 0.295 EIF461* NM_0192761 Hs0018604,m1 2.17 0.00012 3.22 0.079 1.35 0.83 EIF461 NM_0023750 Hs0014055,m1 3.082 0.0000 3.757 0.055 1.09 0.761 FAM126B NM_173822 Hs00545158,m1 3.19 0.01 5.52 0.03 1.26 0.906 FNTA NM_002031 Hs00745591,s1 5.40 0.0001 1.91 0.027 1.12 0.716 GABARAUT NM_002031 Hs0041245,m1 1.18 0.0016 5.03 0.0315 1.07 0.443 GITSCR2 NM_0190225 Hs0025603 m1 2.37 0.00045 1.56 0.021 1.56 0.044 GSN* NM_0190225 Hs002380276 m1 2.56 0.0002	EGR3	NM_004421	Hs00231780_m1	2.11	0.017	_	_	_	_
EI/R3:10 NM_003750 Hs0018670_ml 2.10 0.0034 1.55 0.067 1.29 0.295 EI/F4G1* NM_003760 Hs00191933_ml 2.42 0.0007 3.34 0.035 1.16 0.165 EI/F4G3 NM_00528 Hs00901425_ml 30.82 0.0008 3.77 0.055 1.09 0.761 EI/F3 NM_005228 Hs00557739_ml 3.86 0.0007 2.80 0.001 1.24 0.254 GABARAPL1* NM_031412 Hs00754591_s1 5.77 0.00042 1.83 0.008 1.47 0.525 GABPA NM_006836 Hs00412445_ml 1.18 0.00072 0.70 0.0015 1.07 0.443 GI7SCR2 NM_006836 Hs0041245_ml 1.83 0.002 0.52 0.0021 1.56 0.004 GSN* NM_006492 Hs00252163_ml 2.37 0.000045 1.56 0.0021 1.11 0.312 0.355 IF/FAA NM_002685 Hs00256057_ml 3.30	EIF2B4*	NM_172195	Hs00248984_m1	2.87	0.0026	1.13	0.048	0.58	0.739
Elf-461* NM 198241 Hs00191933_m1 2.42 0.0007 0.34 0.035 1.16 0.165 Elf-4G3 NM 0003760 Hs00186804_m1 2.17 0.00012 3.22 0.0079 1.35 0.83 FXI NM 005238 Hs00091425_m1 3.82 0.0001 5.52 0.03 1.26 0.906 FATA NM 002027 Hs00357739_m1 3.86 0.0007 2.80 0.001 1.24 0.254 GABARAPL1* NM 002031 Hs00745591_s1 5.27 0.00042 1.83 0.001 1.47 0.525 GARA NM 002031 Hs00412445_m1 1.18 0.00072 0.70 0.0015 1.07 0.443 GITSCR2 NM 105710 Hs0041245 Hs00255603_m1 2.37 0.00045 1.56 0.0021 1.56 0.004 GSN* NM 19825 Hs0026507_m1 2.30 0.00027 5.40 0.012 1.28 0.	EIF3S10	NM_003750	Hs00186707_m1	2.10	0.0034	1.55	0.067	1.29	0.295
Elf-4G3 NM 003760 Hs0018604 m1 2.17 0.00012 3.22 0.0079 1.35 0.83 EfS1 NM 005238 Hs00901425 m1 30.82 0.0008 37.57 0.055 1.09 0.761 FMTA NM 0.0037739 m1 3.86 0.0007 2.80 0.001 1.24 0.255 GABARAPL1* NM 0.00142 1.83 0.008 1.47 0.552 GABARAPL1* NM 0.00311 Hs00745591 s1 1.540 0.0001 1.91 0.027 1.12 0.716 GCN/L1 NM 0.0636 Hs00745591 s1 1.540 0.0001 1.91 0.027 1.02 0.716 GCN/L1 NM 0.0636 Hs00745591 s1 1.540 0.0001 1.91 0.027 0.303 1.31 0.375 GRAS NM 0.08425 Hs00255603 m1 2.37 0.000045 1.56 0.0011 1.11 0.312 GRAS NM 0.0553	EIF4G1*	NM_198241	Hs00191933_m1	2.42	0.0007	0.34	0.035	1.16	0.165
ETS1 NM_005238 Hs00901425_m1 30.82 0.0008 37.57 0.055 1.09 0.761 FAM126B NM_173822 Hs00357739_m1 3.89 0.0007 2.80 0.001 1.24 0.254 GABARAP11* NM_002027 Hs00357739_m1 3.86 0.0007 2.80 0.001 1.24 0.254 GABARAP11* NM_002021 Hs00357739_m1 3.86 0.0001 1.91 0.027 1.12 0.716 GCN1L1 NM_002031 Hs0074569_s1_s1 15.40 0.00012 0.70 0.0015 1.07 0.4433 GL7SCP2 NM_015710 Hs00414245 m1 5.49 0.00016 5.03 0.038 1.17 0.807 GNAS NM_08425 Hs00352112_m1 2.67 0.00021 1.56 0.0021 1.56 0.0021 1.32 0.352 GNAS NM_00452 Hs00351315_m1 0.66 0.019 2.67 0.012 1.28 0.255 HiFIA NM_001558 Hs003794121_m1	EIF4G3	NM_003760	Hs00186804_m1	2.17	0.00012	3.22	0.0079	1.35	0.83
FAMI26B NM_173222 Hs0054518_m1 3.19 0.01 5.52 0.03 1.26 0.906 FNTA NM_002027 Hs005739_m1 3.86 0.0007 2.80 0.001 1.24 0.254 GABAAPL1* NM_002031 Hs00745466_s1 5.27 0.00042 1.83 0.008 1.47 0.525 GABAA NM_002031 Hs00745591_s1 15.40 0.00015 5.03 0.038 1.17 0.443 GITSCR2 NM_006425 Hs00255603_m1 2.37 0.00045 1.56 0.0021 1.56 0.004 GNAS NM_080425 Hs00255063_m1 2.37 0.00045 1.56 0.0021 1.28 0.025 GTF2A2 NM_01530 Hs00153153_m1 0.66 0.019 2.67 0.012 1.28 0.255 IFNAR1 NM_002184 Hs0013800_m1 3.34 0.0011 1.67 0.034 1.36 0.617 ILBR NM_002185 Hs00238620_m1 3.34 0.0011 1.6	ETS1	NM_005238	Hs00901425_m1	30.82	0.0008	37.57	0.055	1.09	0.761
HVIA NM_10202/ Hs0035/39_m1 3.86 0.000/ 2.80 0.001 1.24 0.249 GABRAPL1* NM_031412 Hs0074468_s1 5.27 0.00042 1.83 0.008 1.47 0.525 GABRAPL1* NM_02031 Hs00745591_s1 1.540 0.0001 1.91 0.027 1.12 0.716 GCN1L1 NM_06836 Hs0041245_m1 1.18 0.00072 0.70 0.0015 1.07 0.443 GLTSCR2 NM_060425 Hs00055603_m1 2.37 0.000045 1.56 0.0021 1.56 0.0021 1.51 0.0021 1.31 0.375 HFIA NM_00492 Hs0005215313 1 0.66 0.019 2.67 0.012 1.28 0.255 IFNAR1 NM_00055 Hs00370/dm_11 3.30 0.00025 3.02 0.009 1.32 0.853 IL10RA* NM_00155 Hs00233820_m1 3.34 0.0011 1.67 0.34 1.36 0.617 IL3R	FAM126B	NM_173822	Hs00545158_m1	3.19	0.01	5.52	0.03	1.26	0.906
DARBAR/LT NN_0131412 HS0074486_s1 5.27 0.00042 1.83 0.008 1.47 0.525 GABPA NM_002031 HS00745591 s1 15.40 0.0001 1.91 0.027 1.12 0.716 GCN1L1 NM_006836 HS00412445_m1 1.18 0.00072 0.70 0.0015 1.07 0.443 GLTSCR2 NM_015710 HS00412445_m1 5.49 0.0016 5.03 0.038 1.17 0.807 GNAS NM_008425 HS0069276_m1 2.56 0.00037 2.40 0.01 1.11 0.312 GTF2A2 NM_004492 HS00362112_m1 1.08 0.002 0.52 0.039 1.33 0.375 HIF1A NM_001550 HS0037004_m1 1.34 9.87E-66 -	FNIA	NM_002027	Hs00357739_m1	3.86	0.0007	2.80	0.001	1.24	0.254
DABPA NM_002031 HS00/43931_S1 13.40 0.001 1.31 0.027 1.12 0.716 GUTL1 NM_06836 HS00112445_m1 1.18 0.00072 0.70 0.0015 1.77 0.8433 GLTSCR2 NM_015710 HS0014245_m1 2.37 0.000045 1.56 0.0021 1.56 0.004 GNAS NM_08492 HS002526_m1 2.56 0.00037 2.40 0.01 1.11 0.312 GTF2A2 NM_004492 HS0026057_m1 3.60 0.0025 3.02 0.009 1.32 0.853 IFNAR1 NM_00055 HS0037004 1.34 9.87E-06 -	GABARAPL1*	NIVI_031412	HSUU/44468_S1	5.27	0.00042	1.83	0.008	1.47	0.525
BGVR1/ NM_006836 risobri (2443_m) 1.16 0.0012 0.70 0.0015 1.07 0.443 GLTSCR2 NM_015710 Hiso014236_m1 5.49 0.0016 5.03 0.0021 1.56 0.004 GNAS NM_080425 Hiso014256_m1 2.56 0.00037 2.40 0.01 1.11 0.312 GTZAZ NM_004492 Hiso0362112_m1 1.08 0.002 0.52 0.009 1.32 0.853 IF/AA NM_001530 Hiso01530_m1 0.66 0.019 2.67 0.012 1.28 0.255 IF/AR1 NM_000629 Hiso0265057_m1 3.30 0.00025 3.02 0.009 1.32 0.853 IL10RA* NM_001586 Hiso0794121_m1 2.49 0.66 -	GABPA		HSUU/40091_S1	10.40	0.00072	1.91	0.027	1.12	0./10
Dirscriz INM1710 Insoutherstart 3.43 D.010 5.33 D.030 1.17 D.000 GNAS NM080425 Hs00255030m1 2.37 0.000045 1.56 0.0021 1.56 0.0021 GFT2A2 NM004492 Hs00352112_m1 1.08 0.002 0.52 0.039 1.13 0.375 HF1A NM001530 Hs00256557_m1 3.30 0.00025 3.02 0.009 1.32 0.853 IFDAR1 NM_000629 Hs00256507_m1 3.30 0.00025 3.02 0.009 1.32 0.853 IFDAR1 NM_000565 Hs0037004_m1 1.34 9.87E-06 -	GITSCR2	NM 015710	Hs00412445_III1 Hs00414236_m1	5.10	0.00072	0.70 5.03	0.0015	1.07	0.443
BIAD INM_000421 Insolution List Disord Insolution Disord Insolution Disord Insolution Disord Insolution Disord Insolution Disord Disord <thdisord< th=""> <thdisord< th=""> Disord<td>GNAS</td><td>NM 080425</td><td>Hs00255603 m1</td><td>5.45 2.37</td><td>0.0010</td><td>1 56</td><td>0.030</td><td>1.17</td><td>0.007</td></thdisord<></thdisord<>	GNAS	NM 080425	Hs00255603 m1	5.45 2.37	0.0010	1 56	0.030	1.17	0.007
DAT IMI_100003/10_mi L30 D3000 L10 D301 L11 D301 BTF2A2 NM_00492 Hs00362112_m1 1.08 0.002 0.52 0.039 1.13 0.375 H/F1A NM_001530 Hs00153153_m1 0.66 0.019 2.67 0.012 1.28 0.255 I/I.0RA* NM_00158 Hs0038704_m1 1.34 9.87E-06 - </td <td>GSN*</td> <td>NM 198252</td> <td>Hs00609276_m1</td> <td>2.57</td> <td>0.000043</td> <td>2 40</td> <td>0.0021</td> <td>1.50</td> <td>0.004</td>	GSN*	NM 198252	Hs00609276_m1	2.57	0.000043	2 40	0.0021	1.50	0.004
Infinition Infinition <thinfinition< th=""> Infinition Infinition<td>GTF2A2</td><td>NM 004492</td><td>Hs00362112 m1</td><td>1.08</td><td>0.002</td><td>0.52</td><td>0.039</td><td>1.13</td><td>0.375</td></thinfinition<>	GTF2A2	NM 004492	Hs00362112 m1	1.08	0.002	0.52	0.039	1.13	0.375
IFNAR1 NM_000629 Hs00265057_m1 3.30 0.00025 3.02 0.009 1.32 0.853 IL10RA* NM_001558 Hs00387004_m1 1.34 9.87E-06 -	HIF1A	NM 001530	Hs00153153 m1	0.66	0.019	2.67	0.012	1.28	0.255
IL10RA* NM_001558 Hs00387004_m1 1.34 9.87E-06 -	IFNAR1	NM 000629	Hs00265057 m1	3.30	0.00025	3.02	0.009	1.32	0.853
ILGR NM_000565 Hs00794121_m1 2.49 0.06 - <	IL10RA*	NM_001558	Hs00387004 m1	1.34	9.87E-06	_	_	_	_
IL6ST NM_002184 Hs00174360_m1 3.34 0.0011 1.67 0.034 1.36 0.617 IL7R NM_002185 Hs00233682_m1 0.52 0.032 -	IL6R	NM_000565	Hs00794121_m1	2.49	0.06	_	_	_	-
IL7R NM_002185 Hs00233682_m1 0.52 0.032 -	IL6ST	NM_002184	Hs00174360_m1	3.34	0.0011	1.67	0.034	1.36	0.617
JAK1NM_002227Hs00233820_m112.730.00000815.510.041.050.623KHSRP*NM_003685Hs00269352_m11.820.000260.350.00161.220.55MAPK9NM_139070Hs00177102_m11.580.0451.290.050.950.213METTL3NM_019852Hs00219820_m11.300.00010.770.011.170.215MRPL23*NM_021134Hs00221699_m12.620.0010.800.0291.360.79MRPS6NM_032476Hs0066808_m12.750.0251.870.0141.340.451MRRFNM_138777Hs00751845_s18.230.00167.490.0021.350.962MSN†NM_002444Hs00792607_mH4.850.00167.490.0021.350.962MTMR6NM_004685Hs00395064_m16.600.00254.120.0481.120.15NFKB1NM_003988Hs00211582_s158.317.00E-04NR1D2NM_005702Hs0017582_s158.317.00E-04NR1D2NM_005702Hs00198648_m12.920.0017.340.021.490.579NUFIP2NM_020772Hs00325168_m12.370.0012.000.0461.310.929PAPOLANM 032632Hs00413685 m10.620.000210.450.0011.370.672	IL7R	NM_002185	Hs00233682_m1	0.52	0.032	_	-	_	-
KHSRP*NM_003685Hs00269352_m11.820.000260.350.00161.220.55MAPK9NM_139070Hs00177102_m11.580.0451.290.050.950.213METTL3NM_019852Hs00219820_m11.300.00010.770.011.170.215MRPL23*NM_021134Hs00221699_m12.620.0010.800.0291.360.79MRPS6NM_032476Hs0066808_m12.750.0251.870.0141.340.451MRRFNM_138777Hs00751845_s18.230.00042.840.031.220.25MSN†NM_002444Hs00792607_mH4.850.00167.490.0021.350.962MTMR6NM_004685Hs00395064_m16.600.00254.120.0481.120.15NFKB1NM_003998Hs00271582_s158.317.00E-04NR1D2NM_005126Hs0023309_m12.060.00161.560.00060.730.96NTE*NM_006702Hs00198648_m12.920.0017.340.021.490.579NUFIP2NM_02632Hs00413685 m10.620.000210.450.0011.370.672	JAK1	NM_002227	Hs00233820_m1	12.73	0.000008	15.51	0.04	1.05	0.623
MAPK9 NM_139070 Hs00177102_m1 1.58 0.045 1.29 0.05 0.95 0.213 METTL3 NM_019852 Hs00219820_m1 1.30 0.0001 0.77 0.01 1.17 0.215 MRPL23* NM_021134 Hs00221699_m1 2.62 0.001 0.80 0.029 1.36 0.79 MRPS6 NM_032476 Hs00606808_m1 2.75 0.025 1.87 0.014 1.34 0.451 MRRF NM_138777 Hs0751845_s1 8.23 0.0004 2.84 0.03 1.22 0.25 MSN† NM_002444 Hs00792607_mH 4.85 0.0016 7.49 0.002 1.35 0.962 MTIMR6 NM_004685 Hs00395064_m1 6.60 0.0025 4.12 0.048 1.12 0.15 NFKB1 NM_003998 Hs00211653_m1 5.01 0.001 1.28 0.41 NHLH1 NM_005589 Hs00271582_s1 58.31 7.00E-04 - - - - </td <td>KHSRP*</td> <td>NM_003685</td> <td>Hs00269352_m1</td> <td>1.82</td> <td>0.00026</td> <td>0.35</td> <td>0.0016</td> <td>1.22</td> <td>0.55</td>	KHSRP*	NM_003685	Hs00269352_m1	1.82	0.00026	0.35	0.0016	1.22	0.55
METTL3 NM_019852 Hs00219820_m1 1.30 0.0001 0.77 0.01 1.17 0.215 MRPL23* NM_021134 Hs00221699_m1 2.62 0.001 0.80 0.029 1.36 0.79 MRPS6 NM_032476 Hs00606808_m1 2.75 0.025 1.87 0.014 1.34 0.451 MRRF NM_138777 Hs00751845_s1 8.23 0.0004 2.84 0.03 1.22 0.25 MSN† NM_002444 Hs00792607_mH 4.85 0.0016 7.49 0.002 1.35 0.962 MTIMR6 NM_004685 Hs00395064_m1 6.60 0.0025 4.12 0.048 1.12 0.15 NFKB1 NM_003998 Hs00231653_m1 5.01 0.001 1.28 0.41 NHLH1 NM_005589 Hs00231653_m1 2.06 0.00016 1.56 0.0006 0.73 0.96 NTE* NM_00572 Hs00198648_m1 2.92 0.001 7.34 0.02 1.49	МАРК9	NM_139070	Hs00177102_m1	1.58	0.045	1.29	0.05	0.95	0.213
MRPL23* NM_021134 Hs00221699_m1 2.62 0.001 0.80 0.029 1.36 0.79 MRPS6 NM_032476 Hs00606808_m1 2.75 0.025 1.87 0.014 1.34 0.451 MRRF NM_138777 Hs00751845_s1 8.23 0.0004 2.84 0.03 1.22 0.25 MSN† NM_002444 Hs00792607_mH 4.85 0.0016 7.49 0.002 1.35 0.962 MTMR6 NM_004685 Hs00395064_m1 6.60 0.0025 4.12 0.048 1.12 0.15 NFKB1 NM_003998 Hs0021653_m1 5.01 0.001 1.28 0.41 NHLH1 NM_005589 Hs00271582_s1 58.31 7.00E-04 - - - - NR1D2 NM_005126 Hs0023309_m1 2.06 0.00016 1.56 0.0006 0.73 0.96 NTE* NM_006702 Hs00198648_m1 2.92 0.001 7.34 0.02 1.49 0.579 <td>METTL3</td> <td>NM_019852</td> <td>Hs00219820_m1</td> <td>1.30</td> <td>0.0001</td> <td>0.77</td> <td>0.01</td> <td>1.17</td> <td>0.215</td>	METTL3	NM_019852	Hs00219820_m1	1.30	0.0001	0.77	0.01	1.17	0.215
MHPS6 NM_032476 Hs00606808_m1 2.75 0.025 1.87 0.014 1.34 0.451 MRRF NM_138777 Hs00751845_s1 8.23 0.0004 2.84 0.03 1.22 0.25 MSN† NM_002444 Hs00792607_mH 4.85 0.0016 7.49 0.002 1.35 0.962 MTMR6 NM_004685 Hs00395064_m1 6.60 0.0025 4.12 0.048 1.12 0.15 NFKB1 NM_003998 Hs00231653_m1 5.01 0.00027 5.01 0.001 1.28 0.41 NHLH1 NM_005589 Hs00271582_s1 58.31 7.00E-04 - - - - NR1D2 NM_005126 Hs0023309_m1 2.06 0.00016 1.56 0.0006 0.73 0.96 NTE* NM_006702 Hs0198648_m1 2.92 0.001 7.34 0.02 1.49 0.579 NUFIP2 NM_022632 Hs00413685 m1 0.62 0.0021 0.45 0.001<	MRPL23*	NM_021134	Hs00221699_m1	2.62	0.001	0.80	0.029	1.36	0.79
INIT_138/1/1 HSUU/51845_S1 8.23 0.0004 2.84 0.03 1.22 0.25 MSN † NM_002444 Hs00792607_mH 4.85 0.0016 7.49 0.002 1.35 0.962 MTMR6 NM_004685 Hs00395064_m1 6.60 0.0025 4.12 0.048 1.12 0.15 NFKB1 NM_003998 Hs0021653_m1 5.01 0.00027 5.01 0.001 1.28 0.41 NHLH1 NM_005589 Hs00231653_m1 5.01 0.0006 0.73 0.96 NR1D2 NM_005126 Hs0023309_m1 2.06 0.00016 1.56 0.0006 0.73 0.96 NTE* NM_006702 Hs00198648_m1 2.92 0.001 7.34 0.02 1.49 0.579 NUFIP2 NM_0202772 Hs00325168_m1 2.37 0.001 2.00 0.046 1.31 0.929 PAPOLA NM 032632 Hs00413685 m1 0.62 0.00021 0.45 0.001 1.37 0.672	INKPS6	NIVI_032476	Hs00606808_m1	2.75	0.025	1.87	0.014	1.34	0.451
INVI_U02444 INVI_U023555 Invit_U144 Invit_U144 Invit_U02444 Invit_U144 Invit_U1444 Invit_U144 <t< td=""><td></td><td>NIVI_138///</td><td>HSUU/51845_S1</td><td>8.23</td><td>0.0004</td><td>2.84</td><td>0.03</td><td>1.22</td><td>0.25</td></t<>		NIVI_138///	HSUU/51845_S1	8.23	0.0004	2.84	0.03	1.22	0.25
NML. NM_003998 Hs00231653_m1 5.01 0.0025 4.12 0.048 1.12 0.15 NFKB1 NM_003998 Hs00231653_m1 5.01 0.00027 5.01 0.001 1.28 0.41 NHLH1 NM_005589 Hs00271582_s1 58.31 7.00E-04 - 0.96 0.96				4.85 6.60		/.49	0.002	1.35	0.962
NHLM1 NM_005589 Hs00271582_s1 58.31 7.00E-04 -	IVI I IVINO NEKR1		ПSUU392004_MI Но002216521	0.0U F.01	0.0025	4.1Z	U.U48 0.001	1.12	0.15
NR1D2 NM_005126 Hs00233309_m1 2.06 0.00016 1.56 0.0006 0.73 0.96 NTE* NM_006702 Hs00198648_m1 2.92 0.001 7.34 0.02 1.49 0.579 NUFIP2 NM_020772 Hs00325168_m1 2.37 0.001 2.00 0.046 1.31 0.929 PAPOLA NM 032632 Hs00413685 m1 0.62 0.00021 0.45 0.001 1.37 0.672	NHI H1	NIN 002230	Henn271522 e1	5.01		5.01	0.001	1.20	0.41
NTE* NM_006702 Hs00198648_m1 2.92 0.001 7.34 0.02 1.49 0.579 NUFIP2 NM_020772 Hs00325168_m1 2.37 0.001 2.00 0.046 1.31 0.929 PAPOLA NM 032632 Hs00413685 m1 0.62 0.00021 0.45 0.001 1.37 0.672	NR1D2	NM 005126	Hono233200 m1	2.06	0.000-04	1 56	0.0006	 0.73	
NUFIP2 NM_020772 Hs00130645_m1 2.37 0.001 2.00 0.046 1.31 0.929 PAPOLA NM 032632 Hs00413685 m1 0.62 0.00021 0.45 0.001 1.37 0.672	NTF*	NM 006702	Hs00198648 m1	2.00	0.00010	7.34	0.000	1 49	0.50
PAPOLA NM 032632 Hs00413685 m1 0.62 0.00021 0.45 0.001 1.37 0.672	NUFIP2	NM 020772	Hs00325168 m1	2.37	0.001	2.00	0.046	1.31	0,929
	PAPOLA	NM_032632	Hs00413685_m1	0.62	0.00021	0.45	0.001	1.37	0.672

Continued

Table 2 Continued

	GenBank		CFS/ME ($n = 1$	11)	Q-CFS/ME (n=	=6)	Endogenous depression (n=14)	
Gene symbol	accession number	Taqman assay ID‡	Fold difference	p Value	Fold difference	p Value	Fold difference	p Value
PDCD2*	NM_002598	Hs00751277_sH	5.38	0.008	_	_	1.62	0.029
PDCD6	NM_013232	Hs00737034_m1	2.54	0.0002	2.19	0.01	1.69	0.015
PEX16*	NM_004813	Hs00191337_m1	3.98	0.0061	3.32	0.028	0.68	0.776
PGM2	NM_018290	Hs00217619_m1	4.28	0.000001	3.50	0.0014	1.07	0.308
PIK3R1	NM_181523	Hs00236128_m1	4.04	0.005	2.60	0.01	1.14	0.208
PKN1*	NM_213560	Hs00177028_m1	4.58	0.0003	3.95	0.01	1.03	0.887
POLR2G*	NM_002696	Hs00275738_m1	2.71	0.001	1.00	0.039	0.77	0.916
PPP2R5C	NM_002719	Hs00604902_m1	4.65	0.013	8.21	0.045	1.30	0.906
PRKAA1	NM_006251	Hs01562315_m1	4.19	0.0002	2.18	0.001	1.29	0.56
PRKAR1A	NM_002734	Hs00267597_m1	3.55	0.0000004	2.31	0.0001	1.23	0.83
PUM2	NM_015317	Hs00209677_m1	2.73	0.00078	2.33	0.002	1.35	0.82
RAP2C	NM_021183	Hs00221801_m1	6.74	0.013	4.37	0.043	1.46	0.69
REPIN1	NM_013400	Hs00274221_s1	4.51	0.00001	2.13	0.01	1.26	0.41
RNF141	NM_16422	Hs00212656_m1	6.49	0.00000079	7.44	0.0003	1.19	0.411
SELENBP1	NM_003944	Hs00187625_m1	10.57	0.001	7.00	0.02	1.06	0.104
SFXN1	NM_022754	Hs00224259_m1	1.69	0.041	0.69	0.037	1.00	0.24
SHPRH	NM_173082	Hs00542737_m1	0.56	0.02	0.69	0.03	1.00	0.303
SNAP23	NM_003825	Hs00187075_m1	7.00	0.0006	3.17	0.01	1.10	0.132
SORL1	NM_003105	Hs00268342_m1	1.67	4.10E-08	_	_	_	-
SOS1	NM_005633	Hs00362308_m1	1.02	0.001	1.02	0.037	1.27	0.52
TAF11	NM_005643	Hs00194573_m1	1.17	0.001	0.00	0.02	1.40	0.57
TCF3	NM_003200	Hs00413032_m1	2.40	0.03	1.63	0.068	1.29	0.86
TDP1	NM_018319	Hs00217832_m1	3.12	0.001	3.16	0.01	1.12	0.83
TNFRSF1A	NM_001065	Hs00533560_m1	12.37	0.004	18.52	0.03	0.86	0.279
UBTF	NM_014233	Hs00610729_g1	6.38	0.002	2.40	0.011	1.09	0.297
USP38	NM_032557	Hs00261419_m1	3.35	0.01	4.98	0.078	1.43	0.367
WAPAL	NM_015045	Hs00386162_m1	3.94	0.003	3.69	0.026	1.17	0.44
WDR26	NM_025160	Hs00228535_m1	1.36	0.0008	0.71	0.01	1.48	0.95

*Genes found in pilot study.¹³

†Genes found in study using differential display/PCR.⁷

‡Taqman assays were those pre-designed by Applied Biosystems, Warrington, UK.

CFS/ME, chronic fatigue syndrome/myalgic encephalomyelitis.

Leicester, subtype C; London, subtype C, then subtype G; New York, subtype E, then subtypes G, A, C and F; Dorset, subtypes A, F, B. Subtype A was prominent in New York, Birmingham and Dorset; subtype B was prominent in Dorset; subtype C was prominent in Bristol, London and New York; subtype D was prominent in Bristol and London; subtype E was prominent in New York; subtype F was prominent in Dorset and New York; subtype G was prominent in New York; subtype H was prominent in Dorset (figure 2C).

Microbial infections

The presence and titre of specific antibodies (IgM and IgG) to four treatable microbial infections that are well recognised as triggers of CFS/ME were also determined in serum samples; these were EBV, enterovirus, parvovirus B19 and *C burnetii*. The seroprevalence (proportion of subjects who were positive for specific IgG) of each of these infections was typical of the general population: EBV (based on VCA IgG), 88%; enterovirus, 49%; parvovirus B19 (based on viral protein 2 IgG), 74%; *C burnetii* (based on phase I or II IgG), 10%. Of the 11 patients who had *C burnetii* IgG, five were patients whose CFS/ME disease had been triggered by laboratory-documented Q fever.

CFS/ME patients with acute infection with one or more of these agents (IgM or acute phase IgG) were also detected: EBV (based on VCA IgM) (n=3), enterovirus (n=6), parvovirus B19 (n=1), *C burnetii* (based on phase II IgG) (n=12). Of the 12 patients who were positive for *C burnetii* phase II IgG, five had Q-CFS/ME. There were no acute infections detected in the normal group.

Regarding EBV serology, there were also associations between CFS/ME subtype and both EBV VCA IgM titre (p=0.0038) and EBV EBNA IgG titre (p=0.0011) (figure 2D). Using the EBV markers VCA IgM, VCA IgG, early antigen IgG and EBNA IgG, we determined the EBV serostatus of infection for each subject (ie, seronegative, primary infection/re-activation, late phase of infection). Among 111 of these CFS/ME patients, there were 11 seronegative, 61 primary/re-activation and 39 late phase of infection, as compared with the normal group, in which there was one seronegative, eight primary/re-activation, and 19 late phase of infection (χ^2 =9.91, degrees of freedom=2, p=0.007) (figure 2E).

The distribution of CFS/ME patients by EBV serostatus category (seronegative, primary/re-activation and late phase of infection) across the eight CFS/ME genomic subtypes is shown in figure 2E. In the normal controls, the predominant category of EBV serostatus was late phase of infection, whereas in the CFS/ME subtypes, the predominant category of EBV serostatus was primary/re-activation, which was seen in subtypes A, B, C, D, F and H. Subtype G had equal numbers of primary/re-activation and late phase, and subtype E had a predominance of late phase subjects, but also had five seronegative subjects. This distribution was found to be almost statistically significant (χ^2 =25.9, degrees of freedom=16, p=0.055).

EBV-associated genes in each CFS/ME subtype

Within the CFS/ME-associated gene signature of 88 human genes, there were 12 that have recognised associations with EBV infection; these associations have been summarised previously.⁷

Table 3 Fold-difference values for 88 genes in each of eight subtypes (A–H) in 114 subtyped patients with chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME). Genes without values for the subtypes are those for which there was missing data for one or more subtypes. Bold type indicates genes targeted by existing drugs and those CFS/ME subtypes in which fold-difference values of 1.5 were found

Gene	Genbank	CFS/WE subtype							
symbol	accession	A	В	C	D	E	F	G	Н
ABCD4	NM 020323	1.40	0.60	0.81	4.40	10.61	7.02	1.32	0.93
ACTR3	NM 005721	10.17	8.03	4.34	25.73	13.95	6.75	27.57	5.35
AKAP10	NM_007202	3.83	3.25	1.68	8.39	5.59	6.61	4.89	1.64
ANAPC11	NM_016476	2.53	2.66	4.34	3.74	1.59	4.16	2.27	
ANAPC5	NM_016237	1.13	1.26	4.49	1.92	0.78	0.00	2.96	2.95
APP	NM_201413	0.63	1.73	1.42	0.56	4.64	0.84	0.84	2.58
ARL4C	NM_005737	6.62	7.40	2.72	5.85	7.82	8.76	6.68	2.81
ARPC5	NM_005717	2.55	0.81	1.53	5.65	4.87	3.41	4.92	3.01
ARSD	NM_001669	0.64	1.17	1.51	4.60	2.62	0.26	0.12	2.19
ATP6V1C1	NM_001695	1.99	3.80	3.38	2.43	2.06	0.72	3.63	4.55
BCOR	NM_017745	1.42	2.22	1.78	2.30	6.52	1.13	2.65	2.57
BMP2K	NM_198892	8.96	4.83	2.62	16.42	7.76	3.78	11.57	2.06
BRMS1	NM_015399	1.31	4.38	2.44	1.53	4.62	7.06	2.57	3.83
CD2BP2	NM_006110	3.89	1.37	2.21	6.77	4.52	1.33	5.99	3.55
CD47	NM_198793	2.60	6.90	3.37	3.66	4.06	0.95	4.13	2.75
CEP350	NM_014810	3.50	4.47	3.66	5.30	4.50	1.22	5.04	2.04
CITED2	NM_006079	6.43	6.84	1.97	4.95	6.02	4.40	5.50	3.42
CMTM6	NM_017801	3.10	3.70	0.69	10.04	7.81	4.77	1.71	0.73
CREBBP	NM_004380	7.11	1.62	1.09	13.34	5.46	2.61	8.99	3.62
CRK	NM_016823	1.83	5.57	1.26	2.82	4.89	1.03	2.25	2.02
CTBP1	NM_001328	4.95	4.98	1.05	8.62	15.44	3.43	2.42	2.73
CXCR4	NM_003467	13.47	2.18	2.03	28.10	17.57	1.48	10.29	3.57
EBI2	NM_004951	5.67	1.41	2.31	14.93	5.99	0.76	0.42	4.47
EGR1	NM_001955	0.49	2.85	2.42	0.30	0.27	1.00	1.98	2.96
EGR3	NM_004421	0.95	1.33	0.46		5.36		1.39	0.98
EIF2B4	NM_172195	1.44	0.52	1.33	6.00	3.48	0.15	1.69	2.08
EIF3S10	NM_003750	1.43	4.42	2.10	1.72	1.48	1.25	2.83	6.16
EIF4G1	NM_198241	1.13	3.47	3.52	0.99	1.39	2.30	4.53	14.27
EIF4G3	NM_003760	2.40	0.79	0.77	5.46	27.42	1.09	3.62	1.55
EIST	NM_005238	35.12	4.63	4.16	52.65	30.36	17.54	24.17	6.91
FAIVI I ZOB	NIVI_173822	2.04	0.03	0.91	10.18	5.51 E 07	2.59	1.31	0.71
FIVIA		1.39	5.72	2.99	3.88	5.07	2.14 2.50	1.88	4.00
CADANAFLI	NIM_002021	0.00	0.42	21.02	2.93	6.49 5.71	2.00	12 20	5.74
CCN111	NM_006836	0.80	1.40	1 01	0.44	5.25	25.05	1 50	220
GITSCR2	NM_000030	0.00	2.46	0.53	0.44 1 91	5.05	1.13	1.33	0.80
GNAS	NM_080425	1 72	1 13	1.81	3 62	3 18	1.96	3 47	2 20
GSN	NM 198252	1.72	1.10	1.61	3.82	2 36	1.50	3 51	5.81
GTF2A2	NM_004492	0.71	0.53	1.03	0.52	2.00	0.48	1 65	0.87
HIF1A	NM_001530	2 04	0.87	0.82	5 14	4 22	1 66	4 65	1.35
IFNAR1	NM_000629	1.86	0.17	0.79	3.55	5.53	1.41	7.17	0.91
IL10RA	NM 001558	1.12	2.68	0.74			2.31	1.01	1.76
IL6R	NM_000565	2.19		2.47			2.78	2.67	
IL6ST	NM_002184	2.61	1.49	4.81	3.14	3.07	2.67	3.76	
IL7R	NM_002185	1.43	0.91	1.66			1.46	2.06	1.33
JAK1	NM_002227	9.72	7.84	3.29	28.88	9.80	11.17	18.75	6.19
KHSRP	NM_003685	0.42	1.03	0.91	0.61	1.15	0.75	1.07	1.62
МАРК9	NM 139070	1.16		1.62	1.68	1.83	0.00	2.51	
METTL3	NM_019852	0.81	1.38	0.64	1.72	2.92	1.08	1.81	0.68
MRPL23	NM_021134	2.34	0.97	1.23	4.15	4.20	1.56	2.56	2.06
MRPS6	NM_032476	2.03	0.92	2.93	3.05	7.75	1.77	1.95	2.00
MRRF	NM_138777	10.11	13.30	3.96	2.03	9.28	1.33	9.70	7.34
MSN	NM_002444	3.20	1.66	1.81	9.47	7.86	3.13	8.58	2.12
MTMR6	NM_004685	3.71	11.73	2.61	7.73	7.33	2.67	14.97	2.58
NFKB1	NM_003998	3.74	0.91	0.65	8.83	6.51	4.10	7.30	1.55
NHLH1	NM_005589	26.32	37.92	49.09			66.39	51.25	126.29
NR1D2	NM_005126	1.40		2.31	4.57	3.69	1.27	2.50	2.29
NTE	NM_006702	1.75	0.31	0.89	3.92	4.37	1.30	3.87	1.43
NUFIP2	NM_020772	1.55	1.90	1.81	2.83	2.10	1.84	3.50	2.31

Continued

Table 3 Continued

Gene	Genhank	CFS/ME subtype							
symbol	accession	Α	В	C	D	E	F	G	Н
PAPOLA	NM_032632	0.47	0.52	0.29	0.79	4.73	0.58	1.25	0.32
PDCD2	NM_002598	3.83	3.44	2.94	5.00	5.74	5.88	5.36	7.37
PDCD6	NM_013232	1.96	2.72	2.53	2.16	4.79	2.69	2.85	2.16
PEX16	NM_004813	2.10	16.10	2.04	8.88	5.92	0.00	2.90	2.80
PGM2	NM_018290	3.23	3.62	2.16	5.99	5.72	4.89	6.13	3.36
PIK3R1	NM_181523	2.06	4.55	0.58	7.17	7.31	0.95	5.48	0.82
PKN1	NM_213560	2.25	3.76	1.27	6.67	6.09	2.39	8.14	2.84
POLR2G	NM_002696	1.09	5.58	2.06	1.91	6.01	2.60	3.82	2.91
PPP2R5C	NM_002719	2.78	2.62	1.28	9.50	6.14	1.63	7.87	0.84
PRKAA1	NM_006251	2.14	4.10	3.42	3.53	4.17	6.87	7.13	3.11
PRKAR1A	NM_002734	2.05	1.85	2.56	4.14	3.66	2.41	6.35	5.41
PUM2	NM_015317	2.81	1.69	0.87	2.85	5.04	1.49	3.84	2.22
RAP2C	NM_021183	2.69		2.56	4.75	5.35	25.28	10.61	1.95
REPIN1	NM_013400	2.37	3.85	3.12	1.92	6.62	8.53	7.06	6.52
RNF141	NM_16422	3.64	0.64	2.10	9.85	11.45	6.08	10.83	2.09
SELENBP1	NM_003944	7.88	9.51	3.46	22.18	7.54	2.84	7.65	5.70
SFXN1	NM_022754	1.37	3.46	1.58	1.35	1.40	1.67	1.99	1.72
SHPRH	NM_173082	0.82		1.07	0.21	7.17	0.00	0.64	
SNAP23	NM_003825	3.46	0.45	1.89	12.62	13.33	4.15	10.19	1.43
SORL1	NM_003105	1.40	1.91	1.60			2.01	1.52	2.47
SOS1	NM_005633	0.70		0.81	1.69	1.09	0.29	1.61	0.90
TAF11	NM_005643	0.56		1.35	1.05	2.13	0.00	0.21	1.23
TCF3	NM_003200	2.00	0.94	1.08	2.83	3.96	3.54	2.52	2.65
TDP1	NM_018319	1.60	5.50	1.38	4.80	11.55	0.96	4.24	
TNFRSF1A	NM_001065	11.96	4.07	1.36	18.01	13.25	3.30	17.81	2.06
UBTF	NM_014233	2.88	3.59	1.82	6.03	6.46	4.81	10.91	6.44
USP38	NM_032557	2.66		0.71	7.40	4.27	7.18	2.94	1.02
WAPAL	NM_015045	2.97		5.13	3.63	2.78	1.24	6.04	2.97
WDR26	NM_025160	0.84	0.09	0.63	2.18	1.53	0.80	2.74	1.23

The fold-difference values for each of these 12 genes in each CFS/ME subtype/normal were analysed for significant associations using ANOVA. With all 12 genes, there was a trend which did not reach significance (df=89,p=0.119). However, when *GABPA* and *EGR1* were removed from the analysis, the remaining 10 genes showed a striking association with subtype (ANOVA, df=73, p=0.0001) (figure 2F).

DISCUSSION

We have previously reported the differential expression of 88 human genes in CFS/ME and evidence of clinically relevant

subtypes.^{7 8} In the present study, we have confirmed this differential expression in 62 additional and previously untested CFS/ME patients. Combining the previous cohort and the new cohort, we have found evidence of eight genomic CFS/ME subtypes with marked differences in global functioning, clinical symptoms, levels of severity and geographical distribution. The function of these genes and their networks has been published previously.⁷

We have addressed the question of the specificity of these 88 genes to CFS/ME, by testing drug-free patients with endogenous depression. The fact that only five of these genes were



Figure 1 Absolute fold-difference values (mean relative quantity (RQ) in patients with chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME)/ mean RQ in normal controls) for each of 88 CFS/ME-associated genes in eight CFS/ME subtypes (A–H).



Figure 2 (a) Medical Outcomes Survey Short Form-36 (SF36) domain and total scores for each chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) subtype: physical function, physical role (RP), bodily pain (BP), general health (GH), vitality (VIT), social functioning (SF), emotional role (RE), mental health (MH) and total score (Total). (b) Scores indicating occurrence and severity of 11 clinical symptoms for each CFS/ME subtype: headache (HA), sore throat (ST), swollen glands (GLA), cognitive defect (COG), muscle pain (MP), joint pain (JP), muscle weakness (MW), post-exertional malaise (PEM), sleep problems (SLE), fainting/dizziness (F/D), gastrointestinal complaints (GI), numbness/tingling (N/T), spatial span (SSP), verbal recognition memory (VRM). (c) Histogram showing the numbers of CFS/ME patients of each subtype occurring in each of the six geographical locations. (d) Epstein–Barr virus (EBV) antibody titres (viral capsid antigen (VCA) IgM, VCA IgG, early antigen (EA) IgG, Epstein–Barr nuclear antigen (EBNA) IgG) in each CFS/ME subtype and the normal comparison group. (e) Distribution of categories of EBV serostatus (seronegative, primary/re-activation, late phase of infection) in the CFS/ME subtypes, A–H, in CFS/ME (all subtypes combined) and in normal controls. (f) Log (base 2) of fold-difference values of 10 human genes known to be important in EBV infection, in eight CFS subtypes (A–H).

abnormally expressed in these patients, as compared with normal controls, supports the view that CFS/ME and endogenous depression are biologically distinct, and that the psychological features of CFS/ME are in fact secondary to the pathogenesis.

It is particularly interesting that five of six CFS/ME patients with Q-CFS/ME clustered in the same subtype (subtype A). As these patients had had CFS/ME for several years, this finding suggests that they have a common underlying theme, which may be stable for a long time after the onset of disease. In view of this, and as various genes within this human gene signature are closely linked with EBV infection (*NFKB1*, *EGR1*, *ETS1*, *GABPA*, *CREBBP*, *CXCR4*, *EBI2*, *HIF1A*, *JAK1*, *IL6R*, *IL7R*, *PIK3R1*) and enterovirus infection (*EIF4G1*), we tested the serum samples for markers for four treatable microbial infections that are well recognised to trigger CFS/ME (EBV, enterovirus, parvovirus B19 and *C burnetii* (the agent of Q fever)) with the hypothesis that these genomic CFS/ME subtypes may represent host responses to particular infectious agents.

One patient with subtype E had acute parvovirus B19 at the time of sampling. This patient's symptoms were typical of CFS/ME, but this is not unexpected as parvovirus B19 is a recognised trigger for

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CFS/ME.¹³ The importance of testing for these infections is illustrated here, as we have shown previously that B19-CFS/ME is highly responsive to treatment with intravenous immunoglobulin.¹⁴

Six patients had acute enterovirus infections (of undetermined serotype) at the time of sampling, but there was no subtype relationship, as two patients were found to have each of subtypes A, E and G. Enteroviruses have long been recognised to trigger CFS/ME,¹⁵ and they have been detected in the stool¹⁶ and stomach epithelium¹⁷ in CFS/ME patients. Detection in the stomach has been shown to be associated with gastrointestinal symptoms in CFS/ME patients.¹⁷ However, in the present study, patients of subtypes A, E and G did not show gastrointestinal symptoms more often than the other patients.

Twelve CFS/ME patients and one normal subject had IgG to *C burnetii* phase II antigen, suggesting possible acute infection. Five of these CFS/ME patients were among those with Q-CFS/ME. The patients in whom these antibodies were detected had subtypes A, B, D, E and G. Therefore, apart from the patients with Q-CFS/ME (whose CFS/ME disease onset was associated with laboratory-documented acute Q fever), there were no subtype-specific relationships with *C burnetii* antibodies.

Take-home messages

- Expression of 88 human genes was confirmed as being significantly different between patients with chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) and normal controls.
- Gene expression in patients with endogenous depression was similar to that in normal controls.
- CFS/ME patients can be grouped into genomic subtypes which have different clinical phenotypes.
- There was evidence of subtype-specific relationships for Epstein—Barr virus and enterovirus, the two most common triggers for CFS/ME.

The subtype associations with EBV and EBV-linked genes are interesting, suggesting differences in the role of EBV and consequent host responses in the different subtypes. The finding of a noticeably large proportion of CFS/ME patients who were EBV seronegative (10%), compared with 4% in the normal group, was quite surprising given the strong link between EBV and CFS/ME. The fact that five of these 11 seronegative cases were subtype E is interesting, but remains unexplained at present.

It has been recognised for some time that subtypes of CFS/ME exist, and it has been thought that these subtypes may, at least in part, reflect particular aetiological factors.¹⁸ A symptom-based approach has had some success in identifying musculoskeletal, inflammatory and neurological subtypes¹⁹; however, these groups had only minor differences in overall functional severity in contrast with those of the present study.

It is intriguing that it is possible to identify CFS/ME subtypes on the basis of expression values for these 88 genes, and even more so that these subtypes have distinct clinical phenotypes, with marked differences in the occurrence of particular symptoms and their severity. However, what precise sequence of events is involved in the genesis of the gene signatures in each subtype remains to be elucidated. Further work is required to validate and develop these findings.

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Competing interests None.

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Patient consent Obtained.

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