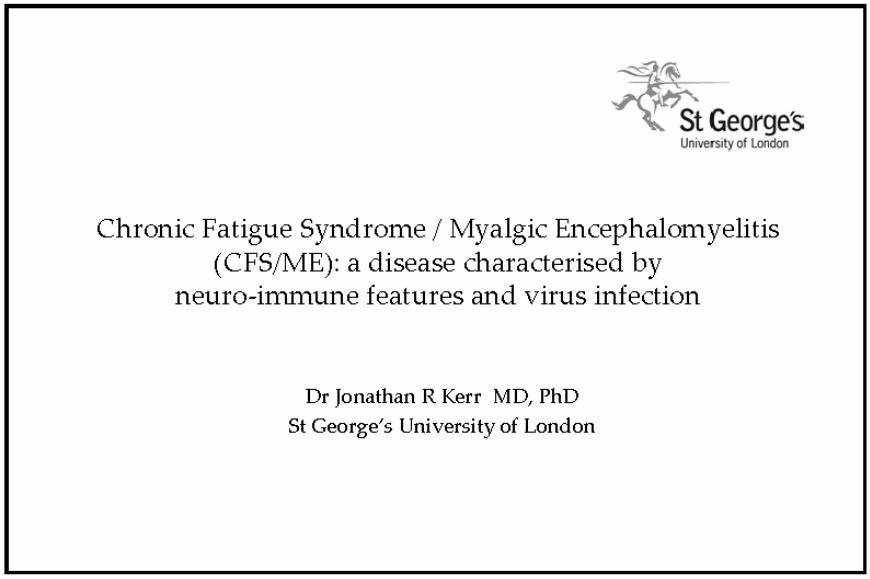
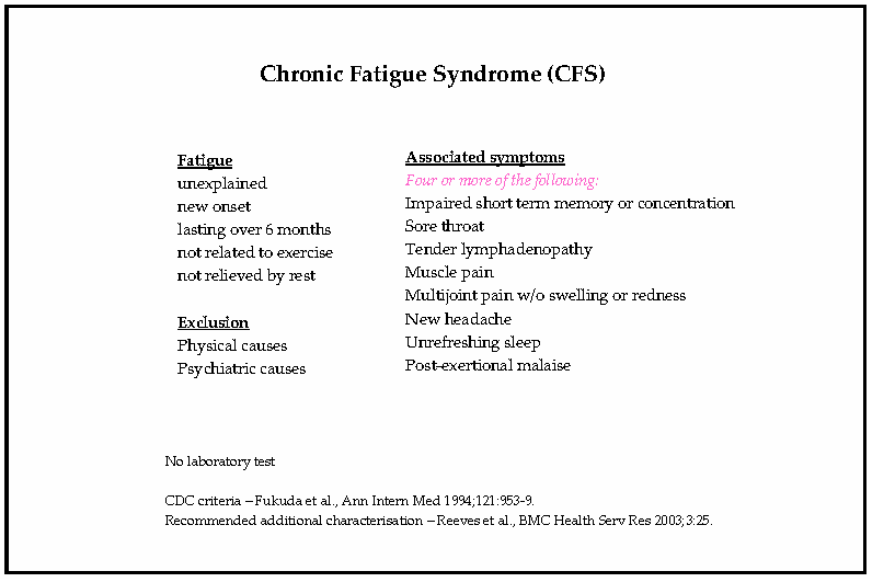
Appendix

Presentation 1 – Jonathan Kerr





Chronic Fatigue Syndrome (CFS)

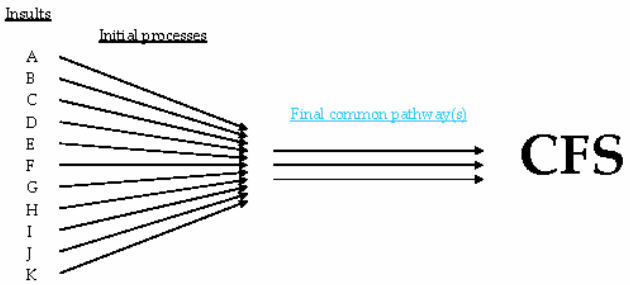
Epidemiology

Prevalence of 0.5%
More common in females (6:1)
Sudden onset
Preceding virus infection ('flu-like illness, outbreaks, specific viruses)
Exposure to toxins, chemicals, pesticides, vaccination
Pre-existing emotional stress

Chronic Fatigue Syndrome (CFS)

Studies of Pathogenesis

Immune system -	↑IC's, ↑IgG, ↑B cells, ↓NK Th2 phenotype cytokine dysregulation / chronic immune activation
Infection -	virus, bacterium
Nervous system -	paresis, visual loss, ataxia, confusion abnormal metabolism of 5-HIAA, A-V, 5-HT, PRL brain scan abnormalities
Endocrine system -	slight ↓HPA axis
Cardiovascular system -	vasodilatation
Psychological function -	depression & anxiety
Genetic predisposition -	deduced from twin studies



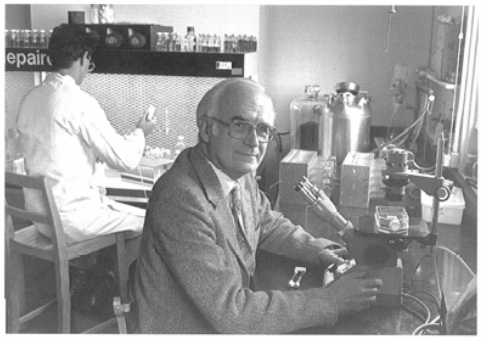
Our Research Strategy

Gene expression signature
Viruses
Protein biomarkers
(Treatments)

How did we develop this strategy?

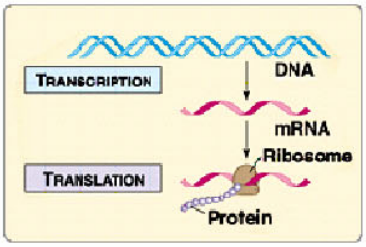
Recognition of need to understand pathogenesis
(human / virus gene expression is important)
(gene screening techniques)

Recognition of need for a diagnostic test
(detection of protein biomarkers)

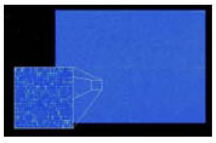


Dr David AJ Tyrrell CBE, FRS

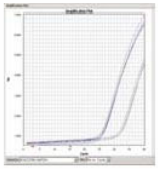
Basic cell processes



Microarray / PCR study

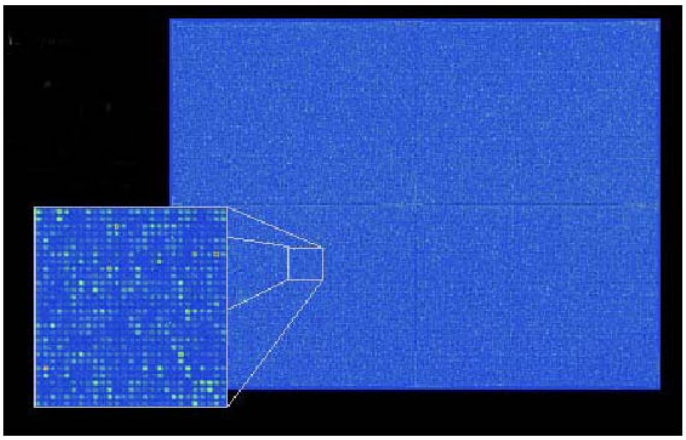


Microarray (~23,000 genes)
27 CFS patients / 54 normal blood donors



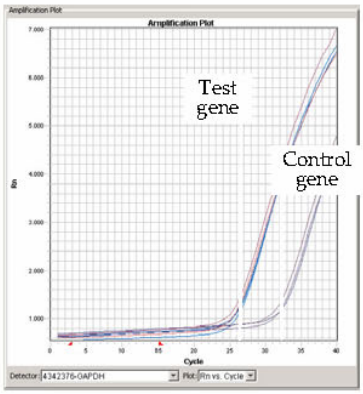
Real-time PCR (182 genes)
57 CFS patients / 60 normal blood donors

83 genes confirmed



Affymetrix U133+2 gene array
47,000 human genes

Real-time PCR



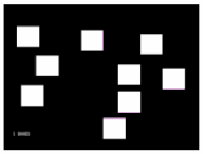
Phase-1 cont.

Phase-1 continued Study

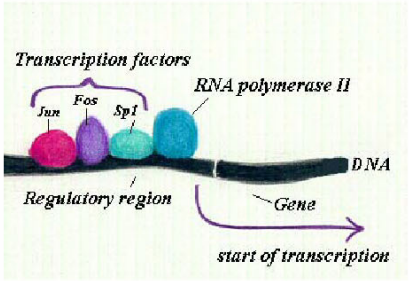
Clinical aspects

1. Diagnosis according to CDC criteria
   (Fukuda et al, 1994)

2. Assessment of health & associated symptoms:
   - CIDI
   - Cantab
   - McGill
   - Chalder
   - MOS-SF36
   - SPHERE
   - Pittsburgh



Gene regulation

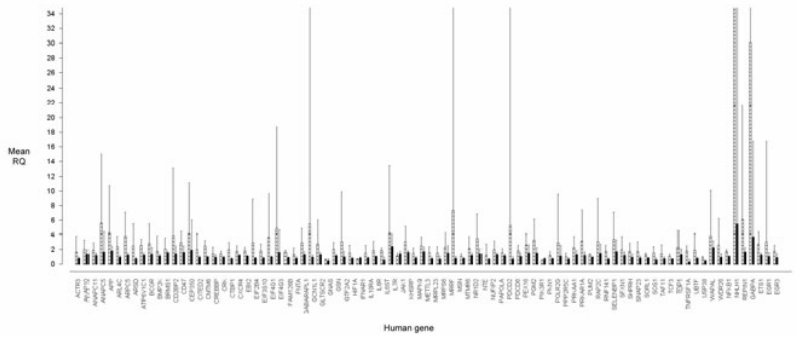


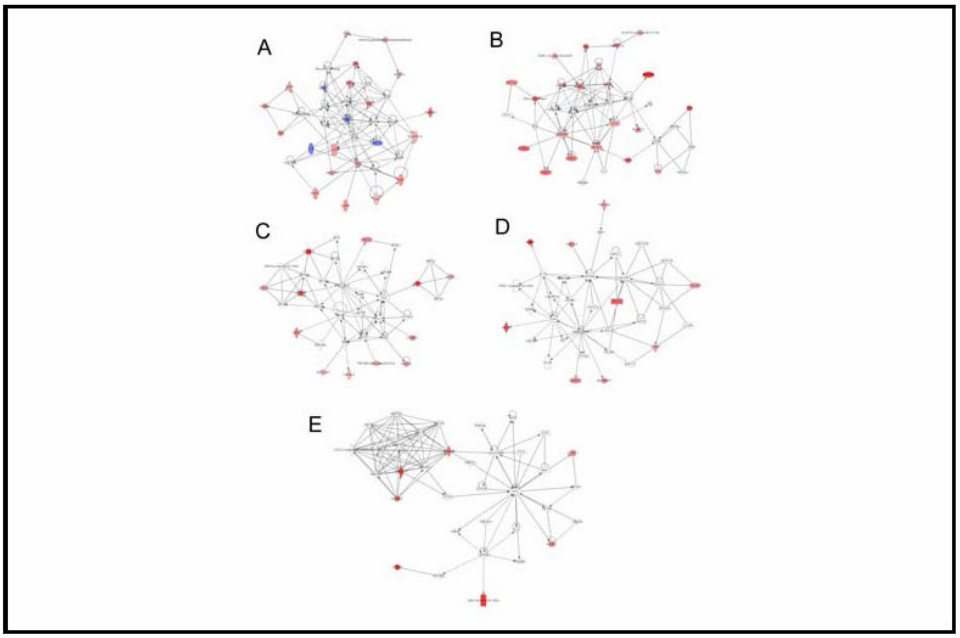
13 transcription factors over-represented

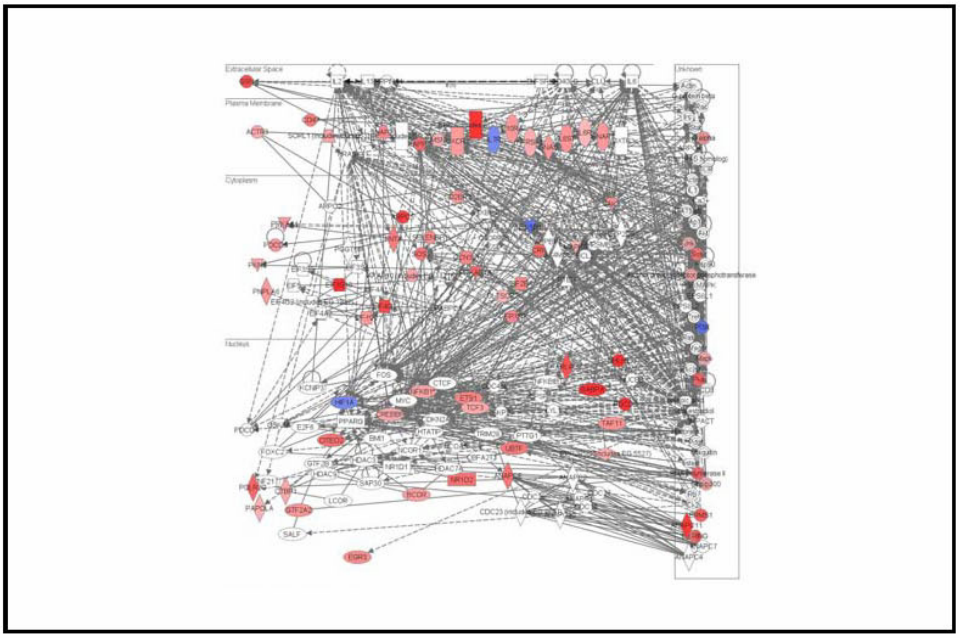
Transcription factors

EGR-1	**2.82**	**0.01480**
EGR-3	**1.92**	**0.01690**
ETS1	**2.11**	**0.00001**
GABPA	**8.06**	**0.00032**
NHLH1	**11.51**	**0.00074**
REPIN1	**3.62**	**0.00001**
NFKB1	**1.59**	**0.00004**
EGR-2	2.26	0.09934
GTF3A	1.27	0.18480
SP1	1.37	0.10086
Egr-4	x	x
REST	x	X
BRLF1		

89 CFS/ME-associated genes




A
B
C
D
E



Diseases and disorders

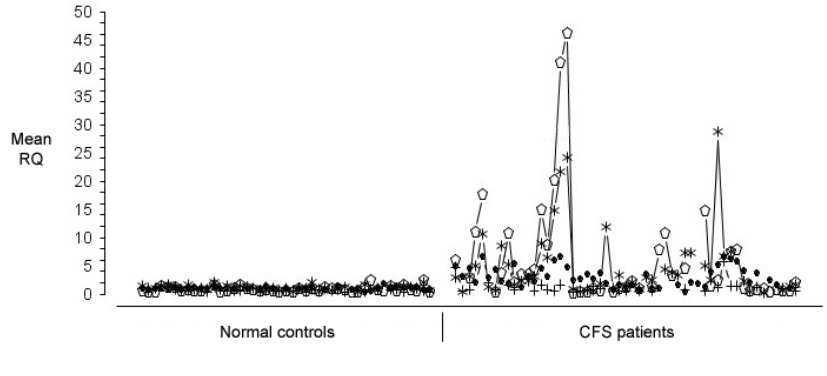
Haematological disease
Immunological disease
Cancer
Dermatological disease
Neurological disease

Molecular and cellular functions

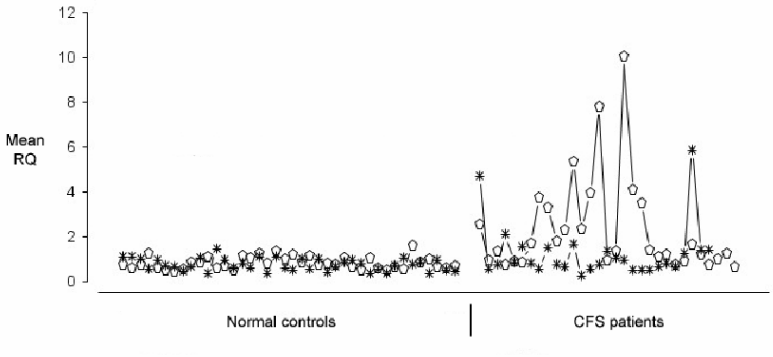
Cellular development
Cell death
Gene expression
Cellular growth and proliferation
Cellular assembly and organisation

Physiological system development and function

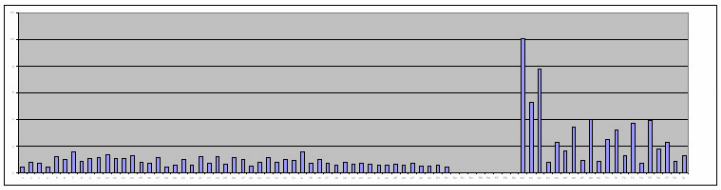
Haematological system development and function
Immune and lymphatic system development and function
Tissue morphology
Organismal survival
Immune response

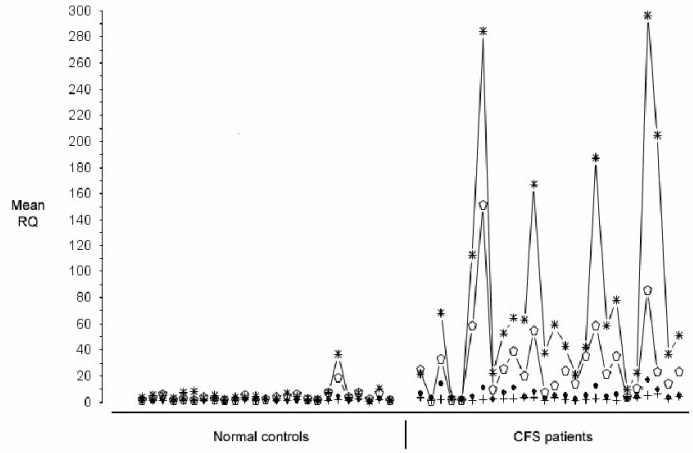


GABARAPL1 (*), MRRF (□), NTE (+), PRKAR1A (•)



EBI2 (□) and NTE (*)





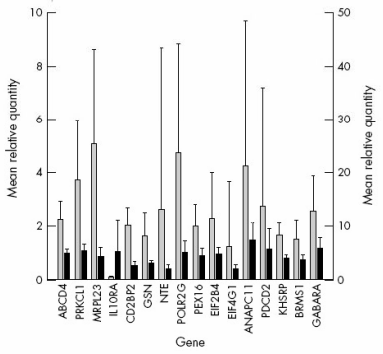
NHLH1 (*), GABPA (□), REPIN1 (•), ETS1 (+)

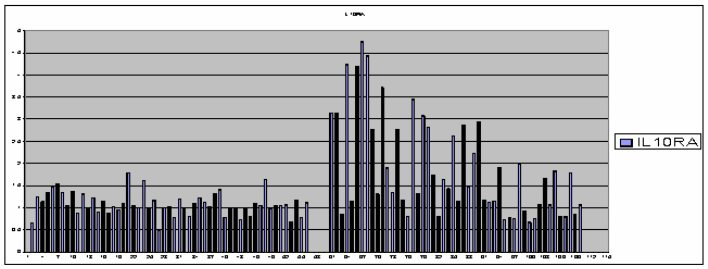
ORIGINAL ARTICLE

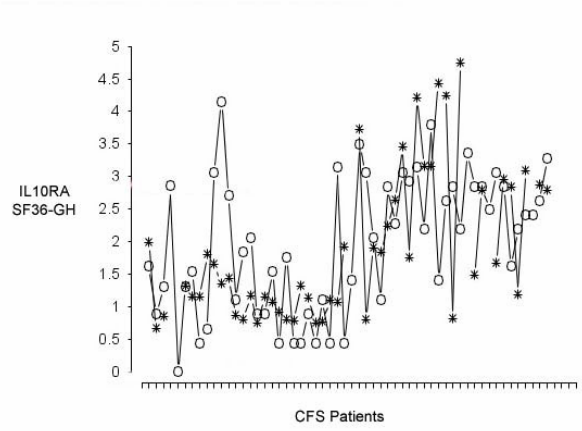
Gene expression in peripheral blood mononuclear cells from patients with chronic fatigue syndrome

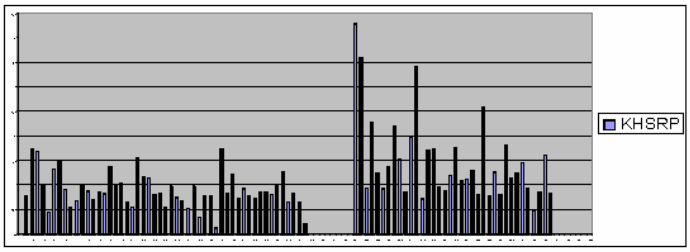
N Kaushik, D Fear, S C M Richards, C R McDermott, E F Nuwaysir, P Kellam, T J Harrison, R J Wilkinson, D A J Tyrrell, S T Holgate, J R Kerr

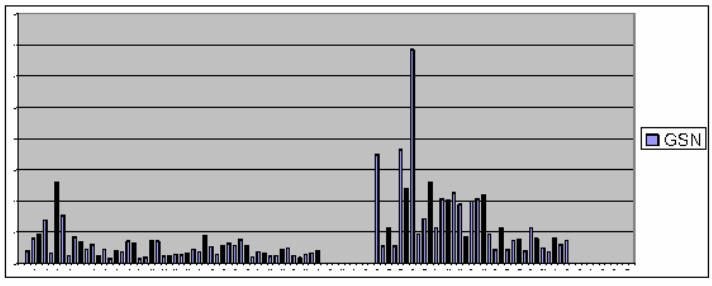
J Clin Pathol 2005;58:826–832. doi: 10.1136/jcp.2005.025718




IL10RA


IL10RA
SF36-GH
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
CFS Patients


KHSRP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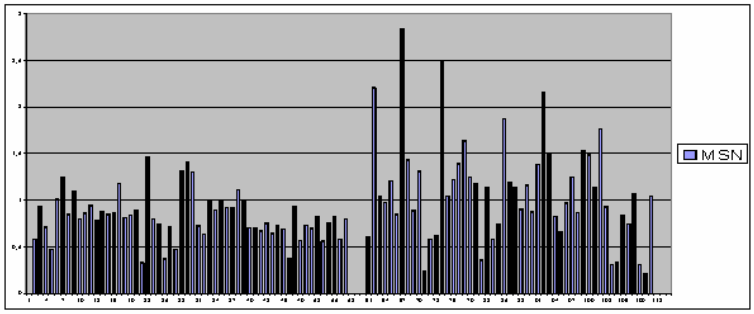
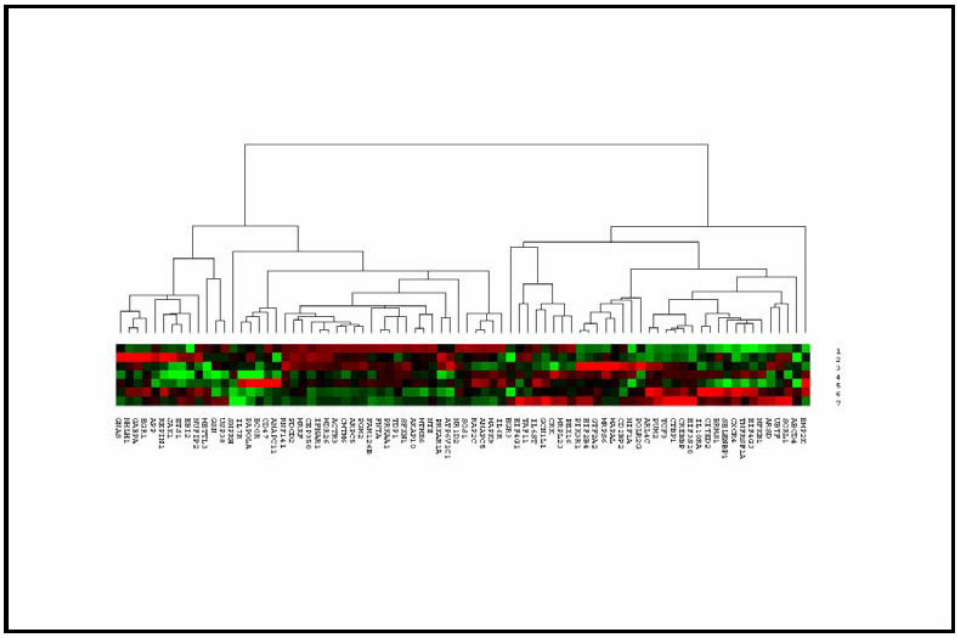
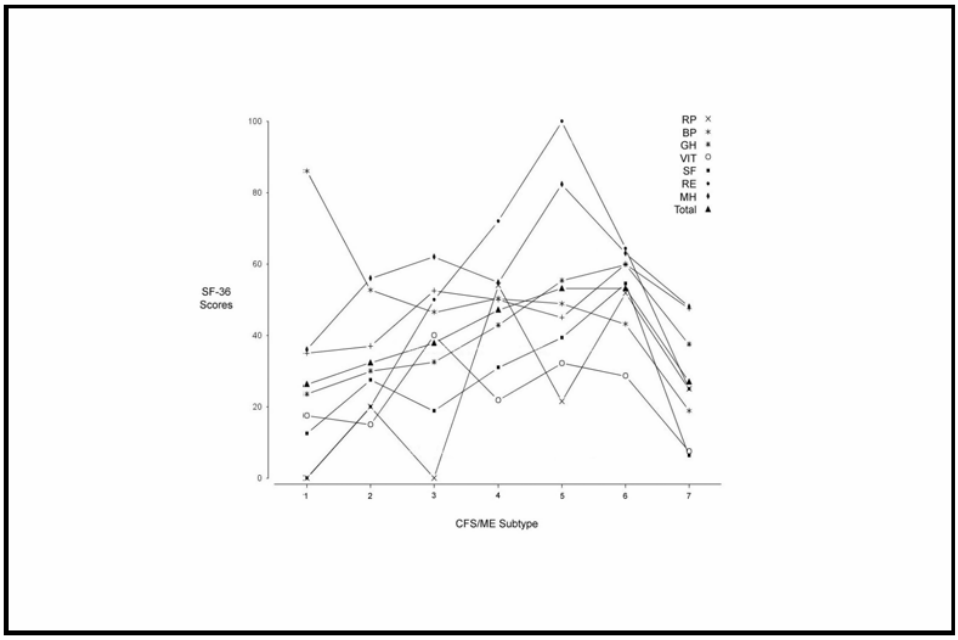
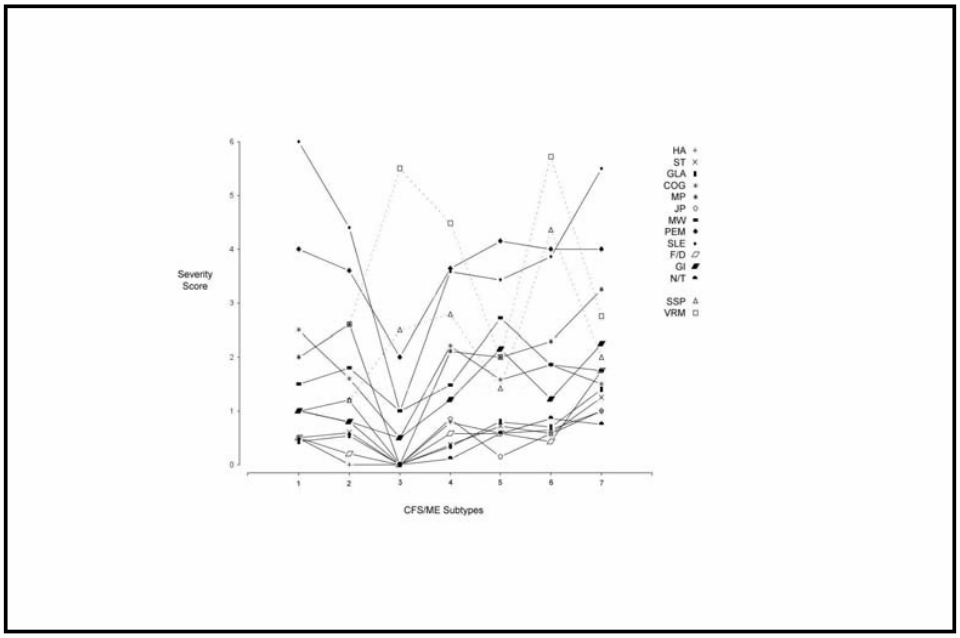


CFS/ME-associated gene	EBV gene	Mechanism
NFKB1	LMP1	Upregulation
EGR1	BRLF1 BZLF1	Upregulation, EGR1 required for reactivation
ETS1	LMP2A	Upregulation
GABPA	LMP2A	ETS1 upregulation leads to GABPA upregulation
CREBBP	BRLF1 BZLF1	BRLF1 and BZLF1 interact with CREBBP
CXCR4	EBNA2 LMP1	Downregulation and reduced CXCR4-dependent migration
EBI2	?	Upregulated 200 fold in EBV infected cells
HIF1A	LMP1	Upregulation
JAK1	?	JAK-STAT activation in PTLD
IL6R	?	Upregulation
IL7R	?	Downregulation
PIK3R1	EBNA2A	Upregulation
BRLF1	BRLF1	**




RP
BP
GH
VIT
SF
RE
MH
Total
SF-36
Scores
100
80
60
40
20
0
1
2
3
4
5
6
7
CFS/ME Subtype


Severity Score
CFS/ME Subtypes
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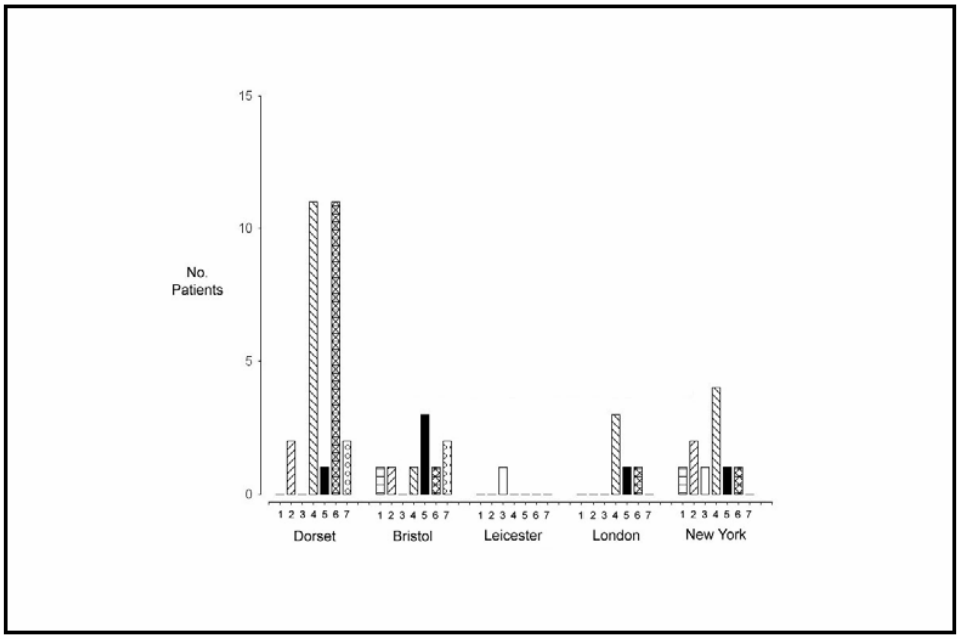
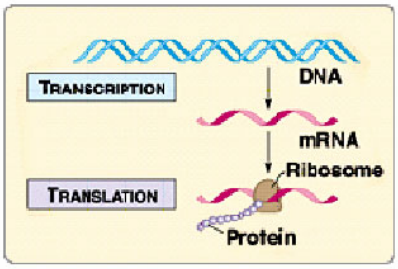
ST
GLA
COG
MP
JP
MW
PEM
SLE
F/D
GI
N/T
SSP
VRM


No. Patients
Dorset
Bristol
Leicester
London
New York

Gene signature of CFS: current priorities

Subtype confirmation
Variation with time
Specificity
? Overlap with GWI
Expression in different WBC subsets

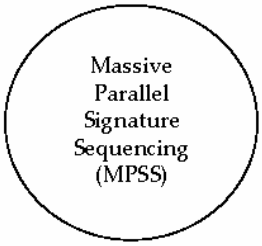
microRNAs



Viruses in CFS

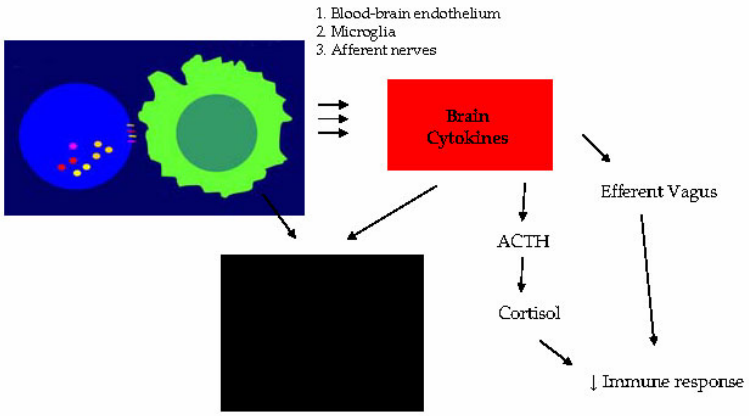
1. Acute / chronic
2. Reactivation

Study of viral gene expression in CFS



Virus genome	MPSS TPM CFS	MPSS TPM Normal	P value
1	24	0	4.22E-03
2	161	59	9.38E-05
3	342	128	1.14E-08
4	342	128	1.14E-08
5	35	12	4.00E-02
6	42	0	1.80E-04
7	926	643	3.90E-05
8	48	10	5.68E-03

Immune system / brain interaction



Proteins

Increased prolactin, HGH, IL-6, TNF-a, IFN-g, TGF-b1, B-microglobulin

Changes in DHEA, DHEA-S, Cortisol, ACTH, prolactin, serum metals, oxidative stress markers, plasma-free tryptophan, melatonin

CSF Corticotropin-releasing factor (CRF) in FM (McLean, 2006)

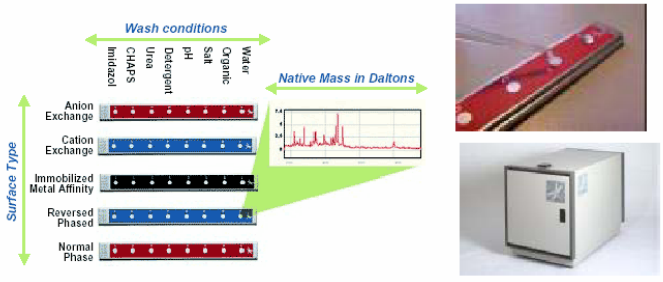
Increased b-alanine secretion in subgroup of CFS pts (Hannestad, 2006)

Vis-NIR spectroscopy differentiates CFS vs normal (Sakudo, 2006)

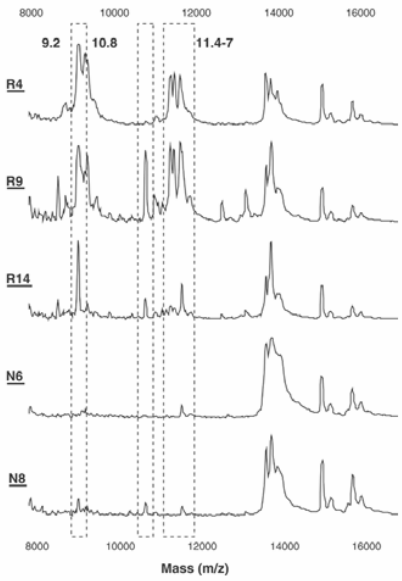
CFS-related proteome in human CSF (alpha-1-macroglobulin, amyloid precursor-like protein-1, keratin 16, gelsolin, orosomucoid 2, pigment epithelium derived factor) (Baraniuk, 2005)

Serum protein biomarkers of CFS

Surface-enhanced laser desorption and ionisation – time of flight
SELDI-TOF



Biomarkers
SELDI-PC



Proteins

15 protein biomarkers
Identification by 2D gel electrophoresis and mass spectrometry
Further CFS patients, Normal persons, and disease controls (specificity)
Select combination which provides best sensitivity and specificity

Take Home Points

Gene signature in CFS - subtypes
Protein biomarkers
Novel viruses
(Novel approaches to specific therapy)

Acknowledgements

CLINICAL COLLABORATORS
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Annette Whittemore, Nevada, USA
Dr David Bell, NY, USA

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